W. David Wick · Otto O. Yang

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The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines

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Preface

It has often been said that nothing in biology is comprehensible except in the light of evolution.[1] HIV/AIDS provides the latest—and most sobering—proof of this adage.

Evolution in the microbial world burst into scientific and public-health consciousness 60 years ago, with the appearance of antibiotic-resistant bacteria. In this well-known episode, the bugs developed resistance to penicillin in a matter of months after it was introduced (around 1947) in the clinic; some bacterial strains are now resistant to every antibiotic we possess. If you become infected by one, you might as well be living in the 19th century. HIV is presumably second only to bacteria in the rate it evolves: roughly a million times faster than mammalian evolution. Only a half-dozen decades after the probable introduction of HIV into the human population (most likely from chimps in Western Africa), variants of HIV have diverged to such an extent that if we were discussing something other than viruses we would call them separate species. But HIV evolution is not just of interest to biologists; it matters profoundly to doctors and their patients. In 1987, the first drug with an impact on HIV was tested in a clinical trial. At first, it appeared that a treatment, if not a cure, was at hand; but, 6 months later, the treated patients were found to be progressing to AIDS as fast as untreated. The virus had acquired mutations, negating the drug's benefit in every subject. It required a combination of three drugs to (partially) overcome the resistance problem.

The immune system fares better than a single drug, suppressing the infection for a decade on average; but the virus eventually learns to evade specific responses and escapes control. The implications of this evolutionary battle for vaccine design cannot be overstated. (After 25 years of research, we still have no licensed vaccine for HIV/AIDS.)

[1] Usually attributed to evolutionary biologist Theodosius Dobzhansky.

The motivation for writing this book derived from a scientific disagreement with our colleagues about why, and how, HIV evolves in a patient after infection. Most published discussions of HIV evolution *in vivo*[2] derive from "population genetics," a field founded in the 1920s by J.B.S. Haldane, Sewall Wright, and especially Ronald A. Fisher[3] (who published the first book about evolution written by a mathematician, in 1930). We will argue that the correct picture derives from a different tradition: that of "ecological genetics." The distinction between these perspectives has to do with enemies. Population genetics postulates that evolution goes on among rival species (or variants of a given species) who compete for niches or resources in a fixed, unreactive environment. The canonical system studied by population geneticists for the last 80 years has been, and remains, fruit flies raised in cages.[4] By contrast, ecological genetics emphasizes that all living organisms have, besides rivals, enemies—i.e., predators and parasites—and escaping them can be the driving force in evolution. In the case of HIV, either the virus or its principal foe—the human immune system—can be regarded as the predator (since in fact HIV targets certain immune-system cells); we prefer to imagine the so-called "killer" T-cells as the predators and HIV as the prey. However you think about it, HIV infecting a human body is not like fruit flies implanted in a jar.

[2] "*In vivo*" is a Latin phrase biologists use to refer to observations or experiments made in living organisms. Here we are contrasting it to "in populations," meaning (for HIV) the study of the epidemic around the globe. Of course, the antithesis of "*in vivo*" is properly "*in vitro*," meaning studies performed in a test tube or glass well.

[3] The same Fisher who introduced the p-value, the exact test, and randomization in clinical trials. Fisher was not satisfied with merely being one of the greatest statisticians who ever lived, but evidently wished to be the Einstein of evolutionary biology as well.

[4] For example, see Nature **467**: 587 (September 2010). The authors report on an evolutionary experiment with *Drosophila*, aka fruit flies, in which deep gene sequencing indicated that "... unconditionally advantageous alleles rarely arise, [or] are associated with small fitness gains ..." the usual conclusion from these types of experiments (see Chapter 1, Section 1.9). We find it remarkable that population geneticists do not entertain the notion that creatures living in cages, isolated from predators, for thousands of generations did not acquire new adaptive mutations in their genomes precisely because little advantage could be thereby obtained. One imagines that evolutionary pressures on free-living fruit flies are quite different.

To state the issue plainly: Fisher made a colossal mistake by leaving enemies out of his thinking and his models.[5] Fisher did have his reasons, of course; indeed, a kind of divorce accompanied the birth of mathematical biology. Ecologists and population geneticists parted ways, each chanting the mantra: "Ecology is short, but evolution is long." That is, changes in an ecosystem (e.g., a new variety of lion moving into your neighborhood, say) are fast (making an impact in your lifetime), but evolutionary changes (e.g., primates developing an upright-walking stance and ability to hurl spears) are slow (taking thousands of generations). Thus, when modeling one kind of change, the thinking went, you could ignore the other. The split has persisted to this day.

But HIV resists absorption into either system. It takes around 3 weeks for the immune system to contain an HIV infection and barely more time for HIV to escape by mutation from a single drug or immune response. The timescales of "ecology" (if we may be permitted to use the term when referring to what goes on in our bodies) and retroviral evolution are identical. Assuming the short-*vs*.-long mantra applies anywhere, it surely does not to HIV *in vivo*.

The historical parallel can be found in the work of field biologists such as E. B. Ford (who studied, among other things, how moths varied their spots to escape predation by birds[6]) later in the 20th century, which developed into the new paradigm Ford dubbed "ecological genetics." In reference to theory, the clash between population genetics and ecological genetics is perhaps most dramatically represented in "Van Valen's law," also known as the the "Red Queen Hypothesis."[7] It was proposed in 1973 by a professor of evolutionary biology at the University of Chicago, Leigh Van Valen.[8] Van Valen's key

[5]In addition, Fisher's "Fundamental Theorem of Natural Selection" was nonsensical, and by his choice to fix demographic population size he missed the possibility of quasi-species. See Chapters 1 and 8.

[6]Ford published the first book on the subject, bestowing its moniker, in 1964 [95].

[7]The reference is to the Alice books. At one point Alice remarks that she is tired of running and the Red Queen replies, "Now, *here*, you see, it takes all the running *you* can do to stay in the same place. If you want to get somewhere else, you must run at least twice as fast as that."

[8]1935–2010; New York Times obit, 10/31/2010. The NYT obituary noted that Van Valen could never get his idea published in an established journal (presumably due to opposition from population geneticists), and had to resort to founding his own and publishing himself. The citation is [299]; a scanned copy can be found on the Van Valen website.

insight was that the Fisherian conception of evolution as a friendly hill-climbing competition was misbegotten. The correct metaphor is military: evolution is an arms race.

Both population genetics and the theory we will expound here are formulated using mathematical models. Unlike population genetics, ecological genetics—because of its more complicated understanding of events—is not generally given abstract formulations. Not surprisingly, the modeling described here is specific to the context of HIV *in vivo* and the immune response. But this is not unusual; mathematicians have been at work modeling biology in particular settings for 9 decades or so. In ecology, models of predator-and-prey appeared in the 1920s, and of pathogen-and-host by the 1950s. Models of HIV infection and the immune response were proposed in the late 1990s. We have used such models in order to, among other things, estimate the number of HIV-infected cells a "killer" T-cell can kill every day, in the body of an infected patient. In 2006, several colleagues and an author used a stochastic version of our model to predict that T-cells alone could abort a retroviral infection, a very controversial conception that was verified a few years later in vaccine experiments in monkeys. Likewise, the motivation for modeling HIV evolution *in vivo* was to generate novel predictions for experiments in animals or interventions in the clinic. The reader will find roughly a dozen such predictions in the pages of this book.[9] Here we mention only the most important one: it may be possible to design a vaccine that blocks HIV from escaping the immune system's clutch.

We wished to write for a heterogeneous audience, including researchers, physicians, patients, teachers, and students; indeed, anyone with an interest in HIV, the immune system, evolution, or modeling in biology or medicine. This desire lead to the inevitable decision to split the book into two parts. Part I contains the biology, description of the models, some easily-grasped formulas, and the conclusions, expressed plainly in the text or in computer-generated figures. There are no prerequisites to read this part. As in any scientific field, technical jargon is unavoidable, so, in addition to defining each term when introduced, we have added a Glossary of frequently used phrases and acronyms after the text. Part II contains the Greek-lettered equations. We have included a brief tutorial about modeling and exercises

[9]See the entries listed under "predictions" in the Index.

in the chapters. We discuss the philosophy of modeling—a topic which can be neglected by the practitioner only at great risk—in the Introduction to Part I.

We wish to thank several colleagues for their help in writing this book: Peter Gilbert, for many discussions about biostatistics, and Fusheng Li, ditto for biological data mining. Finally, W.D.W. thanks the Fred Hutchinson Cancer Research Center and the US National Institutes of Health for supporting in part the research described here (*via* grant 1R01AI05428). The opinions expressed in this book are solely those of the authors and do not represent the views of the National Institute of Allergy and Infectious Diseases or the NIH.

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September 2011[10]

[10]About dates: the bulk of this work was written before 2007. As a result, some of the material, in particular the figures and parameter tables, could be updated to reflect new information from experiments reported after that date. Comments about updating can be found in the Notes to the chapters. However, we are not aware of the demise of any of the principal conclusions of this book on the basis of more recent experiments, although that is a common fate of theories in biology.

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Chapter 1

Introduction

1.1 The HIV Epidemic and Its Origins

In 1984, two groups of investigators—Luc A. Montagnier's at the Pasteur Institute in Paris and Robert C. Gallo's at the National Institutes of Health in Bethesda, Maryland—announced the discovery of the human immunodeficiency virus (HIV in this book, although H.I.V. in the New York Times).[1] The clinical manifestations of a new disease, acquired immunodeficiency syndrome (AIDS), had been observed 3 years earlier, in a population of men who have sex with men, in several cities of the United States.[2] At this writing, HIV has established a global pandemic, among the worst in recorded history; 33 million people are currently infected worldwide, with 2.5 million new infections each year.[3] At least 25 million have died of AIDS. The primary modes

[1]The 2008 Nobel Prize in Medicine was awarded to Montagnier and Françoise Barré-Sinoussi for the discovery of the human immunodeficiency virus and Harald zur Hausen for discover of the human papilloma virus, which causes cervical cancer. The Karolinska Institute's opinion about the discoverers of HIV is controversial, coming after a long and acrimonious dispute between the French and American camps.

[2]A brief account of a cluster of cases in Los Angeles of combined pneumocystis pneumonia and Kaposi's sarcoma appeared in the Morbidity and Mortality Weekly, published by the Centers for Disease Control (CDC), in June, 1981. The combination of a rare form of pneumonia with a rare skin cancer alerted epidemiologists that a new infectious disease may have appeared.

[3]These are revised estimates by UNAIDS, released on November 20, 2007. The current estimate of 33.2 (confidence interval, [30.6,36.1]) million infected replaced an earlier estimate of 39.5 ([34.1,47.1]); the UN agency's revisions reflects lowered estimates primarily of the epidemic in India.

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of transmission at this time are unprotected sexual activity and intravenous drug users sharing syringes. Although the government's top-ranking doctor, Edward Brandt Jr., said shortly after the virus was discovered that he was optimistic that a vaccine would be available by 1987, 20 years later no vaccine against HIV/AIDS has been licensed.

HIV is a retrovirus that primarily targets certain cells of the human immune system.[4] The prefix "retro" refers to an aspect of the virus's lifecycle in the body ("*in vivo*" for biologists, who prefer the Latin): in order to replicate, HIV must first integrate its genes, stored on molecules of RNA, into the host's DNA. This process violates the once-canonical doctrine about the flow of biological information in cells (from DNA into RNA and then to proteins); hence the name. The retroviridae are a ubiquitous family of parasites, or at least fellow-travelers, of vertebrate animals; thus monkeys are infected by various strains of simian immunodeficiency virus (SIV); cats, by feline immunodeficiency virus (FIV); mice, by murine acquired immunodeficiency virus (MAIDS); and so forth. In some cases, these viruses in their natural hosts do not cause disease, but when transmitted to a new host can become pathogenic. A well-studied instance is SIV in sooty mangabeys and African green monkeys, which is tolerated for the animal's lifetime, while injecting variants of this virus into Asian macaques has proved useful for studying simian AIDS. Lessons learned, it is hoped, will be relevant also for human disease. SIV and HIV are also known as "primate lentiviruses" ("lenti" is Latin for "slow"; the appellation refers to the long time between infection and disease).

The HIV epidemic in humans is thought to have begun 50–100 years ago, in at least two zoonotic transmission events. The variety that has spread around the world, called HIV-1, probably originated when an SIV-infected chimpanzee[5] was butchered for its meat, which is consumed in parts of forested central Africa. The accused strain of SIV is prevalent in chimps in southern Senegal and does not appear to make them sick—rendering HIV-1 another, and very important, example of a microbe that became pathogenic after jumping to a new host. A different set of strains also circulating in humans (primarily in West Africa, but also in Europe, especially Portugal, and southwestern India), collectively called HIV-2, probably

[4] "Virus" is Latin for poison or slime; these disease-causing agents—originally called "filterable viruses" because they passed through the finest sieves in the laboratory—were discovered around 1915.

[5] Pan troglodytes troglodytes; the "greater" chimpanzee.

derived from a smaller monkey, a sooty mangabey,[6] possibly from seven separate transmission events. HIV-2 also causes AIDS, but disease is frequently less severe and patients live significantly longer. The virus is also more difficult to transmit, perhaps explaining why HIV-2 is endemic in various regions rather than pandemic worldwide. Co-infection is common in areas where both viruses are prevalent. There is another family of clinically-relevant retroviruses, which cause some cancers in humans, called HTLV (which were discovered before HIV). Because the laboratory and clinical research that has informed our modeling was conducted with various strains of HIV-1, we will limit our discussion in this book to these viruses, which we collectively label simply as "HIV" unless particular variants need be described.

The dynamics of HIV transmission in sexual and injection networks has an obvious bearing on the epidemic and has attracted much interest from epidemiologists, statisticians, and mathematical modelers over the years. The focus of this book, however, is on a different "epidemic": the one in the cells of an infected patient's body, and, in particular, the evolution of the virus in that single infection, during the patient's lifetime.

1.1.1 Notes

For the discovery of HIV: [104]. For its zoonotic origins: [161, 296]. Steve Self and an author wrote one of the first papers about sexual networks and HIV; they explored the impact of "superspreaders" on the problem of estimating vaccine efficacy in a clinical trial: [308].

1.2 HIV *In Vivo*: Part I. Time-Course and Target-Cells

The course of an HIV infection is conventionally divided into three stages: primary viremia, chronic or asymptomatic phase, and AIDS. The first, or primary, phase begins, in the sexually-transmitted case, with a localized infection in a mucous membrane, then spreads to lymph nodes. (Lymph nodes are cell-concentration regions of the lymphatic system, a secondary circulation in the body that organizes and sustains immune responses.) HIV can infect many cell types, but the primary targets of this virus are the

[6] Cercocebus atys.

macrophages and the CD4+ T lymphocytes. Macrophages ("big eaters") are immune-system cells that consume bacteria and matter from dead cells as well as having other immune functions. T lymphocytes, also called thymus-derived or T-cells, are also immune-system cells. The descriptive terms reflect a T-cell's bodily visitations: after generation in bone marrow, as for all blood cells, they transit through the thymus gland and reside for part of their lifetime in lymph nodes. There are several varieties of T-cells. HIV's T-cell targets are dubbed by immunologists "CD4+"; the plus sign means the cells stain positively by a reagent for the surface marker, called CD4. Another kind of immune-system cell, called the dendritic cell (DC), frequents mucous membranes and skin, where it picks up pathogens and transports them to lymph nodes for display to T-cells. HIV may hitch a ride on DCs, but does not reproduce in them. As we write, the question of which cell type is the most-likely portal for HIV's entry into the body—macrophage, CD4+ T-cell, or DC—is still unsettled.

Although macrophages support HIV replication, most virologists believe that HIV is primarily a disease of T cells. We will use the acronym "PIT" in this book for "productively-infected target" cell, by which we shall usually mean a CD4+ T-cell. The adjective "productive" refers to on-going viral replication, and distinguishes PITs from a class of latently-infected cells (discussed in Chapter 2.)

Viruses are obligate parasites, which means that they can reproduce only in their targeted "host" cells. A virus separate from its host cell is a lifeless particle, called a "virion", made up of protein and either DNA or RNA molecules. See Figure 1.1.

The latter, constituting the viral "genome", stores the information necessary to replicate the virus. HIV's genome consists of two strands of RNA. Its life-cycle in CD4+ T cells proceeds as follows. See Figure 1.2. First, a free virion latches on to a CD4 molecule and another molecule called a co-receptor on the surface of the target cell. The virion binds with these cell-membrane receptors and is engulfed into the cell cytoplasm, where it uncoats and releases its genome and certain proteins such as the celebrated "reverse-transcriptase" (RT) enzyme. (RT, discovered in 1970 by Temin and Baltimore, is the molecule that makes retroviruses retro.) Next, RT, in collaboration with host enzymes, transcribes viral genes (from both RNAs; the details are in Chapter 10) into a single strand of DNA. The later penetrates the cell's nucleus, where it is inserted into the host's genome. At this stage, the viral DNA is said to constitute a "provirus".

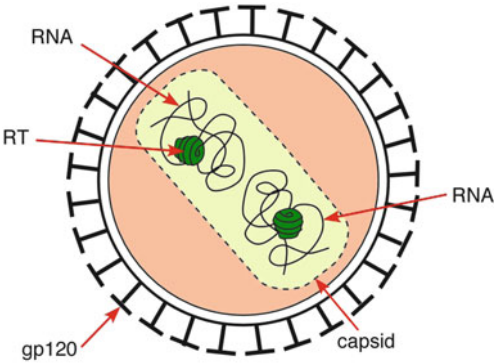

Figure 1.1: Cartoon version of an HIV virion.

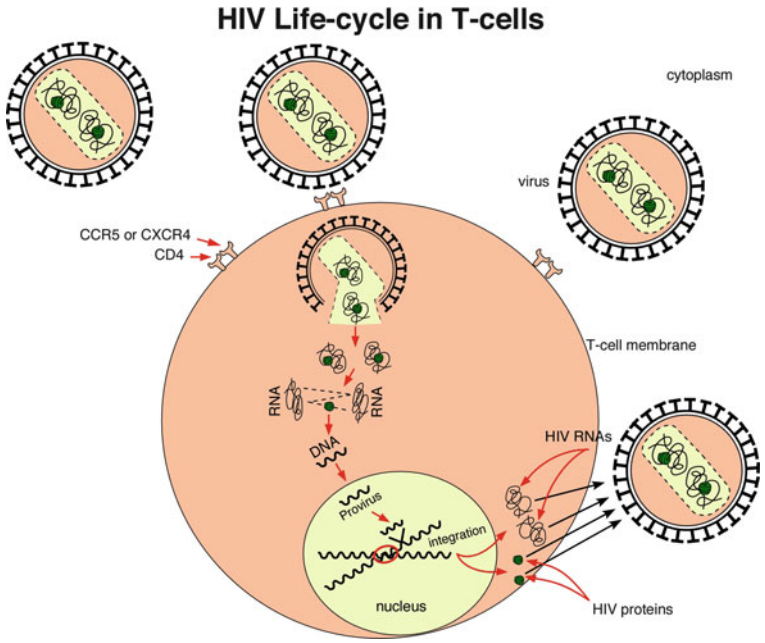

Figure 1.2: Cartoon version of HIV's life-cycle *in vivo*.

The stage is set for the final step in viral replication: synthesizing and releasing new virions. The host's gene-expression system, unable to distinguish self from non-self DNA, transcribes the viral genes into RNA and proteins. These assemble, with some help from host proteins, into new virions. In the last act, either the virions bud directly from the cell membrane, or the cell's excretion machinery releases them, into the extra-cellular medium where they can infect more target cells.[7] The whole process—from initial penetration of the cell membrane to release of the first new infectious virion, often referred to as the "eclipse period"—takes 2–3 days.[8]

Although most of the action occurs where T-cells mostly reside—in lymph nodes and other organs, including the spleen, tonsils, and gut—the amount of virus is usually measured, for practical reasons, in peripheral blood. The level of virus is called "viral load" (VL) or simply "viremia", and is usually expressed as virions per milliliter (ml) of blood. (Sometimes RNA copies is meant instead, which causes confusion because there are two per virion. Perhaps more logical would be total-body burden of virions, but that is not a simple laboratory measurement.) As the infection proceeds, the VL typically reaches ten million or more, peaking in 20–40 days; in this period the patient often reports symptoms similar to that of the flu. This stage is called "primary viremia". At this time the CD4-bearing T-cells in peripheral blood typically drop by around 50 %, from a normal 1,000 per microliter—in immunology, cell concentrations are given "per μl"—to around 500. Due to the many controversies about HIV and T-cell dynamics, which will be a recurrent theme in later sections, it is not known what this implies about the infection.

After the primary stage, the VL typically falls by a factor of 100–1,000 ("2–3 logs"), for reasons that are also controversial and discussed in Section 1.6. The CD4+ T cell population in peripheral blood usually recovers somewhat, but not to prior levels. The stage that follows is called the "chronic", "asymptomatic", or (by mathematicians, rather imprecisely), the "steady-state" period. "Quasi-steady-state" would be more accurate. Characterized

[7]There is also a reported route for virions to pass directly between cells by hijacking the immunological synapse formed between APCs and T-cells or creating a "virological synapse" between two CD4+ T-cells. Although this mechanism for cell-to-cell spread had been demonstrated *in vitro*, its importance *in vivo* is unknown.

[8]The eclipse period was incorrectly estimated in 1996, from data about declining viremia after HAART, to be 1.1 day [237], a figure which made it into many models including some of my own. For discussion of this issue, see the Notes to Section 1.7.

by almost-stable viral loads and CD4 cell counts and lack of AIDS symptoms, this period lasts a highly-variable time averaging 10 years. The onset of the last phase—AIDS—has since 1993 been defined by the Centers for Disease Control as a CD4 count of 200 or less and at least one AIDS-defining diagnosis of an infection, symptom, or cancer rare among immunocompetent persons. The cause of the transition—chronic phase to AIDS—is also unknown at this time. Some conjectures will be discussed in this book.

1.2.1 Notes

Good reviews of the biology of HIV infection include [11, 92, 255, 285]; the last also contains articles on other viruses (such as SIV and LCMV) that establish chronic infections in animals. Direct cell-to-cell spread and the "virological synapse": [152, 244]. CXCR4 expression on naïve T-cells was essential in these experiments and the observation limited to X4 viruses (see Section 1.6), so the importance of this mechanism in primary infection by an R5 virus is unclear.

1.3 HIV *In vivo*: Part II. The Mutation Machine

Perhaps the most striking fact about HIV *in vivo* is its extraordinary replication rate. Unlike some viruses (such as chickenpox or herpes), HIV never enters a dormant or "latent" stage, but reproduces continuously in the body over the whole time-course of infection. The number of PITs in the chronic phase is in the range 10–100 million, and the turnover time is 2–4 days. HIV's mutation rate is also remarkable: it is at least five orders-of-magnitude higher than for DNA-bearing, eukaryotic organisms. The rate was measured in the early 1990s in the test tube, with HIV propagating in immortalized T-cell lines (again, biologists prefer the Latin, and refer to the observation as "*in vitro*", literally in glass) and yielded the average figure: about 0.3 changes per genome per replication cycle. The cause of this error-prone replication is sloppy reverse-transcription of viral RNA into DNA. Now the HIV genome is quite small, even among viruses: about 9.6 kilobases. This yields a probability for substituting a particular nucleotide by another of about 3×10^{-5} per cycle. From these figures it easily follows that every possible HIV mutation is made every day in the body of an infected person, although that does not

mean that any newly-created variant is destined to replace the existing, or "wild-type", strain—an issue that will greatly concern us in this book.

The extremely high rate of generating variants sets the stage for evolution to act in an infected person's lifetime. Indeed, after HIV infection the virus evolves rapidly, changing by as much as 1 % per year in part of the envelope protein. When a variant with a mutation enjoys a replicative advantage, geneticists refer to "positive selection"; and it is frequent in HIV evolution. Selection acts on "phenotypes", or behavioral repertories, which derive from amino-acid sequences in proteins. Each amino-acid is coded in the genome by a triple of nucleotides but there is some redundancy. Nucleotide changes that do not alter amino-acids are referred to as "silent" or "synonymous". When a synonymous change occurs, or a substitution of an amino-acid for another that does not change the function of the protein, geneticists invoke the word "neutrality".[9] Much neutral variation is observed in the HIV genome, which is not surprising given the high mutation-rate, and also many positively-selected events which may reflect the virus improving its reproductive facility in its host cells or expanding its range to new cell-types. But many, even the majority, of adaptive amino-acid changes allow the virus to evade immune pressure. This form of *in vivo* evolution—which we will refer to by the generic term, "escape"—is the central focus of this book.

1.3.1 Notes

The high mutation-rate of RNA viruses was discovered in the 1970s; for the rate in HIV, see [191]. That HIV might mutate and thus escape a T-cell immune response was proposed in 1991 [240]. HIV makes every possible mutation every day: [63]. For a recent review of HIV genetics and AIDS: [220].

The rapid evolution in HIV's envelope protein (Env) in the first year after infection is thought to represent mainly escape from antibodies [261], discussed in Section 1.5. For the frequency of positively-selected mutations, see [70, 334, 335]. That a large fraction are escapes from CTLs: [3, 44, 189]. In one study of four patients for 5 years post-infection, 53 % of 98 a.a. changes were CTL-related [3]. In another recent multicenter study of 75 primary

[9] It is sometimes asserted that only non-synonymous mutations can confer an advantage, but there are situations—involving RNA secondary structure or codon usage—where synonymous changes also affect fitness. Nevertheless, geneticists often use the ratio of synonymous to non-synonymous variation as a measure of the relative importance of neutral "drift" and selection.

HIV patients, a minimum of 20–40 % of amino-acid changes in the Nef and Pol proteins in the first 2 years after infection were probably CTL escape mutations [44].

1.4 The Experience with Drugs

In 1987, only 4 years after the laboratory isolation of HIV, the first drug that had a clinical impact on HIV disease was approved by the FDA. This drug, zidovudine, also called AZT, was but the fifth antiviral drug ever licensed and one of very few to be rationally designed.[10] The drug had been shown, by researchers at the FDA, the National Cancer Institute, and Burroughs Wellcome Co. (the drug's sponsor), in *in vitro* studies to inhibit the reverse-transcriptase (RT) enzyme; only a year later, a clinical trial was halted early after the observation that patients on the drug had lowered viral burdens and better quality of life. At least, that is, for about 6 months. Follow-up revealed that as many patients taking AZT progressed to disease as would be expected in a drug-naïve group. Investigators next tested the virus from progressors and discovered that it had changed—grown less sensitive to the drug. Sequencing revealed that the virus had made three-to-four mutations in the RT region of its genome that had allowed it to escape suppression by the drug. The hope expressed (by, among others, mathematicians) that AZT would prevent AIDS was dashed.

In retrospect, the only surprising part of this episode—besides the unprecedented pace of drug discovery and approval—is the 6 months. Recall that HIV *in vivo* is capable of making every possible mutation every day; hence, one would expect it could evade one drug in a few weeks, if possible at all. Escape from monotherapy with other drugs, often by a single amino-acid-change, is indeed that fast. It turned out that, in the case of AZT, one

[10] A rationally-designed drug is one that was developed on the basis of hypotheses about disease mechanisms at the molecular level, perhaps including detailed X-ray photographs revealing the structure of targeted proteins. By contrast, most drugs in clinical use, even today, were discovered in screening programs and the mechanism of action is often unknown. The earlier anti-viral drugs licensed were acyclovir, amantadine, vidarabine, and ribavirin, active against herpes, flu, and hepatitis. The hurdle in discovering anti-viral drugs is that viruses hijack cellular enzymes to facilitate reproduction, so drugs acting against them are often toxic for the host. Bacteria bring along their own enzymatic machinery and thus are easier to target.

of the amino-acid-replacements the virus made to escape the drug required two nucleotide changes—which is a rarer event.

By 1995, pharmaceutical companies had invented other anti-HIV drugs, especially a new class called protease-inhibitors (which acted against a different HIV enzyme). Two groups enlisted patients in experiments in which they were given three drugs at a time. This regimen—called "triple-combination" therapy, or "HAART" (highly-active antiretroviral therapy)—proved able to reduce viral-loads in the patient's blood streams almost to unmeasurable levels. One of the physician-investigators in these experiments, David Ho, appeared on the cover of Time magazine (as "Man of the Year"), and once again there were predictions that HAART would cure HIV, or at least prevent AIDS.[11] For many patients with access to these drugs, triple-combination therapy has prolonged their lives; but for others resistance eventually deve lops—a scenario that we will encounter again, in another context, in this book.

1.4.1 Notes

For the history of antivirals before AZT see [64]. For development and licensing of the first anti-AIDS drug, AZT, see [337]. For the HAART experiments, see [129, 305].

1.5 The "Killer" T Cells

T (for thymus) cells are part of the "adaptive immune system". The phrase distinguishes the T-cells and B-cells from other immune-system actors that generate the "innate immune response". As the name implies, the adaptive response improves with exposure to the pathogen, for which the host retains a memory; by contrast, the innate response simply detects certain patterns in pathogen proteins or nucleic acids and makes a fixed response. Considering the breadth of the microbial world, storing all existent pathogen-patterns in the human genome is presumed impossible; thus the common conjecture holds that the adaptive immune system evolved in vertebrate animals to defend against diverse enemies: some ancient and some never before encountered.

[11] The assertion was made at the time that 36 months of drug treatment would amount to a cure. This prediction failure was probably due to neglecting the possibility of latency at the cellular level (see Chapters 2 and 10) rather than escape by mutation.

B-cells secrete antibodies that bind foreign proteins in serum; this was the only form of immunity known before the late 1950s, when the role of the T-cell was first described. At that time, the function of the thymus was also unknown. T-cells are specialized to detect foreign proteins that have appeared on the surface membrane of host cells, often due to a viral infection. The way this works is that T cells are equipped when formed with a "T-cell receptor" (TCR) that reacts to a specific "antigen", usually a short peptide. The TCRs are generated by a random genetic process and are extremely heterogeneous (perhaps 10^{15} distinct TCRs are possible, with some random subset made in each person). Cells with TCRs that react to host proteins ("self" in the language of immunology) are deleted in the thymus, leaving for the most part TCRs that react only to foreign antigens ("non-self"). If pathogens are thought of as locks, the adaptive immune system makes boatloads of random keys, almost all of which are useless or have to be thrown away because they open doors in the host. But some will one day fit an infecting organism.[12] After selection in the thymus, the surviving T-cells migrate throughout the body and prepare the defense against invasion. T-cells come in two flavors, indicated by the CD4 or CD8 marker; the former are also known as "helper T cells", and the latter are called "cytotoxic T-lymphocytes" (abbreviated CTLs), or informally, "killer T cells".[13]

Human cells, with a few exceptions (e.g., red blood cells), break down proteins that they have ingested or synthesized as part of house-cleaning and other functions. Most are recycled into other proteins or excreted into blood, but certain short peptides that fit into the central groove of a "human leukocyte antigen" (HLA) molecule are transported to the surface and displayed like flags to T cells.[14] The TCR can only react to peptides presented in a

[12]In 1957, Macfarlane Burnet had proposed the "clonal selection theory" to explain antibody diversity; a few years before, Niels Kaj Jerne, who also coined the term "epitope", had expressed similar ideas. When, around 1962, Jacques Miller and collaborators demonstrated T-cell education by the thymus, it fully realized Jerne's and Burnet's conceptions. Random generation and selection replaced older concepts such as "instruction", meaning that antigen had to be present at antibody or TCR creation; it has been called the Kuhnian revolution, aka "paradigm shift", in immunology.

[13]Some immunologists believe that some CD4s are cytotoxic, while some CD8s are not; this is typical of the field.

[14]The collection of genes that encode these molecules is called the major-histocompatibility-complex (MHC); it is divided into three HLA regions. HLA class I are the molecules that display antigen to CD8 T-cells, and HLA class II display to CD4 T-cells.

complex with a specific type of HLA molecule.[15] One class of HLA molecule is present only on certain specialized immune-system cells called "antigen-presenting cells" (APCs), which present peptide to CD4+ T-cells. These "helper" T-cells in turn provide assistance, in the form of secreted stimulatory factors, to B cells and CD8+ T-cells. Another class of HLA molecule is present on most nucleated cells and presents peptide to CD8+ T-cells. The CD8+ T-cell, when "licensed to kill" (properly stimulated by target and perhaps an APC and/or helper), can secrete molecules that instantly destroy the infected cell—by literally shooting holes in its membrane. CD8s can also secrete other viral-defense molecules called "interferons" and various intercellular messengers called "cytokines"; their role in HIV disease is controversial.

Once activated, a T-cell enters into the cell-cycle leading to division. The result of T-cell activation can be a dramatic expansion of this cell compartment in the body. For example, "naïve" CD8+ T-cells that recognize certain viruses (e.g., influenza), so rare in a person that has not encountered the strain as to be virtually undetectable (less than one in a million CD8s), may proliferate to form 70 % of the compartment a week after infection. Afterwards, most of these cells delete themselves, undergoing a form of programmed cell-death (called, in Latin as usual, "apoptosis"). But a substantial fraction survive and differentiate into long-lived cells that persist for years or decades. These so-called "memory" T-cells, together with B-cells and antibodies, form a quick-reacting line of defense against a second appearance of the foe. (More details of our model of CTL memory and function can be found in Chapter 3.)

Each HLA molecule can present only a limited set of peptides to T-cells. Not surprisingly, evolution has created a diverse family of HLA molecules, and we each have our own repertory.[16] Consequently, none of us can display all peptides recognizable as foreign to our B- or T-cells, which partially explains why we have different outcomes when we get an infection. The peptides that can be recognized by somebody are referred to as "epitopes". For example, epitopes recognized by CD8+ T-cells are 8–15 amino-acids in length. Hundreds of CD4 and CD8 epitopes have been identified in HIV proteins, each presented by one or a few HLA types. The breadth of response

[15] HLA restriction of T-cell epitopes was discovered by Rolf M. Zinkernagel and collaborators in 1974.

[16] For instance, there are three subvarieties of HLA class-I molecules, called A, B, and C; each of us has two of each type, one from each parent. The number of known variants (called "alleles") are, A: 85; B: 185; and C: 42.

to these epitopes varies widely among patients, with some making two or three, and some more than 40 (with an average of around 19).

CD4 "helper" cells also exist in naïve and memory forms and two subsets are of particular importance in our context. Memory CD4s bearing the co-receptor CCR5 are particularly vulnerable to infection by most circulating strains of HIV and are early casualties, especially in the gut. And naïve, HIV-specific CD4s are both possibly required for CD8 memory formation and the perfect target for HIV—attracted to areas of HIV concentration and readily activated by HIV antigens. Damage or loss in this important immune-system compartment may be one explanation for the inability of killer cells to eradicate HIV from the body, as they can for influenza and other infections.

1.5.1 Notes

T-cell function and memory is described in any immunology textbook—but the account changes every few years, as new experiments lead to revised understanding of mechanisms.

1.6 Two Theories of the Chronic Infected State

What controls HIV after infection—that is, brings down peak viremia and establishes the chronic (quasi-) steady-state? Antibodies to HIV are formed late, many months after the primary peak, and do not effectively neutralize the virus. That leaves two possibilities.

In 1996, A. N. Phillips proposed, in the journal Science, a theory asserting the "independence from a specific immune response" of the fall of virus in blood after primary viremia. Phillips incorporated his theory into a mathematical model and demonstrated that his assumptions could indeed reproduce the observed drop to the steady-state. The mechanism was simple: the "predator", here thought of as HIV, consumes so many of its "prey", its target cells, that the production rate of new virions falls. We will refer to Phillips's theory in this book as "target-cell depletion" (TCD). The model was quantitative (much to Phillips's credit) and came equipped with a table of rate-constants (similar to the tables in the modeling chapters of this book). One of these rate parameters caught the eye of an author of this book and resulted in a critique of the theory that was published years later.

The parameter of interest fixed the lifetime of the uninfected target cell at about 700 days. It was known, from fitting models to data generated in the 1995 trials of triple-combination drug therapy, that the infected T-cell survives in the body for about 2 days. Hence the model assumed that infection by the HIV virus lowered the lifetime of the target cell by about the same 2–3 logs as the drop in virus after primary infection. Was this a coincidence?

Subsequent inquires to virologists revealed a widespread, but not universal, belief that the primary targets and producers of HIV are the activated CD4+ T-cells. The requirement for activation (99 % of T-cells in normal, healthy patients are quiescent at any time), stems from an aspect of HIV's lifecycle. The viral genome must first be integrated into the host's; then the cellular translation and assembly machinery can produce new HIV virions. But the host cell must be sufficiently activated to transport DNA into its nucleus, as well as be synthesizing RNA and proteins. Inquiries to immunologists next revealed that activated T-cells persist in that state only for a week at most after they encounter their cognate antigen. This presented a paradox: what cell type was the PIT?

It could not be the resting T-cell, because, although HIV can enter resting cells, its genome is not integrated into the host's, it does not complete its life cycle, and it does not generate new virions (which might burst or otherwise kill the cell, or provoke CTLs to do the deed). It could not be the activated T-cell, because it does not live for 700 days in the body. (If it did, the symptoms of a cold would persist for years.) Macrophages are not candidates either, because HIV does not kill them.

Prior to the publication of the critique by an author and colleagues, another group noted that a TCD model, in their hands, did not seem to yield the observed 2–3 log drop in viremia. We connected the magnitude of the drop in TCD models to the diminished lifespan of the target cell, by a method familiar to mathematical physicists.[17] Both groups concluded that the most likely explanation for the drop was the appearance and effectiveness of anti-HIV CTLs—in other words, a different kind of predator-prey model, with reversal of roles for HIV and immune-system cells. We will label this theory simply immune-control (abbreviated IC), although other immune effectors besides CD8+ T-cells may be involved.

[17]Proving an inequality. A wiseacre once defined a mathematical physicist as a person who knows that 2 + 2 lies between 3.99 and 4.01.

Another argument against TCD, no doubt more persuasive for clinicians than criticisms of models, derives from observation of HIV-infected patients. Cross-sectional population studies such as the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS) have proven a direct correlation between viral load after primary infection and progression to disease. Such a correlation is evidently contrary to the TCD theory: if lower VL results from HIV consuming more CD4+ T-cells, which are themselves important immune-system players, one imagines that the patient would be sicker.

Yet another argument against TCD arises from the "hyperactivation" theory of HIV pathogenesis. After HIV infection, activation markers on T-cells rise as much as fivefold over normal levels, and remain high in the asymptomatic period. It has been argued that T-cell hyperactivation—perhaps one aspect of a general state of immune inflammation—rather than lymphopenia is the primary pathogenic phenomenon in HIV disease. An author of this book pointed out, in 1999, that provided this form of T-cell activation is sufficient to support HIV replication, the elevated level more than suffices to counteract the effect of CD4 depletion—right down to the AIDS boundary. In other words: if the main targets and producers are indeed activated CD4+ T-cells, then target availability may remain constant or even increase in primary viremia. Another phenomenon related to "bystander activation" is the increased CD4 proliferation observed in HIV and SIV infection, which is presumed to be the body's attempt to make up for loss of T-cells (homeostasis). Immune hyperactivation may represent an evolved mechanism on the part of the virus to maintain a supply of cellular hosts.

Immunologists also like to point out that, unlike red blood-cells that transport oxygen, white-cells do not reside in blood. They live in tissues (98 % of T-cells are found there at any time), and only commute by way of peripheral blood. Variation of 50 % in CD4 counts in blood-draws in hospitalized, HIV-uninfected patients over the course of a stay are not unusual. The claim that a drop in CD4 blood counts implies an equivalent loss in the whole body is not a logical deduction. If the number of cars crossing the floating bridge into Seattle one morning dropped by 50 %, it would not imply that the number on Seattle's streets has been similarly diminished. Indeed, a study of CD4 in macaques, sacrificed at various time points after infection by SIV and dissected, found that counts in many tissues actually increased, by as much as threefold relative to control animals, despite the fact that the CD4 count in blood exhibited the usual pattern. The authors of the study

attributed the observed increases to hyperactivation, driving increased CD4 replication in the periphery, and altered tissue distribution. (However, the investigators did not count CD4s in the gut; later studies noted a major depletion there, but at this writing it is unclear whether the virus directly or indirectly kills GI tract CD4s.)

Finally, there is the remarkably small percentage of CD4s in the body that become productively-infected at any time: from 0.01–2 %, depending on tissue examined and disease stage. The scarcity of PITs requires TCD believers to supply some scenario for killing of uninfected bystander cells. In laboratory studies, certain HIV strains induce "syncytia", clumps of CD4s that stick together. But the syncytium-inducing viral phenotype is rarely found in patients except in late-stage disease, where it often appears in connection with a target-cell switch. Early HIV isolates from the primary or chronic phase usually bind a co-receptor called CCR5, together with CD4, in order to penetrate target cells. CCR5 is found on macrophages and a small subset of memory CD4+ T-cells; sometimes the virus evolves a new tropism for cells expressing another molecule, CXCR4, found on the majority of T-cells. (This evolutionary switch from so-called "R5" to "X4" phenotype may be one route to the profound CD4 depletion seen in AIDS; see Chapter 8.) In this connection, a recent observation of super-fast progression to disease caused by an X4 strain of SIV also argues strongly against TCD. In these monkeys, the drop to the steady-state never occurs—despite the fact that the infecting strain can attack all T-cell types (including naïve). Hyperactivation resulting in increased apoptosis is another bystander-depleting mechanism, but, as we already pointed out, it does not bolster TCD.

Other than from modeling, the evidence for TCD remains essentially the observed drops in CD4 and viral load before the steady-state forms, for which other explanations exist. If the reader accepts modeling as a form of evidence, we note that the time-course of viremia and the appearance of HIV-specific CTLs are readily and quantitatively reproduced in a mathematical model, described in detail in Chapters 2 and 3, which does not rely on wholesale consumption by HIV of its target cells.

1.6.1 Notes

Phillips's theory appeared in [239]; for criticism of TCD, see [283, 310]. For the correlation between VL, CD4 count, and disease progression, see [128, 205]. For the hyperactivation theory, see [16, 231, 306, 307]. Increased CD4

proliferation due to SIV/HIV infection: [12, 68, 125, 230, 265, 278]. CD4s may actually rise in some tissues in SIV infection of macaques: [277]. For "syncytia" and the putative R5/X4 switch, see [116, 236]. For the super-fast SIV progressors [193]. For the frequency of PITs *in vivo*, see [122, 124].

Since the 1990s, the debate over TCD *vs.* IC and the identity of the target cell has continued to rage. Most virologists find that HIV does not establish a productive infection in resting T-cells in the lab [55, 127, 280, 290, 342], but some groups believe that the situation is different *in vivo* and HIV can turn resting CD4s into PITs [326, 341]. In 1998 Veazey *et al.* discovered that SIV targets and depletes memory CD4s in the intestinal tract of macaques [300]; see also [183, 198, 227, 243]. Around 2004, the same observation was made for HIV and humans [38, 40, 120, 242]. Damage to the gut may release microbes, driving immune hyperactivation—thus we have a new paradigm for AIDS: it is primarily a bowel disease [39]. It has also been claimed that HIV-specific CTLs are rare in the gut. This combination of conjectures leads naturally to a resurrection of TCD, at least as far as the GI tract is concerned. But other groups reported plentiful anti-HIV CTL responses in the intestines and rectum, at levels similar to blood [139, 221]. It has also been shown that CCR5+ memory CD4s, the category of cells that disappear from the gut, are highly sensitive to being killed by CTL [187]. On the other hand it has not been shown that memory CD4 cells in the GI tract are permanent residents or that they are killed directly by the virus. This extent of controversy is common in virology and immunology; also at this writing, there is no agreement as to the cause of AIDS. (See Chapter 8 for some of the theories.)

1.7 Do CTLs Really Control HIV?

Arguments about models may sway mathematicians, but it took additional evidence from the clinic and the laboratory to convince many virologists that cytotoxic T-lymphocytes really drive the viral load to the fixed point.

The direct evidence for IC now includes the temporal coincidence of declining viremia with the appearance of HIV- or SIV-specific CTLs (they become detectable in blood a few days after viral load peaks); experiments in which CD8s were deleted in monkeys (upon challenge with SIV, the animals had high viral loads, but as CD8s repopulated their viremia declined); and "adoptive-transfer" studies in HIV-infected patients. (In the latter,

HIV-specific CTLs were derived from patients, cloned and amplified by a factor of a billion, genetically labeled, and re-infused back into the patient. The protocol produced a drop in the population of productively-infected cells, which, unfortunately, was transient.) From the laboratory we have the observation of efficient killing of HIV-infected cell lines (HIV-specific CTLs, cyro-preserved from samples of infected patient's blood, can completely suppress HIV in these *in vitro* experiments). Additional direct evidence derives from trials of T-cell vaccines against SIV in monkeys, discussed in the next section.

Indirect evidence can also be cited; for instance, the analogy with viruses that establish chronic infections in other animals, such as lymphocytic choriomeningitis virus (LCMV) in mice. The timing of CTLs becoming detectable a few days after peak viremia and the formation of the steady-state viral and memory CD8 populations is virtually identical to HIV for one strain of LCMV, except for the timing. Control of the LCMV virus has been proven to be dependent on CTLs.

The principal phenomenon addressed in this book—escape from CTLs—also lends significant support to IC. Mutant HIV strains that evade recognition by some CTLs appear as early as a month after infection; if CTLs were impotent at killing (or otherwise controlling[18]) infected cells, they could not create the selection pressure that prompts this rapid evolution. Some of these escapes have even been elicited *in vitro*, by exposing HIV growing in cell-lines to CTLs from the freezer. On the reverse side, the preservation *in vivo* of the function of the HIV Nef gene, which is often lost when HIV is grown in the laboratory, also argues for the power of cellular immunity: Nef partially disarms CTLs by limiting an infected cell's ability to display antigen. (The fascinating story of Nef is presented in Chapter 9.)

More evidence for IC derives from population studies. Investigators in the Multicenter AIDS Cohort Study (MACS) and others demonstrated strong correlations between HLA alleles and disease markers such as viral load (VL), CD4 count, and time to progression. These correlations are difficult to explain without invoking cellular immunity to HIV. Immune control also implies, *inter alia*, that a negative correlation should exist between the strength of the immune response and disease markers. Unfortunately, most studies have been performed on populations of chronically-infected individuals with limited information on the time since acquisition of HIV. Initiation

[18]CD8+ T lymphocytes also secrete various cytokines that may have anti-viral effects.

of HAART also tends to limit the analysis (by censoring the endpoint). Large uncertainty remains as to whether superior T cell responses can diminish VL or delay progression to AIDS. Nevertheless, some studies did find a relation between CTLs and disease status. Some functional aspects of T-cells have been shown to correlate with VL, including proliferative capacity, secretion of perforin (a "killer molecule" that literally perforates cell walls), and number of secreted cytokines (non-lethal molecules that stimulate cellular immunity). Several groups have noted a significant negative correlation between responses to the HIV Gag protein and VL. Gag is a structural protein, part of the capsid—the virion's packaging—and is both frequently recognized by CTLs and possibly more conserved than other HIV proteins. Interestingly, the correlation only appeared in one study when the magnitude or breadth of the Gag response was expressed in *relative* terms; i.e., as a percentage of total CTL response. We will present a theory to explain this observation in Chapter 7.

1.7.1 Notes

For the coincidence of CTL appearance with peak viremia, see [35, 171]. For the CD8 depletion experiments, see [150, 197, 271, 272]. For the CTL adoptive-transfer experiment in HIV-infected patients, see [42]. For CTL function *in vitro*, see [203, 331]. For escapes from CTLs elicited *in vitro*, see [297, 333].

On a population level, HLA has been shown to be a driving force on HIV evolution and HLA-mediated selection of viral sequence is predictive of viral load [213]. The MACS study of HLA and disease markers: [269]. HLA and AIDS: [50]. Certain HLA alleles (particularly, B27 and B57) are associated with better control of the virus, suggesting that recognition of certain epitopes is protective: [8, 9, 22, 89, 112, 163, 164, 209, 288]. Concerning a correlation between potency or breadth of the CTL response and disease markers: studies that found an inverse correlation between T-cell responses and viral load include [23, 24, 34, 47, 56, 81, 115, 226, 245, 273, 324, 340], though others reported direct correlation [21, 196, 339] or no correlation [1, 65, 97, 106, 159, 208]. The surprising positive correlations were often to responses to Nef—leading some to propose that these epitopes are actually harmful. One study suggested that poly-functional T cell responses are better associated with viral control than mono-functional (IFN-γ-based) responses [232]. Interferon gamma is a molecule secreted by activated T cells

that may have an anti-viral effect; it is frequently used as a marker for response. At this time, whether it is proper surrogate for killing ability is unknown. One study in acutely subtype-B infected subjects found that virus-specific CD8+ T cells were correlated with declining viremia in acute infection, but not with control in chronic infection [184]. Assessments of subtype C infected blood donors in Botswana found that the relationships between virus-specific T cell responses and plasma viral load [224] varied across proteins, with a strong inverse correlation for Gag-specific responses (assessed by IFN-γ, and particularly to p24). Zuniga *et al.* [343] observed a significant correlation of Gag responses to VL in a cohort of clade-B infected patients; other reports include [107, 134, 165, 263]. A correlation between number of Gag-specific responses and viral load was also noted in a study of SIV-infected macaques [270].

In 2010, two groups published accounts of experiments [67, 169, 323] with SIV in macaques they interpreted as refuting the concept that CTLs control retroviruses by killing infected cells, as for many other viruses that cause chronic infections. The experimenters applied a CD8-depletion technique in half of the monkeys, followed with a drug-therapy step; the observation was a difference in the slopes of declining viremia between the two groups after drug treatment. We replied in another paper written that year (but whose publication was delayed by journal rejections for 2 years): [318]. We pointed out that the model used to interpret the data, originally published in 1996 [237], was incorrect. Publication [237] claimed estimates of three parameters, but the eclipse period was apparently not estimated by any statistical methodology and was conflated with pharmacological lag, while the two-parameter model without eclipse period used to estimate the others was not suitable for retroviruses. Because of the faulty methodology, all three parameters that the authors claimed to estimate in 1996 were badly biased: the free-virion half-life in blood is not 8 h (it is closer to half an hour); the eclipse period is not 1.1 day (it is closer to 3 days; for modern references, see Notes to Chapter 2); and the "productive-cell lifespan" is not 2.5 days (it is probably longer). The problem with using the two-parameter model became obvious in 2010, as one of the groups estimated the "productive-cell lifespan" to be 0.8–1.1 days, which would not even leave time after the eclipse period for virion production. A second issue concerned the model of PIT dynamics. The authors of the 2010 papers assumed that the "productive-cell lifespan" in a CTL-control model would have to be shortened by a large factor (e.g., 10), but this is incorrect because of the long eclipse period. Killing may not

begin until, e.g., the last quarter of the lifespan of the infected cell, when production is a maximum, and then killing may be virtually instantaneous. Our model (see Chapters 2 and 3 and the cited paper) easily reproduced the experimental outcome (the experimenters found a 5 % difference; our "retrodiction" was 6 %).

In addition, the authors did not realize that, over the month or so that they observed the declining viremia in the drug-treated macaques, the CTLs (which are activated CD8 T-cells with short functional lives) are declining along with the disappearing antigen, so that "productive-cell lifespan" cannot be treated as a fixed parameter in a model (Chapter 3.) We interpret the whole episode as a cautionary tale: beware of simplistic, false models, as they may fit your (limited) data-set just fine, but mislead about what is really going on.

1.8 T-Cell Vaccines

All FDA-licensed vaccines have been approved on the basis of empirical testing—namely, efficacy at preventing infection, proved in a clinical trial. Licensed vaccines for viral diseases are known to generate antibodies, although some (especially the live-attenuated vaccines, such as against varicella and rubella) probably stimulate T-cells as well. Whether T-cells are partly responsible for their efficacy is not known, as no standardized assays of cellular immunity are available for routine use in the clinic. After the identification of HIV as the cause of AIDS in the middle 1980s, the first wave of vaccine research focused on antibodies and by 1994 a candidate based on the HIV coat-protein called gp-120 was ready for testing. The antibodies elicited by the gp-120 vaccine appeared to have only weak ability to neutralize circulating HIV strains (it was developed using laboratory versions) and an NIH advisory panel voted against funding a large-scale trial. Nevertheless, supporters of the concept established a private company, VaxGen, which together with government assistance carried out the first phase-III trial of a vaccine against HIV, which reported in 2003. Unfortunately, the conclusion of the trial was that the vaccine showed no efficacy at preventing infection. (For more about vaccine design and the history of developing HIV vaccines, see Chapter 12.)

Antibodies act instantaneously to neutralize free virions; by contrast, CTLs can only act after target cells have become infected. Hence most

immunologists doubt that T-cells are capable of preventing infection. (However, one author and colleagues published in 2006 a model-based prediction that preventing chronic infection with a T-cell vaccine is possible, see Notes.) Because of these beliefs and the outcome of the VaxGen trial, the goal of vaccine designers shifted in the 2000's to finding a vaccine that can enhance and prolong immune-control among vaccinees who do become infected. With this new (and, in fact, unprecedented) criterion for success, a new measure of efficacy was required. The natural candidate was viral load (VL) after the primary-infection peak. VL predicts time-to-progression to AIDS and correlates with probability of transmission; hence a vaccine that produces a sustained decrease in VL would provide a benefit to the infected individual and to the community.

The plausibility of finding a successful T-cell vaccine is based on studies in the "non-human primate model" (NHP),[19] primarily macaques vaccinated and then challenged with virulent forms of SIV or HIV-SIV chimeras called "SHIVs". In numerous studies, vaccines of various formulations have proved able to lower VL in vaccinated monkeys, relative to controls. However, in 2002 N. Letvin and colleagues reported an important failure mode. An animal that made a predominant response to a single Gag epitope suffered an escape. Gag is a popular choice to include in vaccines, because it is considered more difficult for the virus to alter than other proteins while retaining viability. But in the unfortunate animal, within a year of infection the virus made a one amino-acid change in the epitope, the VL increased a log, the animal developed AIDS and had to be euthanized. All the vaccinated monkeys in this trial eventually suffered escapes and vaccine failure.

Granted the IC theory of the chronic infected state, a vaccine that lowers VL would have to act by generating anti-HIV T lymphocytes, and so it will be labeled here a "T-cell vaccine". The primary motivation for understanding escape derives from a desire to block it—by finding an efficacious T-cell vaccine against AIDS.

1.8.1 Notes

Re preventing infection: it has been verified for other viruses: [52, 228]. But others claim CTLs are "too late and too little" (except in the female

[19] In most of the medical literature a "model" means a disease in a laboratory animal that mimics the human affliction; i.e., *in vivo* rather than *in silico*.

reproductive tract): [257]. A mathematical model predicts prevention of infection by a T-cell vaccine: [316]. VL correlates with probability of transmission: [249]. SIV escapes a T-cell vaccine:[17]. Gag may be more difficult to escape: [194]. For other vaccine citations, see Chapter 12.

1.9 Why Eschew Traditional Population Genetics?

When a fitter variant does replace the existing type, geneticists call it a "fixation" event. In an HIV infection, fixation of escape mutants occurs in primary viremia, but also long afterwards—even a decade later. Understanding the timing and order of these replacement events is also a preoccupation of this work. But why not turn to conventional wisdom for help with this problem?

The subject now called population genetics was initiated in the 1920s by R. A. Fisher and J. B. S. Haldane in the UK, and S. Wright in the US. Among the issues that required clarification at that time were the details of generational replacement, the roles of mutation and selection, and the proper definition of "fitness". Eventually certain choices—presumably derived from reflecting on Darwin's finches, fruit flies, or humans—became canonical in the field; but are these assumptions appropriate when treating viruses infecting a host?

The founders constructed abstract, simplified mathematical models of evolution. (We discuss the philosophy and practice of modeling in the next section.) For population dynamics, they often chose replacement sampling. The "demographic population size", abbreviated N_d, is regarded as fixed, and each new generation is derived from sampling N_d offspring of the previous one. The population size itself is established by some unknown mechanism, such as number of available niches or environmental carrying capacity. Let us make a hypothetical example, for which we apologise in advance to ornithologists, herpetologists, and Galapagos biologists. Imagine Darwin's finches on an island. Each finch eats seeds on the ground and each mated pair of finches requires a twig on which to make a nest. These aspects of the environment are fixed and not the concern of the finch geneticist, any more than is the weather. Fitness is defined primarily relative to other finches: a fitter variant eats more seeds or occupies more twigs, and hence produces a greater

number of, or more viable, offspring. Hence, one subspecies of finch grows more abundant relative to others. The "environment" creates the playing field, but is otherwise unvarying and unreactive—it's the baseball diamond, not the opposing teams.

Depending on the strength of selection, described in traditional genetics by a single number, invariably denoted "s", two evolutionary scenarios can be distinguished. Think of "s" as the increase in reproduction in each generation in a population with a mutation, relative to the existing or dominant variety. If μ denotes the mutation rate at the locus under discussion, with $s < \mu$ we have the "pure-drift" regime, in which less-fit variants can win out by chance, while if $s > \mu$, we have the "selection-dominated" domain, in which the fitter species inevitably overtakes the less fit. Note that this dichotomy is only interesting if typical fitness changes are likely to be tiny, e.g., in the fourth decimal place, because mutation rates (even for HIV) are even smaller. Fisher believed that a typical effect of an adaptive mutation was at least in the third decimal and dismissed "drift", which was championed by Wright. Nevertheless, Fisher's "Fundamental Theorem of Natural Selection" (1930)—stating that, in any population, average fitness always increases—would lead you to believe that by now any possible improvements in fitness must be very small. (Given that evolution has gone on for millions of years, and that fitness—meaning reproduction—cannot increase without bound, any species must by now be close to peak fitness. Therefore, any possible changes must be small.) Many geneticists do indeed believe, on the basis of studies of eukaryotic organisms well-adapted to their environment (e.g., fruit flies raised in cages), that most adaptive mutations yield a tiny fitness advantage. After the development of protein and DNA sequencing in the 1960s and 1970s came the new subject of molecular genetics. The discovery of a large amount of "hidden polymorphism" in proteins led M. Kimura to propose that, at least at the molecular level, most genetic change does not improve fitness at all—a picture that came to be known as the "neutral theory".

But is this a universal paradigm for evolution? Once N_d is fixed, population genetics becomes the study of how gene frequencies change in a fixed population. But Fisher himself remarked in 1930 that selection might act to increase population size in addition to altering gene frequencies. The assertion that if one subpopulation increases by x, then another must decrease by x, is not a consequence of genetics or logic but rather a restrictive assumption about population dynamics, which might be true or false.

And are "environments" really unresponsive to evolution? From the beginning another view about "environments" was expressed,[20] mainly by field biologists. Let us return to the imaginary ornithological scenario. Suppose that on some island finches are rare. Lack of seeds or twigs could explain the rarity, but so could the presence of a variety of snake that eats finch eggs. Perhaps on this island evolving a thicker egg shell is more important then outcompeting rivals for resources; if some subspecies of finch did thereby escape predation, it might soon populate the island, creating some interesting selective effects also on the snakes. Note that, in this situation, the evolutionary pressure might be large, and the environment neither passive nor unvarying.

It was from reflecting on somewhat less exotic examples, such as the number of spots on British moths camouflaging themselves from birds, that "ecological genetics" was born. Ecologists, who study the interactions of populations in the wild (and criticize mathematicians for elaborating idealized theories without leaving their desks), are much more willing to entertain strong selection. E. B. Ford, who laid the foundations of the field in a book published in 1964, maintained that a typical s is higher than .01, a range that will appear frequently in this book. M. Wade, in an encyclopedia article, had this to say about the two paradigms: "The neutral theory of evolution is the antithesis of ecological genetics". The battle over the size of typical selection coefficients and the nature of the "environment" continues to rage to this day.

With respect to HIV *in vivo*, the first question that must be raised about applying population genetics is whether niches are in fact limiting. One of the many surprises in the HIV field was the discovery, made around 1988, that only a small fraction of CD4+ T-cells become productively-infected at any time before AIDS. The niche-limitation picture for HIV in the chronic phase is, of course, TCD. The assumption of fixed N_d for HIV *in vivo* amounts to the statement that nothing that occurs in the genetic makeup of the virus can change the viral load. Ironically, perhaps the first observation about HIV genetics made outside the laboratory was of an escape from a CTL response, followed by an increase in VL, in an infected patient. Another clear case of escape resulting in higher VL was mentioned in connection with

[20]The earliest proposal of "interspecific" rivalry, e.g., between parasite and host, as opposed to Darwin's (and population geneticists') intraspecific competition, may have been due to the 19th century Russian immunologist, Ilya Metchnikoff, a collaborator of Louis Pasteur.

trials of an SIV vaccine; in vaccinated monkeys, a one-amino-acid change in a CTL epitope lead to rapid viral-population expansion and AIDS. In humans, VL drops after the primary peak, then slowly increases during the not-quite-accurately named "steady-state" period. AIDS may be the result of an evolutionary process occurring over the patient's lifetime. Whether this scenario or some other, such as waning of immune pressure or failure of T-cell homeostasis, is the correct explanation for the decade-long progression to AIDS, theorists should not rule out evolution *a priori*.

Secondly, if IC is the correct theory of the steady-state, selective effects are unlikely to be extremely weak. In most patients, due to a phenomenon called "immunodominance" (discussed in Chapter 8) a few clones of CTLs, recognizing a few HIV proteins, do most of the work of controlling the virus. Escaping from recognition of one of these clones implies a large selective advantage. (The analysis of the advantage of escapes will be given in Chapters 5–7.) Unless CTLs make a tiny contribution to control (meaning back to TCD), "s" cannot be in the fourth or fifth decimal place. If IC is accepted, then competition to escape immune pressure is more important than competition for niches, except at the terminal stage of AIDS when CD4+ T-cells disappear from the body. (That case makes an appearance in Chapters 9 and 10.) Like the British moths studied by Ford, HIV is changing its spots to escape a predator—meaning ecological genetics is the right point of view, not population genetics.

Thirdly, HIV's extremely high mutation rate (five orders-of-magnitude larger than for eukaryotes) and the small size of its genome (10 K nucleotides, as opposed to three billion for humans), raises the possibility that HIV can explore a much larger region in sequence-space than would be imaginable for higher organisms. An HIV infection may resemble a cloud of co-evolving variants, a non-classical picture introduced by M. Eigen and P. Schuster in 1977 for RNA evolution and which has become known since as "quasi-species theory". Population genetics, with its fixed demographic population size, can only predict sequential replacements.[21] (Quasi-species makes an appearance in this book in Chapter 8.)

Finally, each transmission of HIV is a migration event. The new host may have a disjoint set of HLA molecules and recognize a distinct group of

[21] Eigen and Schuster were revolutionaries, but not so much because they believed in a high mutation rate (which simply sets the pace of evolution), but rather because they left Fisher-type competition out of their models!

HIV epitopes. If viruses were finches, it is as if we are discussing the moment at which the first pair lands on an island (populated by ravenous snakes). Moreover, there is evidence of purifying selection at transmission—as if only a subvariety of hardy fliers could reach that island. As Darwin belatedly realized, adaptation to a new environment is the most likely explanation for speciation—by the mechanism geneticists now call "allopatric". The species concept is unclear for retroviruses, because of their extremely high mutation rate and the fact that any two variants can recombine through retroviral sex (discussed in Chapter 10), so perhaps invoking allopatry is objectionable. Nevertheless, it is certain that one infected person's virus will be driven farther apart genetically from another's than were Darwin's finches on two islands of the Galapagos. Evolution's gear is certainly not set at neutral.

1.9.1 Notes

Fisher's opinion of the magnitude of selection appeared in 1936 [94]. Ford's book: [95]. Kimura's neutral theory: [168]. M. Wade reviewed the disagreement between population and ecological genetics in [303]. For population genetics applied to viruses *in vivo*, see [267].

1.10 Why Modeling?

Clearly, traditional population genetics is the wrong framework in which to understand HIV evolution *in vivo*. Escape from drugs or immune control can only be understood by constructing a detailed model—which brings us to the role of models in biology.

A model is a mathematical abstraction. Mathematical statistics, population genetics, and theoretical ecology all rely on models, but the form these models take, and the intent of the user, are different. The founders of statistics—particularly, Karl Pearson (patriarch of the correlation coefficient) and his one-time student, Ronald Fisher—did not wish their methods to depend on the validity of anyone's mechanistic theories. In the last decade of the 19th century, mechanism in biology had, to put it mildly, few successes. In addition, there was a popular scientific philosophy at the time called "positivism".[22] Positivists asserted that only directly-observed

[22]It was the precursor of the branch of analytical philosophy that later came to be called "logical positivism."

quantities—in the last analysis, these are sense impressions, like hot, red, or three centimeters down the gel—should appear in scientific discussions. They regarded those who tried to explain phenomena by invoking invisible entities (e.g., atoms, molecules, or genes) as quasi-religious dreamers who flattered themselves by the title "theorist". The positivistic attitude towards mathematical modeling—that it is appropriate for elucidating correlations in today's data set, but should invoke nothing else, for fear of "biasing" the analysis—still prevails in statistics textbooks today.

A mechanistic modeler attempts to explain real events, observed or not, through formulating and solving equations or (since the 1940s) writing computer programs. A model with an adequate degree of realism can be a tool for discovering unexpected scenarios, making novel predictions, and designing new experiments. By contrast, a statistical model is properly restricted to predicting the outcome of the current experiment if it is repeated. We are even cautioned in statistics texts not to extrapolate beyond the range of the present data—which is exactly what the mechanistic modeler is hoping to do. Finally, a statistical method, once perfected, is a finished tool (linear regression is the same algorithm as it was in Fisher's day), while we expect a mechanistic model to grow in realism over time.

If today we are absolved of the sin of believing in mechanism, we can nevertheless distinguish "universal" mechanistic models from models that might actually apply in a specific context. The founders of mathematical genetics aimed at a general understanding of evolution by mating models of population dynamics with hypotheses about mutation and natural selection. Thus they formulated "generic" models containing few details specific to a particular organism or environment. Fisher treated the case of mutation at a single locus and proved his "Fundamental Theorem of Natural Selection" (1930), which stated that average fitness in a population always increases, at a rate proportional to the genetic variation existing at that time.[23] Field biologists objected, wondering how both predator and prey

[23]The analogy to Austrian physicist Ludwig Boltzmann's H Theorem, which proves that entropy in a dilute gas always increases, at a rate proportional to the deviance from a Gaussian law, is obvious. Fisher himself remarked, referring to the second law of thermodynamics, "It is not a little instructive that so similar a law should hold the supreme position among the biological sciences". Boltzmann's atomism was much derided by the arch-positivist, Ernst Mach, and therefore Fisher the geneticist was not an adherent to positivism.

could simultaneously improve their fitness.[24] But another bomb lay hidden in the simplicity of Fisher's set-up, still the basis of much population genetics today: a single population, a single locus, random mating and a static environment. When P.A.P. Moran in 1964 examined the case of phenotypes determined by two loci, he found that average fitness can decrease. Remarkably, ecology, co-evolution, and other complications were not involved; except for permitting more genes in play, Moran's discovery applies in the "standard" genetic model. Theoretical population biology today is replete with overly-simplified, quasi-general, and mostly qualitative models whose suitability in a particular case is difficult to judge.

This brings up the question of qualitative *vs.* quantitative modeling. Qualitative models, in which variables do not have specified units, may be of great interest to the pure mathematician but are of dubious scientific value. The principal concern is the untestability of models with no stated, biologically-relevant, ranges for parameters. The problem appears in the most virulent form for models that exhibit thresholds ("tipping points", *pace* the popular book) between qualitatively-different behaviors. If an interesting transition—say, from a steady-state to extinction—occurs when rate-constant r_1 exceeds 5.0, this mathematical fact is scientifically meaningless if the relevant range for r_1 is actually 10^{-6}–10^{-4}. Moreover, when evaluating a model the first check to make is whether the rate parameters are reasonable. (For a facetious example, in a model of human mortality is the assumed rate 0.1 % per century?) This primary mental process is short-circuited by the phrase "in arbitrary units" appearing in the footnotes to a table or graph. The same criticism applies to the output of models, which are curves of populations changing over time; that the curves have a desired shape with invented parameters does not imply they would also with real parameters. Finally, qualitative models cannot be used for the purpose of estimating unknown rate-constants by fitting output curves to data sets, nor tested against them.

Fitting a model in order to estimate unknown parameters is a standard technique in pharmacology and other areas of biology and medicine. Some of the rate-constants that we will incorporate into a model of HIV infection were derived in this way. But this application of modeling is not without pitfalls. The excitement about deterministic models that can exhibit the bizarre behavior called "chaos" is a cautionary tale. These models can generate endlessly-varying, irregular oscillations, which attracted the interest

[24] Fisher noted the objection, remarking in a lecture in 1953, "Of course, for any particular species, it does not follow that it is gaining on its competitors and enemies. . . "

of ecologists who had observed equally-strange gyrations in animal populations. But in 1976, the American mathematician Stephen Smale proved that quite reasonable ecological models—describing e.g., a predator devouring several types of prey—could generate any curve whatsoever. If we are permitted unrestricted variation of parameters, a general model becomes merely a fancy replacement for a child's Etch-a-Sketch toy.[25] Karl Popper, the Austrian philosopher of science who argued in 1935 that models that can never be falsified are valueless, would have been horrified by such a procedure.

The only way out of this trap is quantitative realism. Modelers must be prepared to defend every equation as implementing a plausible biological scenario, and every rate-constant, whether derived from prior experiments or estimated from new data, must have specified units and ranges. Even then, of course, the model may be simply false; the only honest test is to predict something interesting in a new experiment.

Can we derive our model of immune-control and escape from the ecological literature? Since Lotka and Volterra modeled fish in the 1920s, mathematicians have created a large corpus of work in ecology and some authors have even incorporated evolution into models. Unfortunately for our needs, most work in this area preserves the old "Ecology is short, but evolution is long" assumption (population dynamics occurs on a much faster time-scale than appearance of new forms). Although perhaps appropriate for eukaryotic, DNA-carrying organisms with low mutation rates, this assumption is untenable for RNA viruses *in vivo*. (Recall that, in an HIV infection, every possible mutation is made every day.) Moreover, the models are usually of the "universal", qualitative variety.

We need new models. HIV and the cellular immune response should be regarded as forming a peculiar kind of "ecological" system, in which predator and prey evolution and population dynamics all occur on the same time-scale. Abstract, qualitative, theories created with other scenarios in mind will not be useful in this complicated setting. Only detailed modeling is likely to capture the essential aspects of escape from immune control.

1.10.1 Notes

Pearson's philosophy: [234]. Fisher's Fundamental Theorem appeared in [93] and Moran's counterexample for two loci in [216]. Smale's theorem: [276]. R. May and W. J. Leonard also pointed to the methodological dangers

[25] Etch-a-Sketch is a mechanical drawing toy invented by French inventor Andre Cassagnes and subsequently manufactured by the Ohio Art Company.

inherent in "chaotic" Lotka-Volterra-type models in biology [199]. Popper on falsification: [246]. For the synthesis of ecology and evolution (unfortunately not useful for our purposes): [73, 85, 86, 119, 200].

1.11 Deterministic or Stochastic Models?

In mathematical-modeling jargon, the term "stochastic" refers to the kind of randomness that appears in dice games, as opposed to the sort due solely to uncertainty about some fact (e.g., your teenager's driving may be stochastic, but whether he retained the car keys is another issue). Certain aspects of HIV *in vivo* imply that we need a a stochastic model. These aspects include early events before infected-cell populations become large, the random appearance of new viral lineages, and the possibility that an HIV infection has a low "effective population size".

Deterministic models make sure predictions, by relying on formulas describing the rates-of-change of all variables—which mathematicians call by the archaic name "ordinary-differential equations", abbreviated ODEs.[26] ODEs appeared in the 17th century with Newton's equations for planetary motion, and entered biology in the 1920s with Lotka's and Volterra's treatment of predation among fish.[27] Stochastic models, by contrast, predict future events only with certain probabilities. They made their appearance in 1900, in an attempt by a student of Henri Poincaré to understand price fluctuations in the French government bond market,[28] and 5 years later in

[26]The "ordinary" bit apparently refers to a single independent variable, namely time, as opposed to "partial differential equations", PDEs, which have more independent variables. Unfortunately, this peculiar usage—no one we know of can produce even an historical defense of "ordinary" or "partial"—resists being driven out by some more evocative phrase, e.g., "deterministic rate equations".

[27]ODEs achieved popular recognition with the success of James Gleich's book "Chaos: Making a New Science" in 1987. The type of "chaos" described in that book, that can occur with just three compartments (which we mention several times later for pedagogical reasons), will not play a role in this book. Another kind of "chaos" will, in higher-dimensional infection models: namely, that due to heterogeneity in reproduction.

[28]Louis Bachelier's thesis contained the first mathematical treatment of markets, based on the assumption that price shifts are like random walks. This work is stunning for physicists, as it develops most of the theory of Brownian motion usually credited to Einstein; the shock is partially relieved upon noting the thesis adviser, and learning that Poincaré lectured on Brownian motion at the International Congress of Physics in Paris, in 1900.

Einstein's theoretical explanation for the Brownian motion of pollen grains suspended in water.[29] The founders of population genetics also helped develop the subject now called "stochastic processes".

Fisher *et al.* were interested in, among other things, the rate at which a novel genetic variant would spread through a population. Whether determinism or randomness dominates, they discovered, depends on the product of population size, N_d, and mutation rate at the locus, μ. If $N_d\,\mu$ is of reasonable magnitude, a fitter-variant will steadily replace the existing species over time. Deterministic laws are adequate. If $N_d\,\mu$ is very small, the variant might win out, or be lost to chance happenings. To capture this randomness, mathematical geneticists developed stochastic approximations to evolution called "diffusion" processes (related to Einstein's Brownian motion theory, these processes are continuous versions of the famous drunkard's walk from probability, but with an additional contribution describing the force of selection). They also discovered that reproductive heterogeneity can change the dividing point between the deterministic and stochastic regimes. If heterogeneity is important, the boundary criterion must be restated in terms of an "effective population size", always denoted N_e.

To understand the N_e issue in a human context, imagine two small villages of, say, 1,000 folk each, isolated from each other and in which there exist different habits of mating. In the first town, a republic, citizens take spouses at random, while in the second a king traditionally keeps a harem of 300 women. It is not hard to see that the chance of a mutation sweeping through these populations is different. In the kingdom, who possesses the mutant gene is not irrelevant—whether it is the royal person or a commoner makes a huge difference. Moreover, the descendants of the King will tend to exhibit a certain similarity, reducing the variety that enriches life in the republic. Because of this kind of inbreeding, the founders argued, the population of the kingdom will resemble genetically a smaller population; i.e., N_e will be smaller than N_d.

Other mechanisms besides inbreeding can also produce diminishment in genetic variety; for example, population oscillations and selective sweeps. In genetics texts, N_e is often defined to be "the size of a canonical Fisher-Wright (randomly mating) population that would exhibit the same amount of genetic variance". This definition is not useful for our purposes; instead,

[29] In fact, Einstein admitted that he had no idea whether what he was describing was identical with "the so-called ... Brownian motion"; what he wanted to do was to overthrow Mach's positivistic rejection of the reality of atoms.

N_e in this book will always appear as N_d divided by some measure of the variance in viral replication. (See Chapters 2, 5, 6, and 11.)

From the observed rate of accumulation of mutations in the envelope protein of HIV, A. J. Leigh-Brown proposed in 1997 that the effective population size of HIV *in vivo* is much smaller than its demographic population size: $N_e \approx 100-1000$, as opposed to $N_d \approx 10^7-10^8$ PITs, or $10^{10}-10^{12}$ virions, in chronic infection. Other geneticists objected, on the grounds that the neutral model of evolution Leigh-Brown assumed was not applicable to these sequences, and the dispute has raged to this day. Resolving this controversy is important to understanding the evolution of drug resistance and also to the principal topic of this book: escape from CTLs. A low N_e can greatly delay escape from immune control. See Chapters 5 and 6 for theoretical computations of the magnitude of this effect in model-based and traditional genetics, and Chapter 11 for a proposed experiment that might resolve the dispute.

Besides the N_e issue, there are other reasons that stochastic modeling is essential to understanding escape. The late escapes discussed in Chapter 6 can be explained by a scenario (introduced in Chapter 7) that cannot exist in a deterministic model. Moreover, when multiple mutations are involved, deterministic models can generate mathematical artifacts.

Consider an escape scenario involving mutations at two loci. Each single-mutant variant has a small loss of fitness, but, due to escape from drugs or CTLs, the double-mutant enjoys a large fitness-gain. Let the wild-type form a population of N, and the mutation-rate at the loci of interest be μ. Because of the fitness-loss in single-mutants, they cannot replace the wild-type. Nevertheless, cells infected by single-mutant strains will exist at about $N\mu$ per species. (See Figure 7.5 in Chapter 7.) For HIV, $N \approx 10^8$ PITs and $\mu \approx 3 \times 10^{-5}$, so the prediction is for about 6,000 single-mutant PITs. These variant cells will occasionally generate a cycle or two of reproduction, in which luck might generate the second mutation. For the 1-mutant variants, the simplest ODE model gets it about right, but yields a spurious prediction for the time before the double-mutant replaces the wild-type. (In one case we describe for pedagogical purposes, the ODEs predict five generations to replacement, while the correct answer is 60; see Part II, Chapter 16.) The analogy here is waiting to win the lottery. Imagine that someone gives you a one-dollar lottery ticket each day, with a payoff of US $1 million but an expected value of ten cents (typical of most US state lotteries). Determinis-

tic reasoning in this situation amounts to the belief that today you actually have 10 cents, tomorrow you will have 20 cents, etc., and, if you deposit your "winnings" in the bank, it will eventually grow to that million dollars by the laws of compound interest.

Although the analogy may suffice for the reader, there are two other problems exposed by this example. First, ODEs depend on well-defined rates-of-change, so in biology can only describe "mass-action", meaning events in large populations. But the initial escape variant will infect a single cell. The new lineage may go extinct with a certain probability—but the only probabilities that determinism admits are 0 and 1. (For an exposition of this problem in the same scenario, see exercises 2–5 of Part II, Chapter 16.) Second, computer programs that solve ODEs (discussed in Chapter 4) can exponentially-expand infinitesimal numbers, meaningless as biological populations. Incautiously applying ODEs to escape problems in genetics is like ignoring the warning in all programming texts about explosion due to positive "eigenvalues" or growth rates. (If your program sends you to the land of NaN—Not a Number—and you have successfully avoided division by zero, then an undetected positive eigenvalue is the culprit.) For these reasons, we are suspicious of all ODE models of evolution where more than one locus is involved.

Finally, although perhaps not relevant to escape, there is the question of the probability of infection. Another great surprise in HIV research was the discovery that this probability, from one unprotected sexual encounter, is astonishingly low: less than one chance in 1,000 for discordant homosexual couples (one infected partner and one uninfected), and, although higher for heterosexual couples, still less than 2 in 100. These figures suggest that infection by HIV is a random event; the observation has been confirmed for SIV, in low-dose challenge studies in monkeys. Because of the small probabilities involved, it is likely that the initial number of PITs is small, on the order of one, as opposed to 1,000. Hence, we have another reason that a stochastic process is proper for modeling HIV infection, at least if early events are of interest. The random element in acquisition of infection under natural conditions has an impact on testing drugs and vaccines: the danger

in trials with monkeys with large viral inoculums is that we might dismiss a useful intervention.[30]

For the immune-system, by contrast, deterministic modeling may be acceptable. The initial number of CD8 T-cells specific for a given antigen in the whole body is thought to be around 10^5 (about one-millionth of the CD8 compartment), which suggests that mass-action is allowable on this side. But how many of these precursor cells are co-located with the initial infection, which may transpire on a mucous membrane or a lymph node? The initial battle might take place at this site. This observation prompted several colleagues and an author to construct a stochastic model of CTLs as well as of PITs in early infection. This work lead to an interesting discovery: CTLs alone (in the absence of antibodies against HIV) may be able to abort an HIV infection. Indeed, the authors observed simulated infections in which the PIT population peaked at 1,000 or more but was still driven to extinction by the growing CTL response. In other words, the CTLs win the race, perhaps before the local infection has time to seed other tissues. (Another motivation for this work was to use mechanistic modeling to test the ability of a newly-invented statistical method to overcome a problem of bias in vaccine trials.)

1.11.1 Notes

For a popular account of "chaos" in ODEs, see [109].

Phylogenetic analysis lead to the suggestion that HIV *in vivo* has a low effective population size, [177], although this conclusion has been controversial [268, 275]. That each HIV infection might constitute a form of allopatric segregation (so that early changes are not neutral) appeared in this controversy [268].

Concerning the effective population size and escape, it was mentioned by an author and colleague in [314] in connection with modeling escape from IC; Liu *et al.* also demonstrated, by simulating from a population genetics-type model, that low N_e can delay escape [190].

The debate about deterministic *vs.* stochastic models in biology, other than in population genetics, has raged for a century; e.g., see [18–20, 309, 314].

[30] Trials of vaccines in monkeys often use 1,000 MID50s (1,000 times one monkey-infectious dose that infects 50 % of the time), in order to avoid wasting money and resources.

The cautions about ODEs generating mathematical artifacts are well-known to population geneticists in other contexts; indeed, in 1998 Christiansen *et al.* [59] defined three levels of approximation to the waiting-time problem for the second mutation in a classical Fisher-Wright population. We pursue the first approximation for retroviral sex in Chapter 10.

Even in 2010, papers attempting to explain escape from CTLs by deterministic ODEs were still appearing. Although these efforts are not radically wrong for single-mutation events with large selection coefficients, we do not believe they are correct for other cases (for a more detailed discussion, see the Notes to Chapter 7.) Moreover, ignoring the randomness in infection-chains risks overlooking the most interesting aspects of escape and the impact of vaccines on the phenomenon (Chapters 7 and 12).

For the probability of infection given exposure, see [69, 114, 301]. One theory about the dynamics of the epidemic is that infected persons are much more infectious in the primary stage, where the density of virions in blood and semen is much higher, than are persons in the chronic stage [145]. If true, then the very low probabilities of infection given exposure from discordant partner studies may not be relevant in many at-risk populations; but the issue remains controversial [174, 253, 304].

Stochastic acquisition of infection has an impact on testing drugs and vaccines: [202, 309]. Hence, low-dose studies are needed: [83, 229, 256, 289].

CTLs can abort a retroviral infection: [315, 316, 321]. The latter study was reported at the fall meeting of the HVTN in 2008, in Seattle; an author was in the audience.

Part I

The Biology, Modeling, and Predictions

Chapter 2

The HIV Infection Model

For reasons explained in the Introduction, the model must accommodate new variant populations produced through random mutation (which will appear initially in a single infected cell), possible lineage-extinction, and heterogeneous replication. Multiple genomes, PIT types, and some kind of stochastic process are inevitable components.

The specific type of model we will use is given the long-winded description by mathematicians: a "compartmental, discrete, continuous-time, Markov process". More informally, it is called a "jump process". In this kind of model, the "compartments"—the term was perhaps suggested by the phrase "tissue compartment", from physiology—are collections containing 0,1,2,..., number of entities, which have the same status and potential for change. In our context, the entities will be cells, all having the same infected- or uninfected-status and life-history stage. Hence, their future is alike in probability, although perhaps not in actuality. The dynamics are defined by stating the allowed jumps and the corresponding rates at which they occur. Jumps may create or destroy entities or move them from one compartment to another. Each has a corresponding waiting time, which is a random variable, and so the order in which the jumps occur is also random. The adjective "Markov", derived from the work of an early 20th-century Russian mathematician, means that the probabilities of the jumps are fixed if one knows the current state of all compartment variables. The easiest way to visualize these processes is to imagine how they would be simulated on a computer; the reader might wish to peruse Chapter 4 before proceeding.

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The jump rates in a compartment may be simply proportional to the number of entities, i.e., in our context have the form: per-cell-rate (a constant) times the compartment population. (A familiar example in another context is a statement about incidence of a disease; e.g., "0.1 % of men over 50 are diagnosed with prostate cancer per year" translates in jump-process language into a per-person jump-rate of .001 year^{-1}. If 10,000 undiagnosed over-50 men, defining a "compartment", attended a urology clinic, there would be 10 new cases, on average, per year, which is our "compartmental jump rate". The waiting time before the first cancer diagnosis at the clinic would be 0.1 year, on average, varying from year to year.) If specific rates are constants, the model is said to be linear; if it facilitates growth, it is sometimes called a "branching-process". (Branching processes also describe epidemics, forest-fires, and nuclear chain-reactions.) But a per-cell jump rate in one compartment could depend on another compartment population; e.g., in our full HIV–CTL model, the death rate of PITs will depend on the number of HIV-specific CTLs. If so, the model is said to be non-linear and does not implement a branching-process. Not surprisingly, nonlinear models exhibit more interesting behavior—but are also much harder to analyze.

We now describe the compartments and jumps that define the infection process. A first issue is whether virions should constitute a compartment in the model. After an eclipse phase of about a day, an infected T-cell produces a (highly) variable number of virions: from 50 to 5,000 per day for a few more days of natural lifespan, or until the PIT is killed. Most virions (perhaps 99.9 %) are not infectious, due to sloppy transcription of viral genes or defective packaging of viral and host proteins. Even when infectious, virions have a very brief opportunity to infect a T-cell: the lifetime of a free virion in the body is less than an hour. (If they do not quickly find a target-cell to infect, they are efficiently removed from the blood by the liver and kidneys.) Since virions either infect or disappear quickly, the force of infection at any time is essentially proportional to the number of existing PITs. Hence virions need not form a separate compartment in the model.

A second issue concerns uninfected target cells. Most, but not all, virologists believe that only activated CD4+ T-cells can become PITs. Due to the phenomenon called "hyperactivation", discussed in Section 1.6, the number of targets may be relatively constant, or even increasing, over time. In another theory, revved-up T-cell homeostasis restores missing targets, at least early in infection, before AIDS. Incorporating either scenario in the model would involve us in unsettled questions and add many additional

parameters. For these reasons, we exclude uninfected target cells as a separate compartment in the basic infection model, but we will include them in some discussions in Chapters 9 and 10.

A third issue concerns latency at the cellular level. As HIV infection proceeds, large numbers of infected but unproductive CD4+ T-cells are formed; the mechanism may involve cells being infected at the end of a period of activation, as they are returning to a resting state, or direct infection of resting cells. This "latent pool" of resting T-cells with integrated HIV genomes contributes some infectious virus due to re-activations, which is thought to be the primary obstacle to eradication of HIV by drug therapy. However, the existence of this pool does not likely affect immune response, viral evolution (except for archiving, and occasionally producing, the infecting strain), or escape in untreated patients. Latently-infected cells are probably invisible to CTLs and do not contribute to immune activation. Moreover, a latently-infected cell will likely rest for a long time before being activated again by its cognate antigen (something other than HIV) or the putative bystander-hyperactivation mechanism. Hence the pool probably makes a minor contribution to viral production in untreated patients. Latent cells will therefore not form a separate compartment in the basic model, although we will discuss the accumulation of these cells in Chapter 10.

A fourth decision concerns whether to include all those other cells that HIV can infect, either because a virion can been ingested (by DCs and macrophages), or the cell happens to express the CD4 molecule. For simplicity, and because most production is thought to occur in CD4+ T-cells, we will omit other cell types from the basic infection model.

Finally, there is the question of replication by the infected target cells themselves. Since retroviruses incorporate their genome into that of the host cell, a PIT will likely pass the infection on to its progeny—which could amplify viral reproduction. However, HIV encodes a protein called Vpr and one of its functions is to arrest a T-cell in one stage (G_2) of the cell-cycle. Although this function would seem to be disadvantageous for the virus, it has been argued that the lengthening of this stage (in which more virions are produced than in other stages of the cell-cycle) actually improves viral fitness. For modeling, it means that we do not need to incorporate target-cell proliferation in the infection model.

Ignoring mutation, PIT type, and immune-system killing for the moment, the simplest infection model requires only two compartments. The first, with population denoted X, represents cells in the "eclipse", or infected-target

(IT) phase, before virions appear; and the second, labeled Y, the number of productively-infected-target (PIT) cells. The basic jumps are: transmission of infection to a new target cell (resulting in the "birth" of an IT), progression (IT to PIT), and death of an IT or PIT. The following table schematizes the transitions and rates in the basic process:

HIV Infection-Process Schema

Type	*Jump*	*Rate*
Transmission:	$X \longrightarrow X + 1$	$\iota\, Y$;
Progression:	$X \longrightarrow X - 1$ and $Y \longrightarrow Y + 1$	$\eta\, X$;
Death:	$X \longrightarrow X - 1$ $Y \longrightarrow Y - 1$	$\delta_{\mathrm{IT}}\, X$; $\delta_{\mathrm{PIT}}\, Y$.

In this table, and the rest of this book, lower-case Greek letters stand for rate-constants; they are simply positive numbers. Thus ι, η, δ_{IT}, and δ_{PIT} denote the per-cell rate of transmission, progression, or death of an IT or PIT, respectively. The product $\iota\, Y$ is sometimes called the "force of infection".

Next the model must be generalized to include the additional complexity mentioned at the beginning. In the basic model, only one viral genome will be allowed per PIT. (The generalization to infection by multiple proviruses—integrated viral genomes—will be delayed until Chapter 10.) Only mutations that alter one recognized epitope are included in the model. Hence, the genomes can be identified with sequences of 1's and 0's of length E, the number of epitopes recognized: 1 for wild-type and 0 for mutant epitope. For example, with $E = 3$, the sequence: 1, 1, 1 stands for the wild-type virus, while 0, 1, 0 would indicate that two epitopes had mutated while one was unchanged. With this description, the genomes can be visualized geometrically as the vertices of a cube (hypercube if $E > 3$); there are 8 (2-to-the-power E) in total.

Mutations are primarily due to errors in the reverse-transcription process, i.e., appear just after creation of a new IT. (See Chapter 10 for the details.) Therefore, an IT, and the PIT it becomes, will be infected in the basic model with one, unchangeable, viral genome. We will refer to a "single mutation" as one that changes a 1 to a 0 or vice-versa. Although mutations can produce multiple changes in a genome, such as deletions and frame-shifts, we have lumped these changes into our binary description of an epitope. Thus, "mutation" in our model induces a random walk along the vertices of the hypercube of genomes, with "steps" only to neighboring vertices. (The mutation rate may vary with the locus, as discussed in Chapter 5. The possibility of a "two-step mutation" is discussed in Chapter 7.) With mutations in the model, the force of infection by a particular viral genome is driven also by PITs infected with other genomes, which by a chance mutation during the replication process produce a PIT of the specific type.

Finally, we include different types of ITs and PITs, which represent variation in intrinsic permissivity to HIV replication. A PIT of type k produces virus at rate v_k and a virion generates an IT of type k with probability p_k. Mathematicians call this kind of added heterogeneity "extra-Poisson variation" (EPV), since, if a PIT had a fixed lifetime, and produced virions at a constant rate, the number generated would be a Poisson random variable. (In the popular game of darts, after one player of constant but poor skill has thrown, the number of darts within the outer ring is well-described by a Poisson random variable.) The Poisson law has a very fast drop-off at high numbers (e.g., with mean 10, the probability of 50 is infinitesimal), so EPV accommodates much larger swings in success or failure at reproduction. To implement EPV, we often use the simplest conceivable model, in which PITs come in three flavors. For instance, we can chose: $v_1 = 0$, $v_2 = 1$, and $v_3 = K$ (PITs produce 0, 1, or K virions per unit time) where K may be large (e.g., 10,000) while p_3 is small enough (e.g., .0002) to preserve an appropriate mean. Of course, there are many other candidate distributions for PIT production (unknown *in vivo*), but this choice achieves the desired effect of lowering the "effective population size", N_e (discussed in the Introduction and Chapter 11).

The driving parameter of the process is of course the infection rate (ι), which we derive from the celebrated "basic reproductive number" of the virus, universally written R_0. R_0 originated in epidemiology; it was the primordial "tipping point". There it is commonly defined as the number of secondary infections due to an "index" or primary infected individual, while this person

remains infectious, in the absence of any pre-existing immunity or treatment intervention. The analogy to the *in vivo* situation is exact, if the initial PIT is regarded as the "index" infected cell and the "intervention" ignored is by the immune-system. R_0 and the infection rate are related by a fundamental formula which can be expressed as:

$$R_0(\text{w.t.}) \quad = \quad \text{infection rate} \times \text{mean virion production} \div \text{death rate}.$$

or, in symbols,

$$R_0(\text{w.t.}) \quad = \quad \frac{\iota \times M}{\delta_{\text{PIT}}}. \tag{2.1}$$

We include the abbreviation "w.t." for "wild-type" virus, to distinguish from mutant strains discussed below. M is the sum of v_k's weighted by the p_k's. There is also an unimportant factor involving the length of the eclipse period. (For the exact formula, see Part II, Chapter 17.) It is conventional to work backwards: specify R_0(w.t.) and then, given the other parameters, derive the infection rate from this formula. For reasons explained above, the infection-rate determined in this way ignores target-cell density. If we do wish to discuss the impact of target-cell density, as in Chapters 8 and 9, ι must be replaced by $\iota \times U$, where U stands for uninfected target cells.

We must also specify the basic reproductive number for the mutant strains. The relevant mutations, in addition to escaping immune responses, may also alter "intrinsic fitness". By "intrinsic fitness" we mean growth-rate in target cells, absent any immune response to this virus. Thus the basic reproductive number may depend on genome. We will consider scenarios for which mutations do not enhance intrinsic fitness, that is, for which R_0(mut.) is less than R_0(w.t.), except in Chapter 9. Since we permit multiple mutations per genome, we must allow the corresponding changes in intrinsic fitness to combine in some fashion. The simplest choice is sometimes called a "null-epistasis" model; each mutation contributes a loss-of-fitness factor (f_m), independent of other mutations, which combine in a multiplicative fashion (see Part II). If the factors are not multiplied but combined in some other way, the intrinsic fitness landscape is said to display positive or negative "epistasis", depending on whether the effects reinforce or interfere.

Finally, to fully define the process we must specify the initial conditions: the number of initial ITs or PITs. We discuss this issue in the chapter on simulating from the model.

Thus far, the model defines a multi-type branching-process and the only behaviors it can display are exponential growth or decay of the infected population, depending on the value of R_0. $R_0 > 1$ produces growth on average (extinction is still possible); $R_0 < 1$ leads to decay and sure extinction of the infection. Some strains may be growing out while others are going extinct; that is expected of escape processes. In order to incorporate the CTL response, we add to the death rate (δ_{PIT}) of PITs expressing wild-type epitope "e" another contribution proportional to C_e: the density of the CTL clone recognizing the epitope, introduced in the next chapter. (We use the Greek κ_e for the killing rate, or constant of proportionality.) The antigen that activates these CTLs is proportional to the sum of PITs expressing that epitope. Because the latter feeds back into the killing rate, including CTLs breaks the linearity (or branching-process property) and more complicated behavior ensues.

Table 2.1 records our "standard" values or ranges for parameters of the basic infection process:

Table 2.1: Parameters of the basic HIV infection model

Parameter	Meaning	Value (for rates, per day)
R_0	Basic reproductive no.	4.0–6.0
δ_{IT}	IT non-immune death	0.33
δ_{PIT}	PIT non-immune death	0.33
η	Progression	1.0
ι	Infection	See above
μ	Mutation	3×10^{-5}
K	High-level production	$10 - 10,000$
F_{mean}	High-level mean-fraction	$0.5 - 1$

(The last-mentioned parameter is defined in Part II; it is included to avoid cases where heterogeneity fails to affect survival probability and lower N_e. See Part II, Chapter 22.)

2.1 Notes

A search of the medical database PubMed, maintained by the US National Library of Medicine and the National Institutes of Health, on the string "HIV AND (mathematical model)", on November 22, 2007, at 1:40 pm, Pacific

standard time, yielded 11,549 hits. PubMed does not support adjacency-(phrase)-searches, so this result may be inflated; on the other hand, this result may not include entries with other, equivalent phrases, e.g. "*in silico* method", or papers in pure-math or theory journals. Google, which does support phrase-searching, on 27 November at 3:03 p.m. yielded "about 204,000" hits. Providing a detailed summary of the HIV-modeling literature is therefore not feasible. We will limit citations to papers from which we learned an interesting fact or rate-constant, or with which we have a significant disagreement (beyond the methodological issues raised in the Introduction).

Earlier versions of the infection model appeared in [309, 311, 314]; [309] presented the first stochastic (branching-process) version of an HIV infection *in vivo* (which included reproduction in macrophages but not an immune response). Branching processes appeared earlier in cell biology, in modeling cancer: [215]. A good text in branching processes is [146].

Lifetime of a virion and PIT: [140, 237]. As noted after Section 1.7, the estimates of the first citation cannot be accepted as they were based on a simplistic model of HIV infection.

PIT production: [74, 167].

Eclipse period: Wick was still under the influence of [237] when he wrote early modeling papers and this chapter (probably in 2006); so he adopted 1 day. However, experimentally (*in vitro*) expression of viral RNA is not seen until about 6–12 h [252], viral protein expression is not detectable until about 24–48 h [282, 331], and measurable release of infectious virions is not noted until about 48–72 h [282, 331], so the correct range is 2–3 days. This situation was actually already clear in 1996, from a figure published in [331], which Wick also missed.

Cellular latency: [60, 91].

Vpr arrests the cell-cycle: [110, 126, 126, 133, 254, 262, 286].

Variation in permissivity to HIV replication in target cells due to activation state, exposure to cytokines, or cellular restriction factors: [53, 60, 61, 173, 279].

The range for R_0 in Table 2.1 is controversial. With R_0 in the range 4.0–6.0, peak viremia occurs in our IC model in 20–40 days (see figures in Chapters 4 and 10), which agrees with estimates from studies of primary-infection cohorts where efforts were made to ascertain the exposure event. Rather surprisingly (for us), some authors have argued that R_0 for HIV is 20 or more [90, 185], and for SIV even higher. In the model presented here, $R_0 = 20$ would generate peak infection in a week, which may be correct for LCMV in

mice or SIV in macaques, but not HIV in humans. The disagreement stems from fitting models to VL data, which is almost never available from patients before peak viremia for obvious reasons (the patient has not yet appeared at the clinic), and the TCD-IC dispute. The authors who asserted that $R_0 \geq 20$ used a TCD model with CD4 counts as a surrogate for target-cells, which ignores the activation issue and migration of CD4s from blood into tissues, and they extrapolated back to the moment of infection, despite the fact that no data existed on the initial growth-rate of virus. Concerning *in vivo* evolution, increasing R_0 will shorten the time-scale and hasten early escapes, but otherwise will not alter the principal conclusions of this book (e.g., the Escape Formula, punctuated equilibrium, etc.)

Many additions and revisions to the basic infection model are to be expected. For a model of T-cell activation, including the role of the autocrine growth factor, IL2, see [306]. When the question of whether bystander activation of CD4s *in vivo* actually generates new cells permissible to HIV replication is settled, and rates are measured, it should be possible to ascertain whether hyperactivation results in target cells remaining constant (as in the basic model), decreasing, or (perhaps) increasing during HIV infection.

Note from 2011: as part of a dispute over whether CTLs are really CTLs (see Notes to Section 1.7), we entertained a number of different SIV infection models in which we dropped the assumption that hyperactivation restored targets, in order to capture the rise in viremia after depletion of CD8s. (*Something* has to stop exponential growth in viremia, once immune control is lifted.) The models fit the data in [169, 323] (the papers whose conclusions we disputed) well, suggesting that, at least in macaques infected by SIV, the replacement effect may be absent.

Chapter 3

The CTL Model

T-cells are created continuously in bone marrow and "educated" (selected against recognizing self-antigens) in the thymus. Afterwards, they migrate to lymph nodes and other tissues, where they patrol for cells infected by pathogens. Each T-cell recognizes a specific antigen, which in our context is an "epitope", a short segment of an HIV protein in a complex with an HLA molecule, exposed on the surface of an infected cell. T-cells that have not encountered their cognate antigen are referred to as "naïve" cells; those that have are called "memory" cells.

Both types of cells can exist in a resting or activated state; a naïve cell before encountering its antigen is in the former condition. Naïve T-cells may require contact with an antigen-presenting cell (APC; dendritic cells, macrophages and other cell types play this role) and a CD4+ ("helper") cell in order to become activated. An activated T-cell passes through the "cell-cycle" and divides; this process may continue for a number of cycles. After a certain number of divisions, the progeny cells acquire "effector" status, which for CD8s includes the capacity to kill target cells. Then the cell may die (by a natural process called "apoptosis") or revert to resting; if the latter, in our model it is now regarded as a memory cell. (Some immunologists prefer a model in which effector and memory cells are created in parallel by antigen encounter, and others believe that memory cells can "forget" and relapse to the naïve state.) Memory cells pass through the same sequence, but with faster kinetics, and may not require the collaboration of an APC. Activated cells of either type undergo at least eight divisions without

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having to encounter antigen again. This assumption—sometimes called the "programmed-proliferation" scenario—is an important aspect of our model, as it is the source of a large amplification-factor in the immune response.

The CTL model describes events at the cellular level; hence we do not explicitly represent HLA-peptide complexes, TCR engagement, or secretion of killing factors. The details of these molecular events are abstracted into rate-constants. At this time, too many details of the APC–CD4–CD8 interactions are lacking to include the former pair explicitly in the model; so we absorb contact with these cells into the naïve-cell activation parameter. Nor do we segregate T-cells into tissue compartments such as the thymus or lymph nodes. Rather, model "compartments", in the sense introduced in the last chapter, represent CD8+ T-cells, of naïve or memory phenotype, in distinct life-stages, that recognize distinct epitopes. As before, we will describe the CTL model in jump-process language. We next define the compartments, allowed jumps, and jump-rates.

Ignoring epitope specificity for the moment, the model has one compartment for naïve, resting cells and n_d for naïve cells in the cell-cycle, with analogous compartments for resting or activated memory cells. The different compartments contain cells that have just been generated, or are derived from a certain number of divisions, or have just reverted to resting. Let Z denote the count of cells in a particular compartment. Schematically, the process is defined by the jumps and rates:

CD8 Immune-Process Schema

Type	*Jump*	*Rate*
Birth:	$Z \longrightarrow Z{+}1$	β;
Mitosis:	$Z \longrightarrow Z{-}1$ and $Z_{(+1)} \longrightarrow Z_{(+1)}{+}2$	[Act] Z;
Death:	$Z \longrightarrow Z{-}1$	δ_{NR}, $\delta_{MR}\, Z$; $\delta_{\mathrm{CTL}}\, Z$;
Reversion:	$Z \longrightarrow Z{-}1$ and $Z_{MR} \longrightarrow Z_{MR}{+}1$	$\rho\, Z$.

Here $Z_{(+1)}$ denotes the "next" compartment in the flow of divisions or reversion to resting. The quantity [Act] yields the per-cell activation or meiosis rate. There is one such set of compartments for each epitope specificity. Activation into the cell-cycle of resting, naïve-or-memory CD8s that recognize epitope "e" occurs at rates proportional to the expression of that epitope on PITs, with proportionality factor α_e, or a memory-factor times α_e. Otherwise, for cells in the cell-cycle, [Act] is the inverse of the cell-cycle time. The δ's are cell death-rates (by apoptosis); CTLs promote rather than die except for the finally-differentiated states, at which they can die or revert to resting in the memory compartment. Memory cells react faster, have a shortened cell-cycle, and kill better than naïve cells. The reversion rate (ρ) is chosen so that a fixed fraction ("Revert" in the table below) of CD8s that can no longer divide revert to resting.

The CTLs recognizing epitope e, denoted C_e in the last chapter, are the sum of compartment populations of that specificity: naïve CD8s, which promote to effector status after 4 divisions, and memory CD8s, which promote after one.

Finally, to fully define the model, we must specify the rate of creation ("birth" rate, β) of CD8 T-cells. In order that the immune system maintain itself in a steady-state absent HIV infection, with about 10^5 resting CD8s recognizing each antigen (a figure derived from studies in a mouse, by scaling up), we chose it to exactly balance natural deaths.

We summarize our "default" or standard immune-system parameters in Table 3.1, again with Greek parameters in the left column (ES stands for effector status).

Note that, from the values indicated in the table for n_d and Revert, each activation of a resting cell yields about 13 (2-to-the-power-8, times .05, equals 12.8) more cells; thus the cellular immune-system is, in engineering parlance, a "high-Q" amplifier.

The two choices for the inverse lifetime, δ_{MR}, of an HIV-specific, resting memory CD8 in the table reflect one of many theories about a functional defect in HIV-specific CTLs. The theories attempt to explain the inability of CTLs to clear an HIV infection and hypothesize about a potential vaccine action restoring "proper" functioning. In the "defective memory" scenario, proper, fully-differentiated, HIV-specific, resting memory cells (recently dubbed by some immunologists "central memory" cells) are never created in HIV infection, possibly because of a lack of help by HIV-specific CD4+ T-cells. By contrast, influenza-specific CD8s persist in recovered persons,

Table 3.1: Parameters of the basic CTL model

Parameter	Meaning	Value (for rates, per day)
α_e	Resting activation	10^{-9}–10^{-10}
κ_e	Immune killing	10^{-9}–10^{-10}
β	NR "immigration"	See above
δ_{NR}	NR death rate	.00017
δ_{MR}	MR death rate	.333 or .00017
δ_{CTL}	CTL death rate	.333
Revert	Reversion fraction	.05
n_d	No. of doublings	8
–	Naïve cell-cycles/day	2.0
–	Memory cell-cycles/day	4.0
–	Naïve cell-cycles before ES	4
–	Memory cell-cycles before ES	1
–	Memory speed-up factor	7.0

who are clear of virus, for decades. Since the HIV-specific CD4s are also HIV target cells, which in order to function must come in close proximity to infected antigen-presenting cells, they may be early casualties. Thus, in this theory, HIV is controlled by a self-renewing pool of short-lived, killer T-cells (lately called "effector-memory" cells). Evidence for this scenario includes the observation that, after escape from recognition of an epitope, the corresponding CTL clone disappears quickly.

There are other theories of a CTL defect that can generate time-curves of infection and immune response similar to those we show in the next chapter. For example, defective activation (HIV may provide too little immune stimulation, i.e., in model terms, parameter α is too small; the Nef protein comes into this picture, see Chapter 9), or insufficient killing power (κ is too small). A more provocative theory perhaps derives from another name applied to the family of viruses that includes HIV: "lentivirus". (The prefix "lenti" means "slow".) In this picture, HIV evades elimination by growing too slowly to provoke a sterilizing immune response. Indeed, if we "speed up" the virus by increasing R_0 and decreasing the eclipse period, the CTLs eradicate the infection, but we are no longer modeling HIV. The precise nature of the HIV-specific CTL "defect"—if one exists—does not greatly impact on the escape question.

3.1 Notes

For the programmed-proliferation scenario see [13, 157]. For the precursors that recognize a given antigen (scaled up from a mouse): [26].

A CTL model was described in [314]; an earlier version appeared in [311]. Since then many investigators have proposed alternate hypotheses about CD8 lineages; for example, that the effector *vs.* memory decision may be made at the first division of a naïve cell, by asymmetric cell division, rather than an apoptosis *vs.* memory decision after the last division, as we have assumed. The model will require many revisions over time, but we doubt that they will substantially affect the escape scenarios we describe in this book.

The killing rate in Table 3.1 was estimated from *in vitro* and *in vivo* data by fitting a model [319]. The *in vitro* data, on the suppression of HIV growth in cell-lines by CTLs, was derived in Otto Yang's lab [331]. The *in vivo* data resulted from an experiment performed at the University of Washington and the Fred Hutchinson Cancer Research Center (P.I., L. Corey) [42]. In this IRB-approved clinical trial, HIV-specific CTLs were derived from HIV-infected patients, engineered with a genetic marker, amplified *in vitro* by a factor of a billion, and re-infused into the patient. (In immunology, this classic type of experiment is called "adoptive transfer".) The exogenous CTLs functioned *in vivo*, driving down the number of PITs in blood; unfortunately, the CTLs disappeared in 2 weeks and the benefit was transient. But the experiment successfully perturbed the infected steady-state, providing an opportunity for a modeler to derive an important parameter. The *in vitro* and *in vivo* estimates were comparable in magnitude (granted a plausible re-scaling); another interesting observation is that the killing efficiency of the stored CTL clones on infected cell-lines varied by about a factor of four, with a Gag-specific epitope superior to three other clones recognizing other epitopes. Only a composite of activation and killing could be estimated from these experiments; lacking any experimental data to distinguish them, we usually identify parameters α and κ. (The logic is that both have at least one step in common, namely a TCR–HLA-antigen interaction; but of course due to the other steps, such as degranulation for killing, they may be very different.) For the *in vitro* data, the authors of [319] estimated also a "saturation" (aka "Hill", see Part II, Chapter 18, Exercise 2) parameter, meaning that the activation/killing term in the model was permitted to have a declining slope as a function of the "effector-to-target" (CTL-to-PIT) ratio. However, the ratio *in vivo* is low enough that this extra parameter appears not to play a role.

Chapter 4

Simulating an HIV Infection *In Vivo*

When the HIV and CTL models are combined, we have gone beyond familiar textbook examples of biological modeling. Although mathematics provides insights into the predictions of the model (described in subsequent chapters), to some extent we must rely on computer simulation.

If we are satisfied to implement the whole system deterministically, simulation is easy. The method is basically just updating by rates. First, we chose a small time step, invariably denoted, in the numerical-analysis literature, by "h" (not to be confused with Planck's constant, $\hbar$). This step should be smaller than any biologically-interesting time interval and possibly diminished further to minimize the error inevitably present in all computer calculations (due to approximations and the finite storage of floating-point numbers). For virology and immunology modeling, we usually use $h = .01$ day. If C denotes a compartment population at time t, the update to time $t + h$ is given simply by:

Deterministic Simulation Routine

$$\begin{aligned}(\,C \text{ at time } t+h\,) \;&=\; (\,C \text{ at time } t\,) \;+\\ \{\,(\,\text{change in } C \text{ due to jump } \#1\,) \times (\,\text{rate of jump } \#1\,) \;&+\\ (\,\text{change in } C \text{ due to jump } \#2\,) \times (\,\text{rate of jump } \#2\,) \;&+\; \cdots\} \times h.\end{aligned}$$

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The rates are taken from our tables. All the compartments are updated, then the routine begins again, until a final time is reached. Mathematicians say this procedure approximates "the solution of the system of ODEs" (formulas which specify the rates). Called the "Euler method", it has poor error-control, and a trick due to mathematicians C. D. T. Runge and M. W. Kutta, abbreviated RK4,[1] is used to correct it; see Part II.

If we could take $h \to 0$, we would obtain curves expressing each compartment's complete history. Sometimes calculus yields these curves as explicit formulas. Mathematicians in the 19th and early 20th centuries were adept at finding such solutions, provided the number of compartments was less than three. (This was the case for Lotka's and Volterra's work after the First World War modeling fish populations.) Unfortunately, even if we accept determinism in our context, it is impossible to model HIV evolution and the immune system with two compartments. Nor can we solve our higher-dimensional model by formulas. This is not due to lack of cleverness on our part; one of the major surprises in 20th century mathematics was the realization that non-linear ODEs with more than two compartments simply cannot be solved in the 19th century sense.[2] Even a three-compartment system with quadratic rates can generate trajectories too complicated to be captured by any conceivable formula.[3]

As we pointed out in the Introduction, Section 1.11, we cannot rely on ODEs for any population if it is ever small (as for a new HIV variant). Should we abandon determinism and treat the whole shebang as a pure stochastic process, with random choices for the timing and type of the next jump? Beginning with Bachlier's 1900 treatment of price fluctuations in a market, Markov, Kolmogorov, Feller and other mathematicians learned how to calculate probabilities of various motions in a random process, but for our non-linear, many-compartment system the equations are

[1] Published by Kutta in 1901.

[2] Despair at solving such systems was one of the reasons that mathematicians desired mechanical assistance. The great John von Neumann, sometimes called the "father of the electronic computer", was not looking forward to data bases or the Internet; rather, he once remarked: "the analytic approach to solving non-linear problems had failed."

[3] The meteorologist E.N. Lorenz observed these strange curves in 1963 in a simplified model of the atmosphere. Lorenz's discovery is now dubbed "chaos" and associated with certain metaphors about extreme sensitivity to initial conditions, e.g., that butterfly flapping its wings, causing a hurricane a century later.

intractable. Simulating a discrete jump process directly from its definition is straightforward, however, and can be summarized in the following mock-program:

Direct, Stochastic Simulation Routine

1. Using the rate expressions listed in the tables, compute rates for all allowed jumps;
2. Using the random-number generator (RNG) provided on the computer platform, generate a prediction for the time of the next jump, for each that is allowed, and store these times in computer memory;
3. Pass through the list of projected jump-times and ascertain the smallest one;
4. Make the jump in the corresponding compartment, and re-compute any rates that are affected by the change.
5. Return to step 1.

In step 2, only the jump-times whose rates are influenced by the previous jump need be re-generated; the others can simply be decremented by the time increment of that last jump.

The famous "Markov property"—the essential assumption needed to formulate any dynamical law—reads: "the future and the past are independent, given the present"; i.e., memory of the past is irrelevant. There is only one candidate rule for jump-times that can implement the Markov property, called by probabilists "the exponential law". It originated in physics and is rather inappropriate in biology, as it does not permit an individual to age. For example, modeling human lifespans by an exponential law is like accepting the Neutrino Theory of Death (you live until hit by a neutrino[4]). If you believe this insane theory and an average human lifespan of 80 years, and are confronted by a 79-year-old who demanded to know how long she

[4]Neutrinos—"little neutral ones", named by Enrico Fermi—are ghostly particles copiously created by nuclear reactions in the Sun. Billions are passing through your body at this instant, but you are unaware of it because neutrinos barely interact with matter. The NTD was invented by three inebriated physics students at the University Pub, Seattle, USA, around 1975, and blissfully forgotten by morning.

had to live, you would have to answer: "80 years!" (The neutrinos do not know her age.) The exponential law also has maximal intensity at time zero and a heavy tail; if you modeled a human population of one million in this fashion, there would probably be infant mortality in the first hour and a Methuselah. But the exponential has the practical advantage that to update a projected event-time when it is changed by a jump in another compartment, we need only recompute the rate and draw a new exponential random variable. Otherwise, the task is similar to the problem in statistics of introducing a time-dependent covariate into a survival-distribution, and there are many choices.

Although the direct routine will be used in this book when compartment sizes are small (e.g., less than 300), the barrier to using it in the full model is that intensity at zero age—in other word, the infinitesimal jump-time problem. When a compartment has size 10^{11}, typical of cell biology, the time to the next jump will be very small, perhaps of order 10^{-11} time-units. If 1 day is the basic time-unit, it might be 10^{-6} s.[5] Even with modern PCs, which can perform a billion floating-point operations per second, simulating the process for many model-years would take an annoying amount of real time.

Surely a jump in the next microsecond is only an artifact of the Markov, compartmental set-up? Indeed; the alternative is called an "agent-based" or "semi-Markov" model. Such a process can be considered as compartmental, with each "agent" (in this book, CTL or PIT) defining its own compartment. In an agent-based model, any probability law can be chosen for the next jump-time, including more realistic choices with a cut-off at short and long times (e.g., no cell survives for less than one second, or for longer than 70 years). Due to the interactions, we would still face the problem of how to update these laws after each jump. Moreover, in our context there would be 10^{11} agents, and that is a lot of storage even with modern chips; updating the whole state of affairs would presumably take hours. Compartmental models will remain essential in immunological and virological modeling, at least for a few more spins of Moore's law.

[5]Some improvement in realism accrues by allowing the event to occur after a series of jumps, with independent exponential distributions. This yields a Gamma law, which has zero density at zero age; but, since the first jump must still be recorded, it solves neither the infinitesimal jump-time problem nor the Methuselah problem.

Because the cellular immune system has relatively large populations available, we can use ODE updating for this part. But viral variant populations must be permitted a stochastic evolution, at least until they grow large enough to make mass-action a reasonable approximation. Thus the overall process becomes an unusual mix of deterministic and stochastic processes, transitioning from one status to the other, and interacting through non-linear functions. There are no standard methods to simulate such mixed processes. As a consequence, Steve Self and an author introduced, in two papers published in 2004, a new simulation technique for mixed compartmental processes. The method has a third level of approximation between the pure-jump process and the ODE. The basic idea is to promote a compartment from discrete to continuous status when it becomes reasonably large, then approximate the updating by a technique introduced by the Russian mathematician G. N. Milshtein in 1972 (for a different situation; see Notes). This technique only requires drawing as many normal random variables as continuous compartments (most computer packages include a routine that generates normals), and making some computations. Other issues arising, such as what to do if some compartments are discrete and some are continuous (updated by Milshtein or RK), are also covered in these papers. We elaborated specific criteria for when to switch a discrete compartment to a continuous one, or *vice versa*, which are computed during the simulation. Hence, we will refer to the technique as the "switch method". We implemented the whole routine, pure-jump plus Milshtein plus RK, with switch-over rules, for the combined CTL-and-HIV model, in a program written in the C computer language. With 3 epitopes recognized by the immune-system, 2 infected life-stages of target-cells, 3 PIT-types, and 8 HIV genomes, the total number of compartments is 102 and the routine took about 5 min, on one processor of a Pentium table-top computer (circa 2004), to simulate one HIV infection for 1 year.

Figure 4.1 illustrates the method for an HIV infection, starting with 100 infected-target cells ("ITs") and 10^5 naïve, resting, HIV-specific, CD8s recognizing one epitope, but omitting mutation. (R_0 was 4.0.) We note that the model displays the well-known features of primary infection, such as the pinnacle and plateau in viremia and the time-lag of several days between peak VL and peak immune response. That the model captures the latter without further complications shows that the oft-expressed belief that some other immune-effector cell type must be involved in bringing down viremia is not logical (although neither does it refute the claim that innate effectors such as NK cells, see Chapter 9, may play a role.) The reason that the CTLs

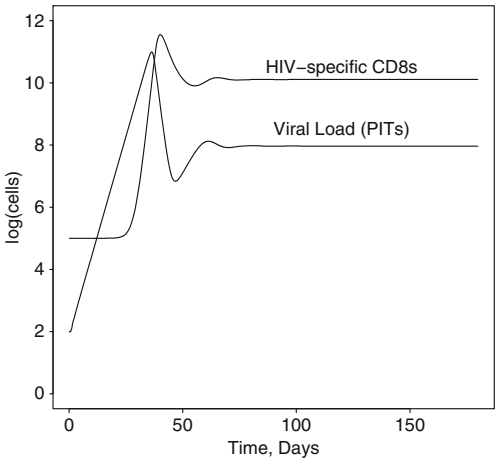

Figure 4.1: An HIV infection, simulated by the "switch method". (100 initial ITs.)

peak later but still suppress the virus is simple: they are working effectively before they attain their maximal memory population. The exact timing of the peaks depends on rate-constants, chiefly R_0 and activation; for example, upping R_0 to 6 pushes the VL peak back about 10 days.

Figure 4.2 shows the result of simulating from the completely-deterministic version of the model (updating all compartments by RK) with the same assumptions; clearly the switch-method is not essential here. However, with one initial IT the situation changes; in the stochastic model some infections go extinct, as shown in Figure 4.3. (In one of four runs, the infection died out. The extinction rate is discussed in the next chapter and Part II.) Figures 4.4 and 4.5, for which we included three epitopes and allowed mutation, illustrates escape—and the necessity of stochastic modeling when multiple viral variants may exist.

4.1 Notes

Mathematicians have created a large literature on approximations (especially by "diffusions") to jump processes, and computer scientists a similarly large corpus on simulation. But when faced with the problem of simulating an

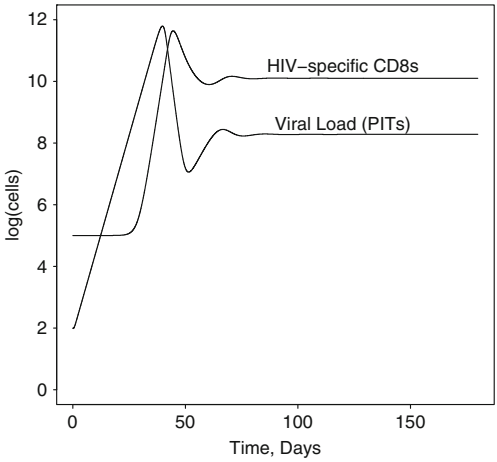

Figure 4.2: An HIV infection, simulated entirely by ODEs. (100 initial ITs.)

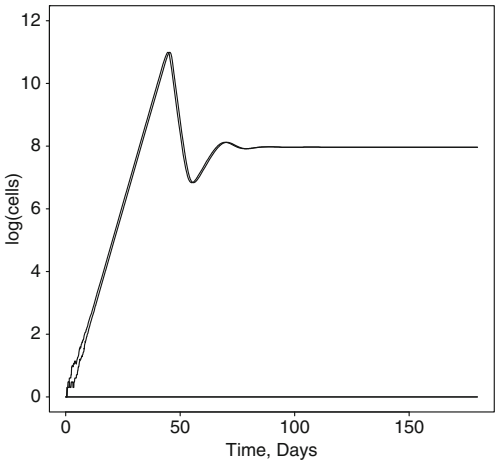

Figure 4.3: An HIV infection, simulated by the "switch method". (1 initial IT; 4 runs.)

infection that might start with a single cell and fluctuate over 11 orders of magnitude, meanwhile stimulating and being limited by another highly-variable cell population, we did not find a published solution and had to start from scratch. Our "switch method" is described in [312, 313].

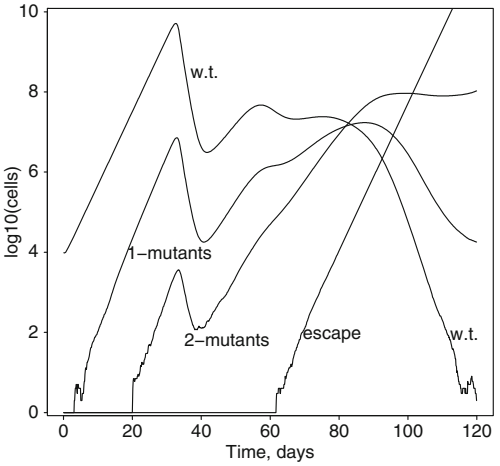

Figure 4.4: An HIV infection, including mutation. (100 initial ITs; 3 epitopes; no EPV.)

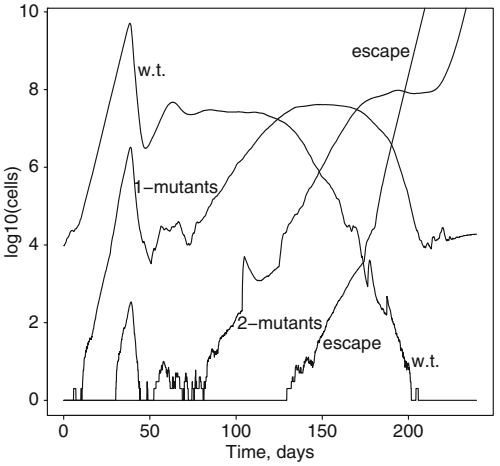

Figure 4.5: An HIV infection, including mutation. (100 initial ITs; 3 epitopes; moderate EPV.)

The combined model is said to be "whole-body, uniformly-mixing", because CTLs and PITs located anywhere in the body are assumed to have the same probability of encountering each other. The distinction is with a model in which CTLs and PITs may be localized to some tissue, e.g. a mucous membrane or lymph node; see [316] for further discussion of this issue and its possible consequences for a preventive vaccine. The killing-rate parameter derived from [319] (discussed in Chapter 3) must be converted from laboratory units (the estimate of [319], up to a factor of 4, was around 0.3 microliter cell^{-1} day^{-1}), to the whole-body context. The laboratory value must be multiplied by a volume factor: roughly $1/(5.50.10^6)$; 5×10^6 for 5 l of peripheral blood and 50 for the fact that only 2 % of T cells reside in peripheral blood (98 % reside in tissues). Hence the small magnitudes of the parameters α and κ in Table 3.1 in Chapter 3, which reflect the small probability of a CTL and a PIT coming together. In a model with "biographic" localization, these parameters might be much larger, but to extend the model to the whole body we would require more parameters describing "emigration" from one tissue to another. These have not yet been measured.

Chapter 5

The Escape Formula

Since the 1930s, geneticists have invoked the metaphor of a "fitness landscape" to describe the selective advantages conferred by varying genes in a static environment. The environment of HIV *in vivo* is not static, but dynamic and reactive, so topographical imagery is dubious. Also unlike classical genetics, we must distinguish two contributions to viral fitness: one relating to the ability of HIV to grow in its target cells, independent of any immune response, and another reflecting immune pressure. Both appear in a heuristic formula for the rate of escape from CTL control, which we describe in this chapter.

The situation of interest is that one HIV strain is being held at steady-state, and we wish to know the rate it can escape by a particular mutation that diminishes or abolishes recognition by CTLs of a particular epitope. (As in previous chapters, we will refer to the controlled strain by the name "wild-type", even it differs from the infecting strain.) The formula expresses the expected number of times per generation that a new HIV lineage with the escape mutation appears and avoids extinction. The formula is a product of factors, which we describe in detail below.

In symbols, it reads.

$$\mathrm{ER} \;=\; N_e\,\mu\,(\,\mathrm{REA} \;-\; \mathrm{LOF}\,)^{+}\,. \tag{5.1}$$

The meaning of the superscript + is that the escape rate per generation due to this one mutation is positive and equal to the product of these factors if REA is greater than LOF, and otherwise zero.

Next we discuss the meaning of these quantities. The effective population size, N_e, of the number of producing cells was mentioned in the Introduction

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The Escape Formula

The Escape Rate Equals:

Effective Population Size, times

Mutation Rate, times

(Relative Escape Advantage minus

Intrinsic Loss-of-Fitness).

(and will be further discussed in Chapter 11); it may be lower than the demographic population size N_d, the true number of PITs. The assumed reason is heterogeneity in replication; in fact, the exact formula (derived in Chapter 20) contains a factor of N_d divided by the variance in production (the variance of the number of viable virions produced by a PIT in its lifetime). The justification for writing the ratio as N_e is presented in Chapter 6; it derives from traditional genetics, where it is related to the inbreeding definition.

The mutation rate (μ) represents the probability, per cycle of replication, of generating the particular escape variant. The simplest kind of mutation, called a point-mutation, alters a nucleotide in the HIV genome; to yield an escape it must also change an amino-acid in an HIV protein that affects CTL recognition of the epitope. As explained in Section 1.3, point-mutations are mainly due to error-prone reverse transcription. Other types of mutations, such as frame-shifts and deletions, can also produce escape and are covered in the formula. (Recombination, that can bring mutations together, is discussed in Chapter 10.) The mutation rate, as defined here, must be carefully distinguished from the rate of generating a successful mutant lineage—one that avoids extinction; the former is measurable in the lab, while the latter is

only defined *in vivo* and depends on many factors not realizable in the test-tube. The formula expresses the second probability, not merely the chance of changing an amino-acid.

By "relative escape advantage" (REA) we mean the fraction of killing escaped. REA = 1 would mean complete abolition of immune killing by CTLs recognizing the given epitope, and REA = 0 no change. Finally, the intrinsic loss-of-fitness (LOF) affects the variant's ability to replicate in HIV's target cells, independent of the immune response. LOF = 1 would mean inability of the new lineage to expand, even if there were no killing by CTLs, while LOF = 0 means growth at the same rate as the wild-type, in that same immune-free situation. (There are also some unimportant constants of order one in the exact formula we have omitted in this discussion; see Part II for the details.)

Generally, at least until late in the escape story, there will be more than one epitope recognized. If more than one of these responses can be escaped by mutation, and if we neglect the small chance of the virus making several of these mutations in one round (discussed in Chapter 7), the overall escape rate is the sum of the contributions for each individually.

We derived the Escape Formula by considering the probability that a lineage goes extinct in a branching-process (BP). A BP is a stochastic jump process in which the dynamics of entities of a given kind (here, cells infected by a given viral lineage) is independent of other kinds (cells or lineages). A BP that can grow (called, in the probability literature, "super-critical") has a dichotomous fate: it either expands forever or goes extinct. As we remarked in Chapter 4, the full HIV–CTL process does not define a BP. The independence assumption is not satisfied, because, for example, one lineage may express the same epitope as another; hence these PITs will stimulate and be killed by the same clone of CTLs. Nevertheless, for each appearance of a new variant strain, the extinction-or-growth decision is likely to be made quickly, while the variant population is less than 1,000 PITs. The CTLs will be relatively unperturbed (note the activation parameter in Table 3.1), and the BP-approximation will be satisfactory.

The essential idea in the derivation is "effective reproductive number", $R_{\text{eff.}}$. By this we mean the expected number of offspring PITs of a certain PIT, given the current immune environment. For example, granted that the wild-type is held at steady-state before any escapes, $R_{\text{eff.}}(\text{w.t.}) = 1$: each wild-type PIT generates one other, on average, before dying. This is simply the definition of steady-state. The first step in deriving the formula is a

computation of $R_{\text{eff.}}(\text{mut.})$, the effective reproductive number of the variant with an escape mutation. In the second step, the probability of escape is related to $R_{\text{eff.}}$. For example, in the simplest branching-process model of HIV growth (neglecting the eclipse period and heterogeneity), the extinction probability is simply the reciprocal of $R_{\text{eff.}}(\text{mut.;m})$. If $R_{\text{eff.}}(\text{mut.;m}) = 4$, the extinction probability is 0.25, so that the escape probability is 0.75; if, as is conceivable for escaping minor responses, $R_{\text{eff.}}(\text{mut.;m}) = 1.01$, these probabilities are .99 and .01, respectively. The explanation for the appearance of N_e in the Escape Formula is that heterogeneity in replication can greatly increase the extinction-rate. Suppose that a mutant PIT makes 0, 1, or 10^3 offspring before dying, with probabilities .4965, .5 and .0035. Then its $R_{\text{eff.}}$ is again 4.0, but the probability its lineage goes extinct is now about .998. See Part II, Chapter 22, for the math, but the reason is intuitive: a PIT of this type tends to have 0 or 1 offspring most of the time, so its line is likely to be extinguished unless saved by a rare, and prolific, descendant. Hence, what we called extra-Poisson variation (EPV) in Chapter 2 can substantially delay the time-to-escape.

In subsequent chapters we will call the difference in the last factor: REA–LOF, the "effective selective advantage", denoted ESA. ESA replaces the classical selection coefficient ("s") of population genetics, which is not well defined because REA is a function of the entire immune and viral environment existing at that time (discussed further in Chapter 7), and not simply attached to the mutation. In general, the REA for an escape depends on how many epitopes are still wild-type, by how much they stimulate CD8s, and how well these kill PITs. If, for example, there are three epitopes recognized, and the corresponding CTLs make equal contribution to killing PITs (unlikely), then each REA $= 1/3 \approx .33$ and an ESA is positive for a mutation escaping one of these responses provided the LOF at that locus is $< .33$, i.e., the variant loses less than 33 % of its replicative capacity. With each escape, the REAs and corresponding ESAs of the remaining loci change (we will study this dynamics in Chapter 7).

5.1 Notes

With respect to prediction and testing, the Escape Formula has the advantage of incorporating only observable quantities. The mutation rate at each locus can be measured in *in vitro* propagation experiments. The CTL responses

in vivo (the densities we called C_e) can be detected in blood by a variety of techniques. Estimation of the killing potentials (the parameters κ_e) was discussed in the Notes to Chapter 3. Methods have been developed for reliable estimation of LOF [31, 105] associated to a mutation *in vitro* (in cell-lines); and, for some instances, *in vivo*—namely, transmission of escape strains to an HLA-incompatible recipient [178].

Chapter 6

Early and Late Escapes

The immune-control hypothesis, as we explained in the Introduction, leads to a very different picture of HIV evolution *in vivo* than that of traditional population genetics. In this chapter, we compare the explanations for escapes derived from the two approaches.

6.1 Explaining Early Escapes

For purposes of discussion, let us label an episode an "early escape" if a variant strain, that has altered or deleted recognition by at least one CTL clone, replaces, or at least grows to rival, the wild-type by 2 years post-infection (p.i.). Figure 6.1a and b, shows two examples: escapes in Gag and Env epitopes, both complete by 8 months p.i. Dozens of similar examples can be found in the literature, see Notes.

Classical genetics, in the deterministic regime, provides a formula for the frequency of wild-type and variant strains during such an episode. (See Part II, Chapter 21.) The formula involves only time (usually expressed in generations), the mutation rate μ at the locus of interest, and the selection coefficient, s. As usual in population genetics, it is assumed that if the variant grows by x, the wild-type had to drop by x (because the sum is assumed constant), so only one population need be shown. We show the wild-type curves. We first checked that this formula accurately predicts simulated replacement curves using the fixed-N_d, classical genetic model; see Figure 6.2. (The figure shows the w.t. frequency. We assumed the deterministic, strong-selection regime; $N_d = 10^7$; $\mu = 3 \times 10^{-5}$; and $s = .03$.) We included the

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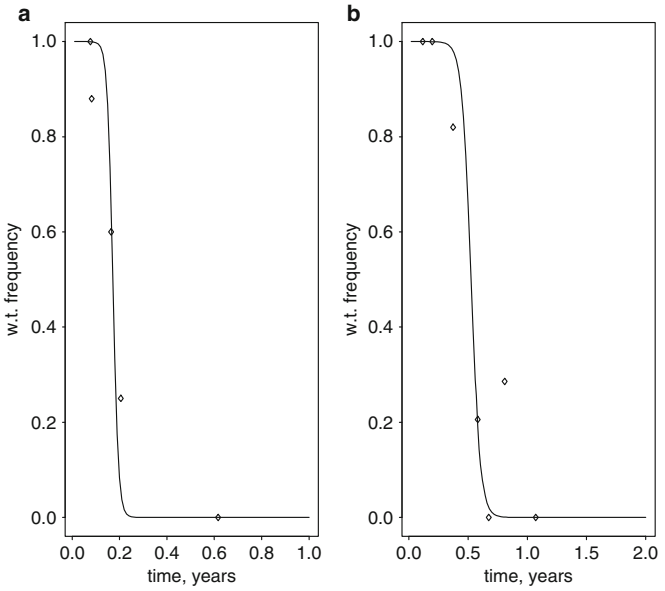

Figure 6.1: Two early escapes, in Gag and Env, and fitted curves.

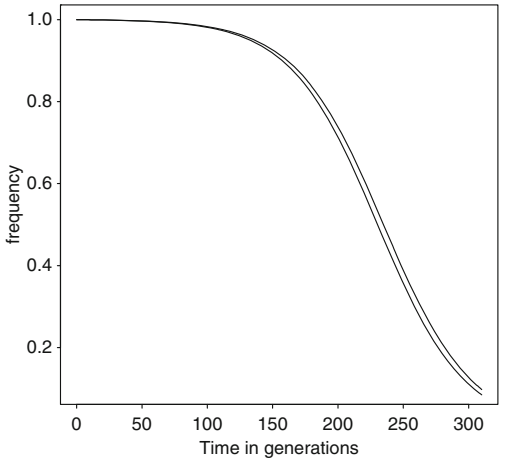

Figure 6.2: Escapes in the classical model; deterministic regime.

best-fitting curves using this formula in Figure 6.1; for early escapes, they fit the data fairly well. The classical selection coefficient, s, is often estimated in this way and reported in the literature. But we will argue in Chapter 7 that there is in fact no "s" uniquely associated to an escape mutation. A fit of this single-locus model (if it does fit) can be spurious and misleading.

Although a full HIV–CTL model has too many free parameters to undertake a formal model-fitting exercise with data from only a few sequences and time-points, it can generate similar patterns. Figure 4.4 showed the viral populations in a typical simulation (without EPV) as mutants with little intrinsic loss-of-fitness escape. Figure 4.5 showed how moderate extra-Poisson variation (EPV, resulting in a lower N_e) can slow down the process, by about twofold in this case over Figure 4.4.

6.2 Late Escapes and the "Fall-Off-a-Cliff" Shape

One group of investigators searched for CTL escape mutants in stored blood samples from five subjects followed from the middle 1980s to the early 1990s in the Multicenter AIDS Cohort Study (MACS). The patients were effectively untreated and had uncontrolled viremia. By sequencing multiple viral clones from different time-points, the investigators were able to document escape from CTLs as late as 12 years p.i. For several patients, the viral genotype-frequency curves had a surprising shape. See Figure 6.3a, which shows the wild-type frequency from patient 'B' of their study; panel (b) of this figure shows another late escape from a different study. In the particularly striking example of patient B, the virus had a wild-type amino-acid called threonine ('T' in a.a. sequences), at the seventh position of a CTL epitope in Gag p17, in 10/10 clones sequenced at two time-points prior to, and at, 3.35 years p.i. At 3.54 years p.i., the threonine was replaced by a valine ('V') in 12/12 clones, and remained 100 % V for three additional samplings (at 4.66, 5.67 and 6.73 years). A CTL-response assay revealed that the T-to-V mutation conferred a 70 % drop in recognition of the epitope. Moreover, the CTL clone recognizing the wild-type epitope peaked simultaneously with the mutation, then disappeared.

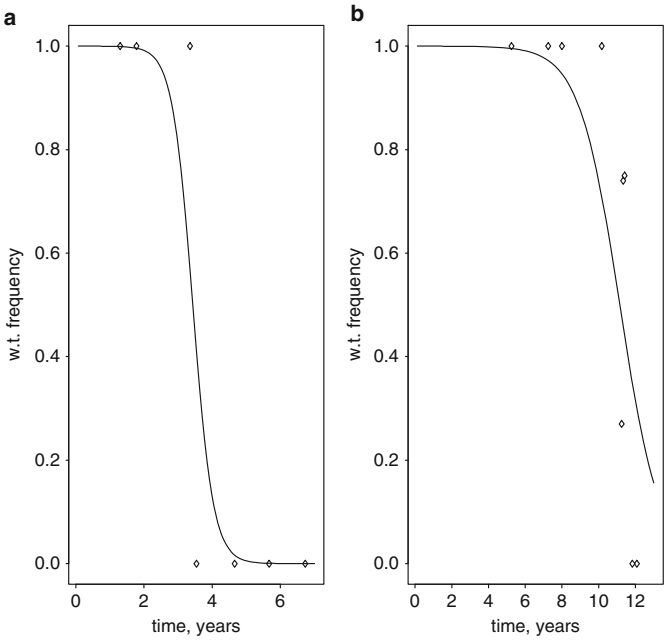

Figure 6.3: Late escapes and fitted curves. (**a**) patient 'B'; (**b**) patient '007'.

6.3 Can Classical Genetics Explain Late Escapes?

For late escapes, such as for Jamieson *et al.*'s subject B, the fit of the deterministic formula is poor, the best curve under- or over-shooting the wild-type-frequency when it falls; see Figure 6.3. The reason of course is that the one free parameter—selection coefficient, s—cannot explain both the long stability period and the speed of the sweep. The simplest way to see this is to note that a tiny, positive s would result in a gradual substitution of variant for wild-type, which in about half the time to fixation would be about half-complete; if escapes can look like a fall off a cliff, this explanation cannot work. (For a more sophisticated calculation, see Part II, Chapter 21.)

Besides the fall-off-a-cliff problem, a biological difficulty with the deterministic model arises from the long time before the replacement. A delay of

many years can only be explained in this paradigm by a very small selective advantage; e.g., for patient B in Figure 6.3a (escape in 3.5 years), the best fit was $s = .0091$ day^{-1}; for patient 007, Figure 6.3b (11.2 years), the best was $s = .0025$ day^{-1}. These small selective coefficients require a peculiar coincidence of effects derived from viral and host biology, as can be seen by contemplating the Escape Formula. The quantity taking the place of the traditional "s" is, up to trivial factors, the difference REA–LOF.[1] The REA term is derived entirely from the host immune response, while LOF refers solely to the growth-rate of virus in target cells. HIV entered the human race fairly recently, after perhaps millennia of SIV evolution in monkeys; it is conceivable that nature arranged such a coincidence. Nevertheless, mathematical explanations based on coincidences in parameter values (sometimes called "knife-edge" theories) are methodologically suspect. Moreover, tiny positive selection coefficients imply a virtually flat fitness landscape—which might describe the evolution of fruit flies raised in glass jars, but for pathogens *in vivo*, it is not plausible. (Jars, after all, lack immune systems.) By contrast, small *negative* selection coefficients readily arise from escape sequences in a single patient's lifetime (discussed in the next chapter).

Can classical genetics explain sudden escapes by invoking the small-N_e, stochastic regime? We next determine what level of reproductive heterogeneity would be needed. With heterogeneity, N_e replaces N_d in the definition of the drift-regime boundary, which becomes $N_e\,\mu < 1$. Mathematicians developed diffusion processes, derived from Einstein's Brownian motion theory with an additional deterministic "'drift", to describe the random evolution of gene frequencies in this situation. But the fall-off-a-cliff shape, with highly variable time-to-the-fall (see Figure 6.5 below), is not reminiscent of the paths of a diffusion. Moreover, the extreme variation we have to consider renders doubtful the approximations used in the derivations. Therefore, we resort to a simpler branching-process calculation.

In the stochastic regime, mutants appear sporadically. Once a favorable mutation occurs, the new lineage may grow to fixation or die out. Before frequencies change appreciably (altering the immune response), the mutant lineage can be treated as a branching-process, with mean number of successful offspring $1 + s$ and variance-to-mean ratio VMR. Both quantities can

[1] If $R_{\text{eff.}} = 1 + s$ and s is small, then, up to an unimportant factor, ESA $= 1 - 1/(1 + s) \approx s$; see Part II, Chapter 20.

be derived from the offspring distribution and the sampling hypothesis (see Part II, Chapter 21). Using these numbers we find

$$\text{Probability of outgrowth} \quad = \quad \frac{N_d\,\mu\,s}{\text{VMR}}. \tag{6.1}$$

Now N_d/VMR is approximately the inverse probability of two offspring having the same parent, which is the inbreeding definition of N_e; hence, we can rewrite this formula as

$$\text{Probability of outgrowth} \quad = \quad N_e\,\mu\,s. \tag{6.2}$$

Once the successful mutant lineage appears it grows exponentially at rate $1 + s$. Thus, to explain a long stable period, conventional genetics can assume N_e small (because VMR is large); by taking s large, it can also explain a sudden replacement, which is indeed seen in Figure 6.4. (The figure shows w.t. frequency curves in two runs. We assumed the stochastic, strong-selection regime; $N_d = 10^7$; $\mu = 3 \times 10^{-5}$; $s = .3$; and VMR $= 10^5$.)

A typical untreated HIV infection involves 10^7-10^8 PITs. For one (successful) mutant to replace the wild-type in 0.2 year (36 generations) requires

$$(1 + s)^{36} \quad = \quad 10^8, \tag{6.3}$$

which yields $s = 0.66$. For the wild-type to remain stable for 3 years requires

$$N_e\,\mu\,s \quad \approx \quad .002. \tag{6.4}$$

With $\mu = 3 \times 10^{-5}$ and $s = 0.66$, $N_e\,\mu\,s = N_e \times (2 \times 10^{-5})$. So $N_e = N_d = 10^8$ is ruled out, as is the "compromise" candidate, $N_e = 10^5$. The drift-selection regime of classical theory would require $N_e = 10^2$, perhaps six orders-of-magnitude below the demographic population, to explain this observation without other hypotheses. (We discuss these other hypotheses in Section 7.4.)

6.4 Late Escapes in the IC Model

If one accepts immune-control of HIV, it would be inconsistent to explain late escapes by arranging to have tiny positive selection coefficients in the model. But that is not necessary. Figure 6.5 illustrates replacements in a scenario we

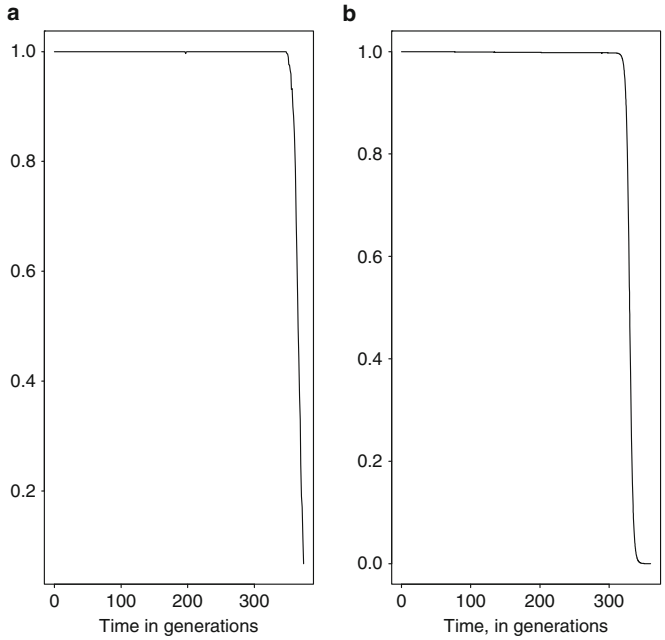

Figure 6.4: Escapes in the classical model; stochastic regime.

call "Gag immunodominance" (see Chapter 7); the sweeps are greatly delayed and the curves exhibit the "fall-off-a-cliff" shape. (Parameters: $\delta_{MR} = .33$; $E = 3$; α for the three epitopes were $1, 0.2, 0.1 \times 10^{-10}$, yielding REAs $0.75, 0.16, 0.08$; LOF for the three single mutations were $0.9, 0.2, 0.1$; so, by the Escape Formula, all selection coefficients were negative.) The explanation for the late replacement in this scenario is presented in the next chapter.

As we saw in the last chapter, heterogeneity in viral replication can also greatly delay escape in the IC model. But to explain late escapes in this manner is equally *ad hoc* as postulating tiny selection coefficients; besides, it presents the same conundrum as in the last paragraph of the previous section (N_e would have to be implausibly low).

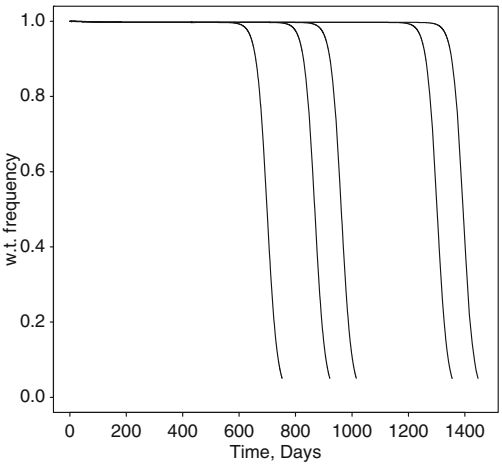

Figure 6.5: Late sweeps in the "Gag-immunodominance" scenario.

6.5 CTLs and Selective Sweeps: The "Overkill" Scenario

In classical, population-genetics type models, replacement of the existing species by a fitter variant is a simple consequence of competition. But in immune-control theory, the mechanism is different. Suppose that multiple CTL clones exist that recognize the wild-type virus, and a mutant has escaped recognition by one of these clones. As the mutant strain grows, its unmutated, wild-type epitopes contribute antigen that stimulates T-cells. The CTLs that still respond now divide more frequently and increase in numbers. The increased killing destabilizes the wild-type and its population crashes. In other words, after escape mutants appear, the CTLs literally kill off the previous strain. This scenario—a complete selective sweep with out competition for resources—is unique to CTL-control theory (although presumably the phenomenon is known to ecologists in other contexts). The sweep may occur when single-mutant variants arise, or it may be delayed until a dual-mutant appears. For 1-mutants, co-existence (as in "quasi-species" theory; see Chapter 8) may or may not be possible, depending on parameters. We saw hints of these possibilities in Figures 4.4 and 4.5.

Ignoring theories for the moment, we note that the concept of "selective-sweep" cannot be entirely accurate for HIV *in vivo*, because of the phenomenon of "latency." For HIV, latency refers to the establishment of a pool of resting T-cells with integrated HIV genomes (as opposed to the "clinical latency period" which exists for certain other diseases). Latently-infected, resting CD4s archive old virus and when activated can become PITs. (This is thought to be the primary obstacle to eradicating HIV with drugs. Patients who start but then interrupt drug therapy often suffer a recrudescence of wild-type virus.) Thus, even after apparent "sweeps," it is likely that investigators sequencing many genomes will still detect the original virus.

When the final escaper (that has deleted recognition of all epitopes) appears, immune-control has ceased and the overkill mechanism becomes irrelevant. After this event—which, in one theory, constitutes AIDS—target-cell depletion would eventually limit viral growth and competition for the remaining CD4s would diminish or eliminate rival species.

6.6 Notes

Early escapes from CTLs: [36, 51, 147, 153, 163, 248].

Rouzine *et al.* [267] derived the classical formula for escape curves that were fit to the observations in the figures.

Patient B took part in the Multicenter AIDS Cohort Study (MACS) [147]. Other researchers have documented CTL escape mutations appearing up to decade or more after infection [112].

Concerning N_e: further discussion and references appear in Chapter 11.

Chapter 7

Understanding Escape

In this chapter, we discuss consequences of the Escape Formula and interesting discoveries we made by simulating escapes under the immune-control model.

7.1 Evolutionary Scenarios Unique to the Immune Control Hypothesis

Imagine for a moment that we are not virologists or immunologists, but rather field biologists, studying mice in a prairie field in South Dakota. Two kinds of predators eat these mice: voles and eagles. Voles are common and eat a lot of mice; in fact, vole predation is a key factor limiting the mouse population. Eagles are rare and consume but the occasional mouse. Let us further postulate that two mutations may appear in mouse lineages: one that makes mice taste bad for voles, and one that changes a mouse's hair texture, making it harder for eagles to see them. Both mutations may have a downside for the individual affected, perhaps reducing a mouse's attractiveness to mates. Now in this situation it is easy to deduce that, if sexiness-changes are identical, the first mutation to sweep through the population will be the one that renders mice unpalatable to voles. Indeed, at the outset the second mutation may even have a negative selective balance (i.e., the advantage of escaping the rare raptor may fail to overcome the reduction in reproduction). After the vole-evading mutant population appears, one would expect to find more mice, fewer voles, and more eagles around that field—the latter

W.D. Wick and O.O. Yang, *War in the Body: The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines*,
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because eagles that catch more mice can raise more eaglets.[1] Eventually a new steady-state will form, in which eagles have become the "top predator" on mice and control their numbers. It may now be the case that the mutation that lets mice evade notice by eagles becomes favorable, and a second sweep might occur.

Returning to virology/immunology, recall that the differences: REA–LOF, in the Escape Formula express "effective selective advantages" (ESAs) of viral mutations in the presence of an immune response. A first and important observation is that an ESA cannot be evaluated without knowing the entire immunological "environment" existing at that time. Moreover, as the populations of variants (making up the viral "quasi-species") change, so will the stimulus to CTLs and these immunological responses. If the reader insists on a classical "s" attached to an escape mutation, then it must be accepted that it might depend on the timing and order of other escapes as well as the total expression of antigen by all other variants existing at the time. Needless to say, the existence of selective effects that vary with everything makes for novel and interesting scenarios, as the fanciful story above suggests.

If the mutation rates at different loci are the same (in fact they often differ), the virus will probably escape first *via* the mutation with the largest positive ESA (if one exists). Even with a relatively small ESA, this first escape will occur rapidly. For example, suppose one escape mutation has an ESA of 0.01, and that it is the largest of all relevant mutations. In the "fairly high N_e" scenario, $N_e \approx 10^5$ PITs; assuming $\mu \approx 3 \times 10^{-5}$, from the Escape Formula the escape rate is: $10^5 \times 3 \times 10^{-5} \times 0.7 \times 0.01 = .021$ per generation. (The 0.7 is due to the nuisance factor mentioned in Chapter 5; see Part II, Chapter 20.) Assuming a generation time of 2 days, the expected delay before the successful escaper appears is about 100 days. In the "no EPV" scenario (meaning $N_e \approx N_d \approx 10^8$) or if the ESA is much higher, the escape lineage appears almost instantaneously. There will be a lag before it can supplant the wild-type's (if possible). After the first escape, all the REAs increase and there can be a cascade of further escapes (discussed in the next section).

Concerning that lag: the Escape Formula is derived from evaluating $R_{\text{eff.}}$ for the mutant, so the initial growth rate can be derived from it (see Part II, Chapter 20). However, predicting the exact time to replacement is

[1] Reflect again on the peculiar neglect of this possibility—a mutational sweep generating multi-species fluctuations—in traditional population genetics, which eschews ecology.

complicated, as it involves the changing pressure from the immune system, which eventually slows growth of the variant to zero provided it still expresses a wild-type epitope. The lag time would also be affected by competition for target cells, and hence may differ between early and late disease. (The validity of the Escape Formula itself is not affected by hypotheses about competition, although the magnitudes of the terms in the formula may be; see Section 8.9.) In our simulations, which omit competition for reasons explained in the Introduction, the lag before replacement, if it occurs, is usually a few months; if an ESA is fairly large (as for escaping a dominant response), it can be almost as short as the time from infection to primary peak (around 20 days). Co-existence with a mutant strain is also possible; see Section 8.3.

If no ESAs are positive, escape is prevented, or at least, as we shall learn later in this chapter, greatly delayed. As a third consequence of the formula, a response to many epitopes is likely superior at preventing escape to a response to one or a few epitopes, no matter how potent the CTLs are at killing PITs. For example, if all LOF = 0.2 (a 20 % loss of fitness) and all CTL clones have equivalent size and potency, at least five are required to prevent escape. Unfortunately, despite hundreds of identified CD8 epitopes in the HIV genome, due to the phenomenon called "immunodominance" (discussed in Section 8.1) in most patients the virus is controlled by a few dominant responses. (Up to 42 responses have been observed in a single patient. It is not known at this time whether the number of epitopes recognized is inversely correlated with rate of progression to AIDS, as one would naturally conjecture.)

The Escape Formula makes another interesting prediction if one epitope is both relatively conserved and immunodominant. Such epitopes often exist in the Gag gene. For purposes of discussion, we now put a subscript "m" on the quantities when referring to several mutations. In light of the Escape Formula, we take "relatively conserved" to mean that LOF_m is large for the mutation, and "immunodominant" that the corresponding epitope has the largest REA_m. ("Conserved" is more commonly used for nucleotides or amino-acids that are not observed to vary in databases of HIV sequences. Of course, these sequences are taken in population studies and so are influenced by many factors, including those in the formula. "Immunodominant" has a technical meaning in immunology that refers to CD8 responses detected with certain reagents; our definition is idiosyncratic as it includes a functionality factor.) In this situation, escape from the dominant response

will be prevented (or greatly delayed) if $\mathrm{LOF}_m > \mathrm{REA}_m$ for that mutation. Importantly, escape from the subdominant epitopes will also be prevented (or delayed), even if the relevant mutations have smaller LOF_m's, because the corresponding REA_m's are diminished. (A killing term coming from the immunodominant response appears in the denominator.) For example, with $E = 3$ the following situation would suffice (let the epitope and corresponding mutation in Gag be labeled number one):

$$\begin{aligned} \mathrm{LOF}_1 &= 0.8, & \mathrm{REA}_1 &= 0.7; \\ \mathrm{LOF}_2 &= 0.3, & \mathrm{REA}_2 &= 0.2; \\ \mathrm{LOF}_3 &= 0.2, & \mathrm{REA}_3 &= 0.1. \end{aligned} \tag{7.1}$$

(We amplify on immunodominance and the exact conditions preventing stepwise escape in Section 8.1.) The possible relevance to the correlation between Gag-dominance and viral load mentioned in the Introduction arises if patients in these cohorts who made a predominant response to Gag were protected from escapes. That the correlation should only exist between *relative* Gag dominance (as opposed to overall CTL response) and VL is a prediction of the formula.

The Escape Formula suggests yet another scenario not familiar from classical genetics. Suppose virus-specific, equipotent CTLs recognize 10 epitopes and some ESAs are positive. After each escape, the dominant strain and the number of epitopes still recognized has changed, so the REA terms have to be re-computed; with these assumptions they will follow the pattern: 1/10, 1/9, 1/8,.... (The LOF may also change, as they represent the intrinsic fitness loss caused by making a new mutation in the currently-dominant strain. This is the phenomenon called "epistasis"; see Section 8.2.) If a mutation in one epitope has $\mathrm{LOF} = 0.2$, five escapes in other epitopes must precede its loss. A researcher who sequenced only this one epitope might be surprised by its stability. Using our CTL–HIV model with $E = 4$ ($E = 10$ requires keeping track of 1,024 strains), and random choices of the LOFs, scenarios appeared in which final escape was delayed for up to 4 years. See Figure 7.1. (The figure shows a histogram of escape times; there were 100 simulations per panel. Panel (a): times of first replacement, wild-type by any mutant strain; panel (b): times of final replacement, by a strain with no wild-type epitopes. We chose multiplicative LOF, aka "zero epistasis", with f_m's chosen independently and uniformly on [0,0.6]; no EPV; all other parameters as in the tables; and the program halted at 4 years.)

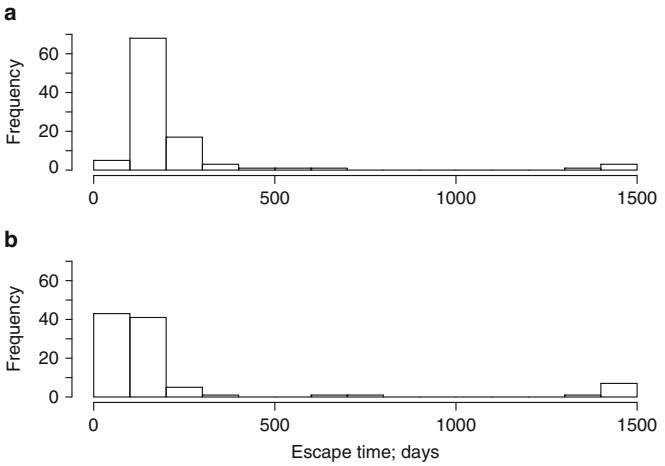

Figure 7.1: Escapes with $E = 4$ and random choices of LOF.

I hope by now that the analogue of the mice/vole/eagle scenario has occurred to the reader. It is, of course: HIV as the mice, CTLs recognizing the immunodominant epitope as the voles, and CTLs recognizing the subdominant(s) as the eagles. After escaping the immunodominant CTLs, the viral load should rise and the virus will now be controlled by the formerly-subdominant CTLs. Escapes from the latter are probable; but before the first escape, they may be impossible. Thus it is likely that escape sequences are predictable, as we will see in the next section.

7.2 Climbing in the Fitness Alps

In the popular imagination, evolution climbs a mountain, ceasing only when it attains the pinnacle (humans, in our conceit). This perhaps-dubious metaphor was introduced by Sewall Wright in the 1930s; at about the same time, R. A. Fisher proved his "Fundamental Theorem of Natural Selection" stating that average fitness in a population always increases.[2] Is this

[2]Wright thought there might be several peaks, on which subpopulations can become trapped. P. A. Moran later dismissed the whole description, entitling his paper with a two-locus counter-example, "On the nonexistence of adaptive topographies".

"fitness landscape" concept at all useful to understand escape from immune control?

A curious consequence of the topographical picture is that evolution might be predictable—because the path to the top might be unique, the only one that evolution can traverse. In this section we ask whether the pattern of replacements (i.e. the order, without reference to duration) and the final situation can be predicted from the Escape Formula. The formula states the rate of escape for a given immune "environment", viral population, and candidate escape mutations. As escapes proceed, that environment changes, as does the makeup of the viral population. We will assume that the latter has a dominant species existing before, and sufficiently-long after, each escape. This scenario is compatible with classical genetics (by its "fitness sweeps"), but not with a rival, the so-called "quasi-species" theory, see Section 8.3. For our HIV–CTL model, as we remarked in Section 6.5, after some escapes CTLs can quickly kill off the existing strain, so it is at least plausible.

We will need information about the parameters appearing in the Escape Formula. The killing potential of CTL clones varies greatly (TCR avidity for antigen appears less important for κ_e than epitope specificity), as does response *in vivo* (avidity, pre-cursor frequency, immunodominance and presumably other factors determine C_e). Intrinsic LOF due to mutation is also highly variable (some escape mutants revert quickly if transmitted to a host with different HLA type than the transmitting subject, indicating substantial LOF *in vivo*; others are stable, indicating little LOF). Lacking quantitative estimates of these parameters, let us adopt uniform or biased "priors", as in Bayesian philosophy. That REA and LOF be chosen independently seems reasonable, as they reflect different aspects of host and virus biology. Restrict attention to the case where one mutation deletes recognition of one epitope, identifying mutations with the epitopes they alter. Thus we will switch to a subscript "e" in place of "m". Let ζ_e stand for immune pressure on epitope e (i.e., $\kappa_e C_e$); these are chosen at random and fixed. Because the iteration will proceed through possible sequences of mutations, and the meaning of "LOF" may depend on how many sites are already mutated, it is necessary to fix a choice of LOF for all situations at the outset. For example, if we choose the multiplicative case ("zero epistasis") of Chapter 2, we randomly preselect the f_m's and fix them.

To mimic a stepwise-replacement process, we delete the epitope with the largest ESA at each step and recompute the Escape Formula, as fol-

lows. Let $\mathcal{E} = \{1, 2, \ldots, E\}$ denote the set of wild-type epitopes recognized initially by CTLs. The algorithm begins by defining a variable epitope set, $\mathcal{E}'$, and initializing $\mathcal{E}' = \mathcal{E}$. Compute the initial $\text{REA}_e = \zeta_e / \sum_{e' \in \mathcal{E}'} \zeta_{e'}$. Then:

The Iterative Escape Algorithm

1. Compute the ESAs: $\text{REA}_e - \text{LOF}_e$, for $e \in \mathcal{E}'$. If any are positive, find the largest, record its index, and delete it from $\mathcal{E}'$. Otherwise, halt.
2. If $\mathcal{E}' = \phi$, halt. Otherwise ...
3. Re-compute the REA_e's and LOF_e's for the epitopes remaining in $\mathcal{E}'$, and return to step 1.

The prediction is the ordered list of deleted epitopes and the final set remaining (possibly empty). The iteration is repeated many times (drawing new parameters before each run from the Bayesian priors), and the probabilities of outcomes analyzed.

Figure 7.2a shows the probability, with flat priors, that at least 3, 5, or 8 remain when the algorithm halts, as a function of the number of epitopes initially recognized. We can imagine that these responses were generated by a vaccine; then the figure suggests that to expect 3 after initial escapes, we should deliver 8. Figure 7.2b shows the situation if the distribution of LOF's was biased to have mean 0.2; now the suggestion is to include at least 12. (We defer the discussion of whether a vaccine could plausibly generate such responses to Chapter 12.)

Needless to say, there are many reasons to doubt these conclusions, besides not knowing the parameters. We chose multiplicative LOF ("no epistasis") to make the preceding figures, but that is not restrictive as the method is readily adapted to other intrinsic-fitness landscapes. We assumed that, as one strain replaces another, the *relative* sizes of the remaining CTL clones (i.e., the fractions of the overall response) remain fixed. As the viral load fluctuates, so will the *absolute* sizes of the CTL clones still active; but the *relative* sizes may reflect intrinsic aspects of CTL biology. Finally, escapes may not follow the predicted sequence, because either steady-state conditions do not predominate between escapes or chance rules over determinism.

These issues demonstrate again the value of constructing a detailed, stochastic model. We simulated the process using the combined HIV–CTL model, with $E = 4$, random choices of avidities (the parameter called α

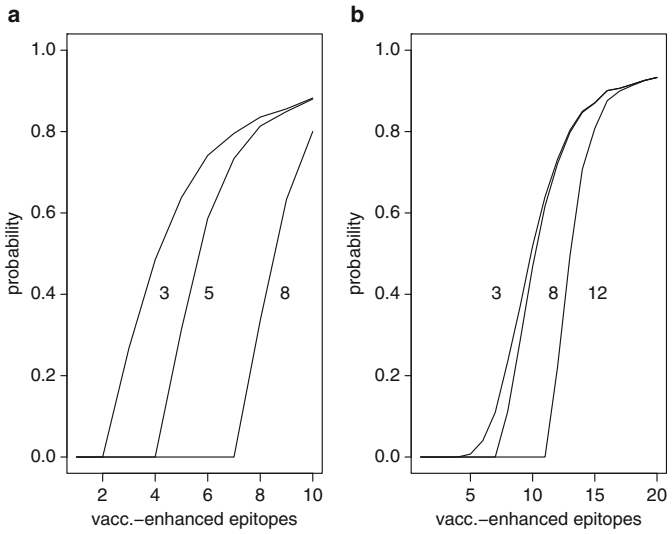

Figure 7.2: Epitopes remaining when the escape algorithm halts. (**a**) flat priors; (**b**) biased to mean 0.2.

in Chapter 3; chosen independently for different epitopes and varying by a factor of 5), and random LOF's ("zero epistasis", with f_m's chosen independently and biased to have mean 0.38). The routine recorded the C_e's at time 50 days as a surrogate for response. We observed two scenarios. In about half the runs, a sequence of single-epitope-loss replacements occurred; in 100 % of these cases the algorithm correctly predicted the exact sequence of escapes. But, in other runs, the sequential-replacement picture broke down: multiple-loss mutants appeared at low frequency and one eventually surpassed the wild-type. Nevertheless, in 19/20 of these runs the iteration correctly predicted the initial epitope lost and the final population structure. When the iterative algorithm failed, according to the simulations, it tended to be conservative: it predicted fewer epitopes would persist (so if the simulations were run longer the two methods could come into agreement). But it also generated one anomalous prediction (too many epitopes persisting). With $E = 6$, 3/5 patterns agreed while 2/5 were conservative at 2 years. An additive fitness structure gave similar results. Finally, we did not incorporate EPV; a low N_e would both delay and randomize the order of appearance of escape mutants.

For purposes of discussion in the next section, it will be interesting to examine also the ESAs when the routine halts with exactly three epitopes remaining. Let PE3 be the percent of iterations ending with three epitopes, and AESA be the average maximum ESA (smallest in absolute value) of a remaining epitope. Adopting uniform priors for the ζ's and the LOFs, here are the results from 10,000 iterations of the algorithm:

E(initial)	PE3	AESA
4	25.4	$-.032$
5	15.5	$-.019$
8	1.45	$-.0013$
12	.02	$-.000016$

Thus, as the initial number of epitopes increases, the magnitude of the final ESAs decrease. We will see next that $E = 3$ and small, negative ESAs may not imply evolutionary stability.

7.3 Punctuated Equilibrium

In our simulations, we noted that the time-to-escape can be anywhere from a few months to many years, depending on viral and immune parameters. The controlling Escape Formula distinguishes the cases; however, for three or fewer epitopes, the situation is more complicated than a cursory examination of this formula would indicate.

Although the formula asserts that the probability that a mutant lineage with negative ESA grows out is zero, that does not mean that such variants can play no role in escape. Although incapable of supplanting the wild-type, since $R_{\text{eff.}}(\text{mut.;m}) < 1$, the 1-mutant populations will achieve a steady-state size of about $N_d\,\mu$, where N_d is the (true) number of PITs. Consider the case: $E = 2$, single-mutants have negative ESAs, and double-mutants have positive ESAs—as computed from the Escape Formula, updated for whatever environment exists with a 1-mutant dominant strain. Then a double-mutant will appear in about $1/(2\,N_d\,\mu^2)$ generations, and the lineage grow out after a possibly longer time.[3] If however $E > 2$ and the double-mutants also have

[3]That longer time is: $1/(2\,N_e\,\mu^2\,\text{ESA}_2)$, where ESA_2 is the ESA of the double-mutant, plus the lag to grow to the size of the wild-type. Thus EPV can delay this double-escape, as per its usual role in this story.

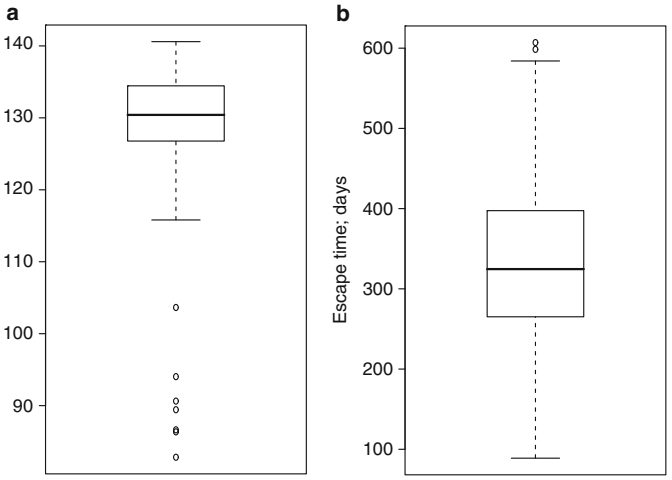

Figure 7.3: Surprising escapes, even with negative ESAs.

negative ESAs, their numbers will not exceed order $N_d\,\mu^2 < 1$ and so a further replacement event would appear to be essentially impossible. Call it the "Rule of Three". Nevertheless, even with ESAs unfavorable for escapes, we sometimes observed replacement episodes in our simulations. For the critical case, $E = 3$, with CTL clones of identical immunogenicities and killing efficiencies as epitopes are lost the REAs pass through the sequence: 0.33, 0.5, 1.0. Assuming a simple dependence on number of mutations, let LOF$(k : n)$ denote the loss-of-fitness of a strain with k mutations relative to one with n. The replacement-stability boundary with equivalent epitopes corresponds to LOF$(1 : 0) = 0.33$, LOF$(2 : 1) = 0.5$ and LOF$(2 : 0) = .667$ (yielding vanishing ESAs). (See Part II, Chapter 23.) But when we arranged LOF$(1 : 0) = 0.34$ and LOF$(2 : 0) = 0.67$, which imply ESAs of 1- and 2-mutants slightly negative, escape nevertheless occurred in some runs, albeit delayed a year or more relative to the situation on the other side of the boundary. See Figure 7.3. (The figure shows distributions of replacement times with $E = 3$: (a), LOF$(1 : 0) = 0.2$ and LOF$(2 : 0) = 0.4$, (b), LOF$(1 : 0) = 0.34$ and LOF$(2 : 0) = 0.67$. Other parameters were as in tables.) Figure 7.4 shows a similar box-plot of escape times in a "Gag immunodominance" scenario, arranged to have slightly negative ESAs in 1-mutants.

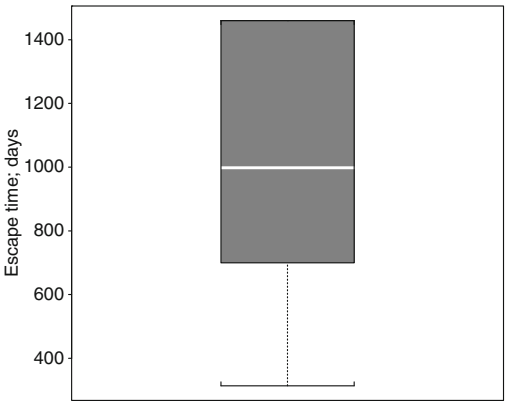

Figure 7.4: Escape times in the "Gag immunodominance" scenario.

(100 simulations; the program halted at 4 years; 27 had yet to escape. Same parameters as for Figure 6.5.)

The explanation for the fuzziness of the escape threshold is perhaps surprising. See Figure 7.5. In the upper panel, the 1- and 2-mutant lineages grow slowly over time, setting the stage for the entrance of the 3-mutant. (Of course, it not possible to ascertain from this figure whether the 1- and 2-mutant populations are truly growing slowly, or merely equilibrating slowly.) Further inside the favorable domain, the mutants are better controlled (lower panel). The slightly-negative ESAs in this example might have been created from a sequential-escape series beginning with $E > 3$, but that ended with three epitopes, as discussed in the previous section.

Let us define a "sequential-replacement" escape sequence to mean that a single-mutant variant is born with $R_{\text{eff.}}(\text{mut.;m}) > 1$, the variant population grows and replaces the wild-type, and the process continues, so that sampling over time and sequencing would generate a history of successive replacements. The special escape route when $E = 3$ can generate a variant with multiple mutations that appears spontaneously, apparently without mutant ancestors. (They exist at too low frequency to be picked up by sampling, as in Figure 7.5. In Figures 6.5 and 7.3, the escape-strain invariably mutated all three epitopes before it grew past the wild-type.)

Note that this is not a case of deleterious mutants persisting because they are nearly competitive with the dominant strain, as in quasi-species

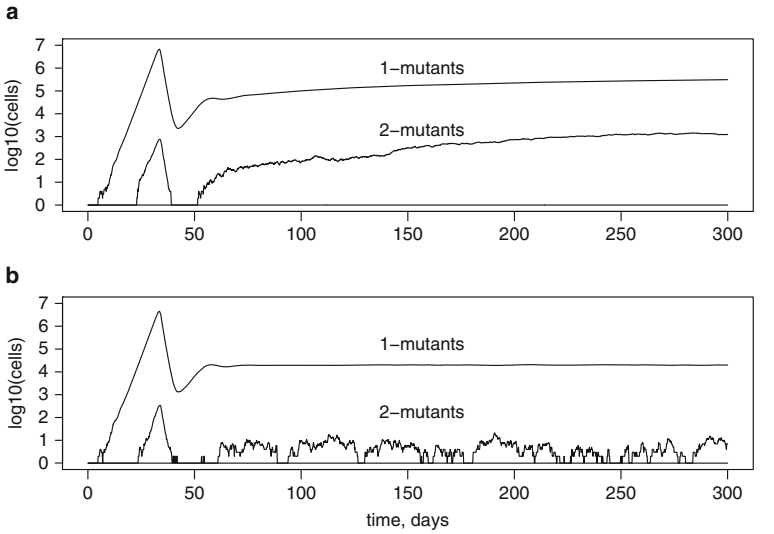

Figure 7.5: The explanation for "punctuated" escapes.

theory, nor of "balanced selection", as in traditional genetics. (Each 1- and 2-mutant lineage is going extinct, but the populations are being continuously regenerated by mutation.) Nor are nearly-neutral variants replacing the wild-type by chance, as would be predicted in neutral theory for a population with a small N_e. (We did not incorporate EPV when making the figures, so N_e was not small.) We noted in the previous section that, when the virus escapes by sequential replacements, the sequence can often be predicted from knowledge of immune and viral parameters (although some variation remains in the timing). By contrast, this other escape process is intrinsically random and more akin to the scenario described in 1972 by Stephen Jay Gould and Niles Eldredge that they called "punctuated equilibrium" (abbreviated PE from here on). Gould and Eldredge proposed PE to explain gaps in the fossil record and invoked the spatial separation of sampled sites from the scene at which the intermediate steps in evolution took place. Curiously, the "punctuated" escapes in our viral simulations occurred despite the lack of any "spatial" segregation in the model; even with much sampling and sequencing, the missing links would still likely be missed, simply because of their low frequency.

Even with more than three epitopes recognized by CTLs, we can expect an accumulation of variants with one or two mutations whose ESAs are not too negative. This observation suggests a "burst and pause" pattern for escapes from CTLs. In the "burst", positive ESAs drive early escapes. The "pause" represents a period in which all escape mutations have negative ESAs. Finally, late escapes result from PE and mutations whose ESAs have turned positive, due to alterations of their respective REAs. (Again, this scenario could not appear in classical population genetics, because of the assumption of an unreactive "environment". Of course, any pattern of escapes could always be arranged *ad hoc* in such models, by suitable choice of positive selection coefficients.)

7.4 The Rivals to Punctuated Equilibrium

One popular hypothesis invokes compensation: a neutral mutation appearing first, somewhere else in the genome, might compensate for loss-of-fitness in an escape mutation. Since the time to fixation of a neutral mutation is $2\,N_e$ generations (in conventional genetic models), with the higher estimates of N_e this time could be years, even decades (although it need not progress to fixation to facilitate escape). In the case of patient 'B' of Chapter 6, the investigators did not observe other Gag substitutions that fixed before or simultaneously with the escape mutation; but a compensatory mutation outside the sequenced region cannot be ruled out. Examples of compensation of HIV mutations are known, but if not proven the explanation becomes unrefutable, like the old canard that the youthful Wolfgang Mozart could not have written his early music without help from father Leopold. PE, as it appears in our setting, can also be thought of as proceeding through compensations—but the other mutations may be in unrelated proteins and exert influence, not through subtle biochemical interactions, but because fitness is a function of the entire immune response.

Another suggestion invokes another kind of compensation: simultaneous changes, in one replication cycle, with no or canceling fitness-losses. The probability of two specified nucleotides changing in one replication cycle is 9×10^{-10}; with 10^8 PITs ("high N_e") and an ESA $= .1$, the Escape Formula yields an escape rate of $\approx 10^8 \times 9 \times 10^{-10} \times \times 0.7 \times .1 \times 0.5 \approx 3.1 \times 10^{-3}\,\mathrm{day}^{-1}$. The waiting time is about 330 days; possibly relevant, although not if ESA $= .01$ or N_e is actually much smaller than N_d. (Recall from Section 1.4 that two

nucleotide changes required to change an a.a. in RT was invoked as the explanation of the lag of 6 months to a year before escape from the drug AZT.)

One rival to PE we do not entertain is the proposal that each escape mutation has its own selection coefficient, all are positive, but some are very small (e.g. in the third decimal place, or smaller.) Our reasoning was presented in Section 6.3; in particular, we objected to an assumed near-equality of two terms in the Escape Formula as representing a "knife-edge" coincidence. Our "punctuated" late escapes also appear near a threshold, but the knife need not be as sharp: the difference between the terms can be in the second decimal. Moreover, we presented a mechanism by which small, negative ESAs might have been created: by a sequential-escape series beginning with $E > 3$ epitopes but that ended with three (see Section 7.2).

Finally, as we saw in Chapters 5 and 7, a low effective population size can greatly delay escape. With the exception of Figure 4.5 and those in Chapter 11, the figures in this book were generated from models that did not incorporate EPV (implying N_e was essentially the same as N_d), compensation, or simultaneous mutations, in order to study the role of other genetic mechanisms.

7.5 Notes

Some of the consequences of the full fitness-landscape were discussed previously in [314], although at the time of writing the formula was not yet in the final form.

The killing potential of CTL clones varies greatly (avidity for antigen appears less important for κ_e than specificity, [332, 333]), as does response (avidity and a host of other factors determine C_e). Intrinsic loss-of-fitness due to mutation is also highly variable (some escape mutants revert quickly if transmitted to a host with different HLA type than the transmitting subject, indicating loss-of-fitness *in vivo*; others are stable, [99, 178]).

Concerning evolutionary determinism in retroviruses *in vivo*: Draenert *et al.* [77] presented a fascinating study of HIV infection in two monozygotic (identical) twins and another brother, all of whom were infected through drug injection. The twins were infected first, from the same source, and the brother later, again from shared equipment. The first two had the same clinical course; moreover, "viral evolution... demonstrated a striking degree of

similarity", with identical escape mutations in three of four epitopes to which CTL responses declined. The brother was infected by a CTL-escape virus, which did not revert. In the same immune environment existing in the twins, including shared HLA haplotypes, the CTL responses were virtually identical; the authors concluded that "CTL responses are determined by HLA and viral sequence despite the fact that the rearrangement of the TCR is stochastically determined..." Even Env mutation and humoral responses showed parallel evolution. This amazing determinism lead the authors to propose that, in vaccine design, we might desire to immunize "not only against prevalent strains, but also against what HIV will evolve to become under immune selection pressure."

Punctuated Equilibrium: [111].

We know of only one candidate for escape with sudden multiple epitope loss, patient 'B' from Jamieson *et al.*'s survey of the MACS cohort described in Section 6.2. In patient B, an escape in RT (a.a. 311, K $\longrightarrow$ R) appeared nearly simultaneously with the escape in Gag, shown in Figure 6.3. The RT mutation appeared in 80 % of the sequences sampled 2.4 months before the Gag mutant fixed, at 3.54 years; i.e., 5 % of the time-span since infection. It is suggestive of the stochastic route, but, as the investigators did not sequence whole genomes, the mutations might not have appeared in the same viruses.

Re compensation: see [100, 163, 238]. For the time to fixation of a neutral mutation see [168].

As of 2007, single-locus analysis, assuming determinism and independence of escape rates at different loci, prevails in the literature. (Some authors at least put the mutations in at random, but this does not exculpate them from the sin of ignoring stochasticity in the infection process.) The reliance on deterministic ODEs to describe escape from one response may not be problematic, provided the selection-coefficient is large and the infection dynamics is homogeneous. Because of HIV's extraordinary mutation-rate, a hundred to a thousand single-mutant PITs might be created each day in an infected patient, so the waiting-time to a successful escaper will be short. But with a small selection coefficient or significant EPV, the extinction probability might be .9998, and now a deterministic treatment of the escape rate is off by orders-of-magnitude.[4]

[4]That "super critical" branching processes, that have $R_{\text{eff.}} > 1$, nevertheless can go extinct has been known for 80 years. That heterogeneity increases the extinction rate is perhaps less well known, but was pointed out in [314].

For multiple loci, the pitfalls in this kind of analysis derive, as in population genetics, from the simplicity of the set-up and circular reasoning. A one-parameter model cannot fit late escapes, as we saw in Chapter 6, see Figure 6.3. As we have seen in this chapter, escapes occur sequentially and cannot be evaluated as though everything else remains fixed. PE is of course impossible in a deterministic model of evolution. (A phrase like "although $R_{\text{eff.}} < 1$, a PIT may occasionally make a few offspring..." is meaningless in an ODE description.) Moreover, many authors seem oblivious to the possibility of negative selection coefficients (what we have called negative ESAs). In this restricted view, if an escape occurs early, the associated "s" is large; if late, it is small but still positive. From the frequency of escapes requiring years to reach fixation, some authors have concluded that CTLs barely impact on HIV disease. But consider the hypothetical case that, in some lucky patient, no escapes occurred in, say, 20 years after infection. Applying the single-locus method, the selection coefficients would come out virtually zero, and we would have to deduce that this patient's T-cells applied little or no pressure—overlooking the possibility that the patient enjoyed a robust immune response, firmly corralling the virus. By ruling out negative selection coefficients at the outset, the authors already ruled out immune control, rendering the approach circular.

Finally, even if one insists on small positive selection coefficients for late escapes, a tiny "s," as we pointed out in Section 6.3, would be a consequence of a near-coincidence of two terms in the Escape Formula and does not imply that the corresponding REA_m is small. Even if some ESAs should prove small and positive, that fact does not imply that CTLs are, in toto, impotent to control HIV. Indeed, in our model the contribution of CTLs to PIT mortality is about 70 %, with the rest due to natural lifetime (represented by the parameter δ_{PIT} in Chapter 2); nevertheless, small ESAs appear in many examples in this book. We should also remark once again that explanations based on "knife-edge" parameter coincidences are suspect in biology.

Chapter 8

More Topics About Escape from Immune Control

8.1 Immunodominance and Escape

A problem that has vexed vaccine developers in the HIV field is that some subjects make only a few CTL responses to HIV proteins, despite hundreds of know epitopes delivered by the injection. (In natural infection, the average number of responses is around 19; as many as 42 have been observed in a single patient.) Many theories have been elaborated for this surprising and, from the insights about escape arising from the Escape Formula, unfortunate phenomenon called "immunodominance". For example, some antigens may be more immunogenic (in model terms, have a larger CTL activation parameter, labeled α in Chapter 3) than others. Factors affecting activation include the propensity of the antigenic peptide to fit into an HLA molecule, the rate of processing and delivery of the HLA-peptide complex to the cell's external membrane, and the "avidity" or strength of binding of the TCR to the complex. Due to the amplification factor in the immune system (a consequence of the programmed-proliferation, or PP, assumption), a relatively small change in the activation parameter can generate a large difference in CTL clone size. Figure 8.1 shows the difference resulting from a factor of four in α in the "normal memory" scenario. ($R_0 = 4$; $\delta_{MR} = .00017$ day^{-1}; other parameters as in the tables; the darker line represents the minor epitope.) With the "defective memory" assumption about the putative CTL-defect, there is an even more dramatic drop-off of the minor response, to a level below

W.D. Wick and O.O. Yang, *War in the Body: The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines*,
DOI 10.1007/978-1-4614-7294-0_8,

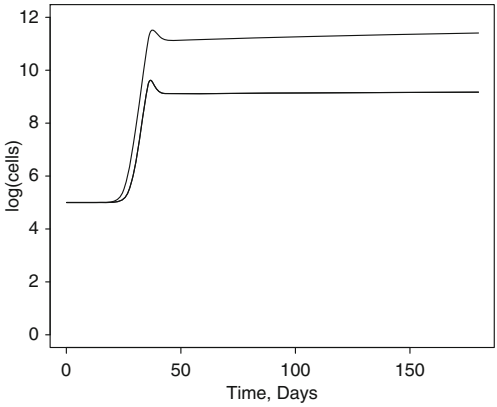

Figure 8.1: Immunodominance by varying immune activation ("normal memory" scenario).

pre-infection equilibrium. See Figure 8.2. ($\delta_{MR} = .33$ day^{-1}.) This is caused by PP and also the short lifespan of "resting" memory cells in this scenario, which do not receive sufficient stimulus to maintain their numbers. Some viral proteins are expressed earlier than others and have more time to stimulate CTLs. Figure 8.3 demonstrates that a 12 h difference between "early" and "late" also generates immunodominance. (Normal memory; other parameters as for the previous figures.) Figure 8.4 illustrates the early-vs.-late effect in the "memory defect" scenario. An example of an "early" protein is Nef, described in Chapter 9. More complicated theories of immunodominance postulate intracellular competition among antigens (to sequester HLA molecules or to be transported to the cell's surface); intercellular competition among APCs to take up antigen or stimulate T-cells; or that early expression of one epitope means the PIT is killed before late epitopes appear.

What are the implications of immunodominance for the boundary between stability and escape, ignoring the PE scenario? Consider the critical case of three responses, one relevant mutation locus per epitope, and that mutation totally abrogates recognition of the epitope. Then there is a simple criterion on the sum-of-losses in fitness that must be satisfied for there to exist a set of favorable immune parameters that would prevent escape, namely:

$$\text{SOL}: \quad \text{LOF}_1 + \text{LOF}_2 + \text{LOF}_3 > 1. \tag{8.1}$$

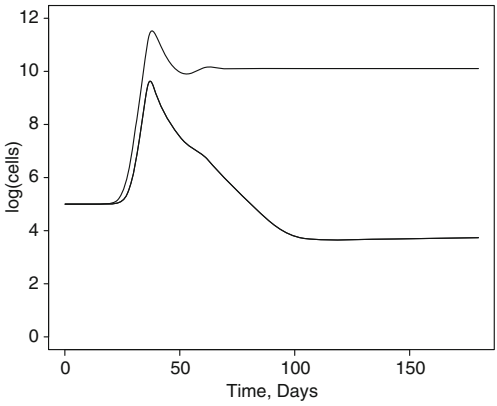

Figure 8.2: Immunodominance by varying immune activation ("defective memory" scenario).

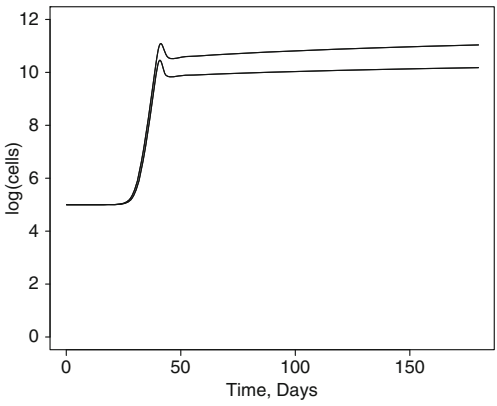

Figure 8.3: Immunodominance by early expression ("normal memory").

This condition is a simple consequence of the Escape Formula (see Part II, Chapter 23). Thus, if there exist three mutations, each with at most a 30 % intrinsic loss-of-fitness in replication, and can delete all the responses, escape is certain. (I.e., at least one response will be lost; whether the virus will proceed to delete all three depends on assumptions about epistasis, discussed below.)

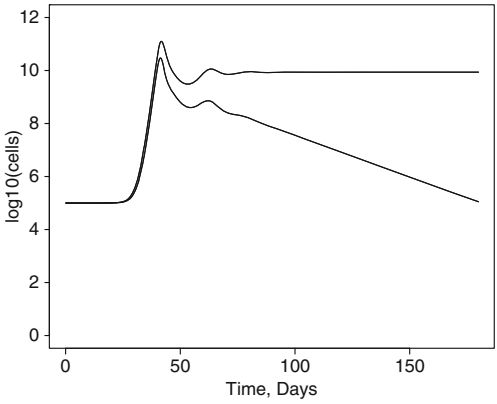

Figure 8.4: Immunodominance by early expression ("defective memory").

Granted this condition is satisfied, we can further examine what the Escape Formula says about the stability boundary. Consider the simplest interesting scenario: first CTL response dominant, equal responses (and functionality) in the subdominant pair, and the relevant mutations in the subdominant epitopes have equal LOFs. Analysis reveals the following cases (see Figure 8.5 and, for the argument, Part II, Chapter 23). Let us call DR the dominance ratio $(\kappa_1 C_1)/(\kappa_2 C_2)$ (which, for equal killing efficiencies, is just the ratio of CTL clone sizes) and $\mathrm{SOL} = \mathrm{LOF}_1 + 2\,\mathrm{LOF}_2$. Immunodominance implies $\mathrm{DR} > 1$; for stability to be possible, $\mathrm{SOL} > 1$ by (8.1); obviously $\mathrm{SOL} < 3$. The analysis reveals that, if escape from the immunodominant epitope is possible at all (i.e., $\mathrm{LOF}_1 < 1$), DR cannot be too high. But otherwise immunodominance may be substantial. There is another restriction in one case: when $\mathrm{LOF}_2 < 1/3$, implying $\mathrm{SOL} < 1\,\frac{2}{3}$, DR cannot be too low, either. In this case, immunodominance is actually beneficial, by preventing escape in the subdominant epitopes. Figure 8.5 shows the stability region (between the curves) as a function of LOF_1, for fixed $\mathrm{SOL} = 1.3$. We have truncated the figure at $\mathrm{LOF}_1 = 0.9$ so that the lower curve is visible; in fact the upper curve tends to ∞ as $\mathrm{LOF}_1 \to 1$ while the lower boundary stays finite.

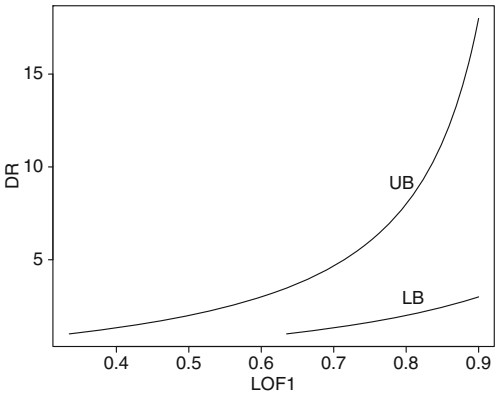

Figure 8.5: A stability (no-escape) domain with three epitopes and immunodominance (SOL = 1.3).

8.2 Epistasis and Escape

Geneticists use the term "epistasis" to refer to the impact on fitness of combinations of mutations in a single genome. The case where fitness-effects multiply is usually referred to as "zero epistasis"; if fitness is enhanced or diminished relative to this rule, it is called positive or negative epistasis, respectively. There are many theories about the role of epistasis in the evolution of sex and other matters outside our purview here. More interesting for our topic is the possibility that a combination of mutations that escape multiple CTL responses might also abolish viability—which would be a consequence of negative epistasis. But such a fortuitous scenario cannot be taken for granted (and has not been reported for CTL escape mutations, to our knowledge).

In traditional genetics, which ignores ecology, fitness is simply a matter of number of offspring and their ability to occupy niches (or exploit food resources). The analogy to the standard concept is what we have called R_0(mut.), the basic reproductive number of the strain with mutation(s). We saw, in Chapter 2, how interactions of mutations can be accommodated in R_0(mut.). But if we reflect also on the impact of the immune system, it is not clear that the traditional "epistasis" concept is well-defined. Nevertheless, it is interesting to investigate the role of epistasis in escape.

In order to include the impact of the immune system, we must consider effective reproductive numbers. The quantity that best expresses "full fitness" gain, call it "FF" for this section, relative to the wild-type is $R_{\text{eff.}}(\text{mut.}) - 1$, since (by definition of steady-state) $R_{\text{eff.}}(\text{w.t.}) = 1$. The quantity that best expresses "intrinsic fitness" gain is $R_0(\text{mut.}) - 1$, since $R_0 = 1$ one means no growth. Let us denote it "IF" in this section. In addition, let "KR" stand for "killing ratio", meaning the fraction of CTL killing still active for the mutant virus, relative to the non-immune death rate. Then we have the proportionality (see Part II, Chapter 20, Exercise 4; this fact is a part of the derivation of the Escape Formula):

$$\text{FF} \propto \text{IF} \quad - \quad \text{KR}. \tag{8.2}$$

Consider first the intrinsic fitness, IF. If the individual changes combine by the rule (used in many simulations; see also Part II, Chapter 17)

$$R_0(\text{mut.}) - 1 \quad = \quad (\, R_0(\text{w.t.}) - 1\,) \times (\, 1 - f_1\,) \times (\, 1 - f_2\,) \times \ldots, \tag{8.3}$$

where each f_m indicates fitness loss independent of other loci, we have the multiplicative rule. For example, if $R_0(\text{w.t.}) = 4.0$ and $f_m = 0.2$ for each mutation, IF follows the rule: 3×0.8, 3×0.64,...; i.e, it is a geometric series. This generates (according to genetical convention) "zero epistasis". However, KR is not multiplicative. If the killing rates do not vary with epitope, it is just proportional to the number of wild-type epitopes remaining on the virus; i.e., arithmetic, not geometric. Hence, escaping multiple responses appears to involve, in some sense, "positive epistasis". (One survey of HIV mutations did find widespread positive epistasis, but the variants were escaping drugs, not CTLs, and fitness was assessed *in vitro*.)

But this logic properly applies only if we ignore the changing "environment", which is established by all viral species and all CTLs present at each time. As we pointed out in Section 7.1 (recall the parable of mice, voles, and eagles), as this "environment" shifts, a mutation that had a negative ESA and could not produce a growing lineage can acquire a positive ESA and the lineage expand. We are tempted to declare that all such examples represent "ecological epistasis." But since epistasis traditionally refers to fitness change assuming a fixed environment, "ecological interactions" is better. Perhaps "epistasis" should be reserved for the impact of combined mutations on what we have called intrinsic fitness.

It is also of interest to ask whether epistasis in this restricted sense is involved in the special case of $E = 3$ and the boundary of escape, discussed in

the previous section. It turns out that the no-replacement boundary (which the virus can overstep by the punctuated mechanism) in this case corresponds to negative epistasis. (See Part II, Chapter 23.)

8.3 Quasi-species and Escape

In 1977, M. Eigen and P. Schuster proposed an alternative view of evolution in the "RNA world", postulated to precede the appearance of DNA and the first cell. The idea was applied to viruses in the 1990s. The authors suggested that the replacement paradigm of classical genetics (one dominant species existing at most times, with occasional selective sweeps) should in these contexts give way to another picture: a cloud of co-existing variants, evolving as a whole to maximize reproduction.

Presumably, among the motivations for the quasi-species theory were the discovery of very high mutation rates in RNA viruses and an analogy to thermodynamics. The theoretical underpinning of thermodynamics is the "ergodic hypothesis", which asserts that the system sojourns across the space of states that is accessible, given constraints such as the walls of a container and the conservation of energy. Analogously, a population of RNA viruses might be able to explore the entire hyperspace of viable genomes. Eigen even noted a possibility analogous to a phase transition: as a solid melts beyond a critical temperature, so the wild-type might be destabilized at a sufficiently high mutation rate, sinking into a sea of almost equally-fit variants.

Clearly, whether this picture is correct depends not only on the mutation rate but also on the fitness landscape and the importance of competition. In a landscape with a Mount Rainier surrounded by a sea-level plain, the less-fit variants would be too rare to form a quasi-species, while with Hobbesian competition the weaker would be quickly eliminated. Moreover, the quasi-species theory was developed mathematically using rate equations (ODEs), which we have criticized as inapplicable to multi-locus genetic problems. Nevertheless, if fitness changes are small, and competition not too intense, then the quasi-species concept will apply at least to some variants. Indeed, at the genome level, an HIV infection does behave much like a gas passed through a nozzle, then expanding to fill a container.

Could a dispersal of variants with neutral or nearly-neutral mutations contribute to escape from immune control? Yes, by the mechanism called compensation, discussed in Section 7.4. What about a cloud of escape-mutants?

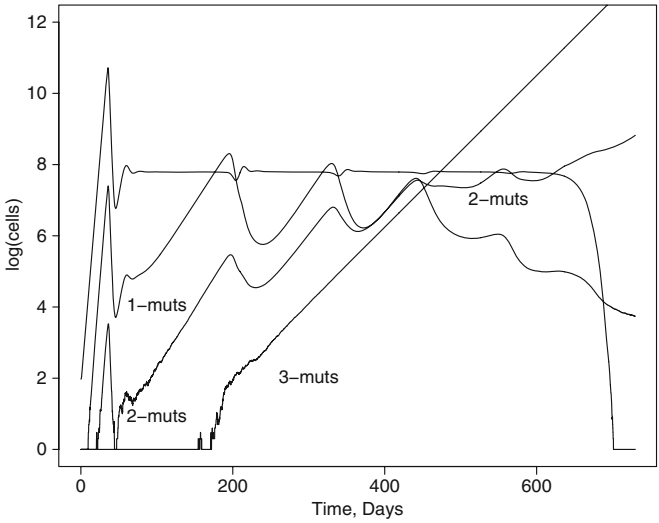

Figure 8.6: Co-existence . . . but not with the dual mutant. We have included the ultimate escaper, with 3 mutations, in this figure, but it does not affect the co-existence issue as (with $E = 3$) it cannot stimulate CTLs.

Classical genetics predicts sweeps, while quasi-species theory emphasizes co-existence. It turns out that our model lies somewhere in between. Co-existence of escape mutants and wild-type in a steady-state is possible for single-mutant variants for a range of parameters, but co-existence with dual mutants is impossible. When the variant that has deleted two epitopes escapes, the wild-type goes extinct. See Figure 8.6. The addition of competition to the model would require replacing "steady-state" by "quasi-steady-state" for small competition, while for intense competition—e.g., for CD4 T-cells in late AIDS—co-existence would be replaced by sweeps. (For the mathematical treatment of the co-existence problem, see Chapter 24 of Part II.)

8.4 Muller's Ratchet and Escape

As part of a discussion in the 1930s of that age-old question, "why sex?", R. A. Fisher and H. J. Muller pointed out that, in a population reproducing sexually, favorable mutations that occur in different lineages can be

brought together, but in an asexual population they can only be combined sequentially (i.e., a second mutation occurring in an offspring of one possessing the first, and so on). In 1964, Muller proposed another reason why sex mattered: the "ratchet". Operating in a sufficiently-small population lacking recombination, Muller's ratchet moves it downhill: disadvantageous mutations accumulate, overall fitness deteriorates, and the whole group might go extinct. (Muller assumed disadvantageous mutations are more common than beneficial mutations and, due to "drift", strains bearing the former would fix by chance.) If Fisher, in his Fundamental Theorem of Natural Selection, claimed everybody is getting better, Muller's riposte was that some are degenerating.

It is implausible that HIV in a single patient loses fitness over time (see Section 8.9 below), although the ratchet might operate in tissue-confined, isolated populations of viruses.[1] Or this effect could apply to the small numbers of variants with one or two mutations that precede the fixation of the 3-mutant in the PE scenario, increasing the likelihood that some relevant population goes extinct beforehand. However, Fisher's and Muller's arguments depended on the usual population-genetics assumption of intense intraspecific competition (if one variant population increases by x, then another must decrease by x), for which we have expressed doubt where HIV *in vivo* is concerned. Moreover, these populations are continuously refreshed by new mutations, rather than maintained by drift. Besides, HIV is not really an asexual organism. (See Chapter 10 for discussion of sex among retroviruses.)

8.5 Viral Load and Escape

Using the combined HIV–CTL model, we simulated the infection through several escapes, assuming that different epitopes had different "immunogenicities", abilities to activate CTLs. (The parameter varied is α in Chapter 3, for each epitope.) Figure 8.7 shows a typical trajectory of viral load as evolution

[1] It is commonly held that parasite and host adapt to each other, finally attaining a mutually-beneficial equilibrium; if so, this process implies diminishing virulence on the parasite's part. But evolution must occur on both sides, by modulating reproduction and mortality, and so it requires many generations for both. There have not been a sufficient number of human generations since HIV entered the population for this scenario to have any plausibility.

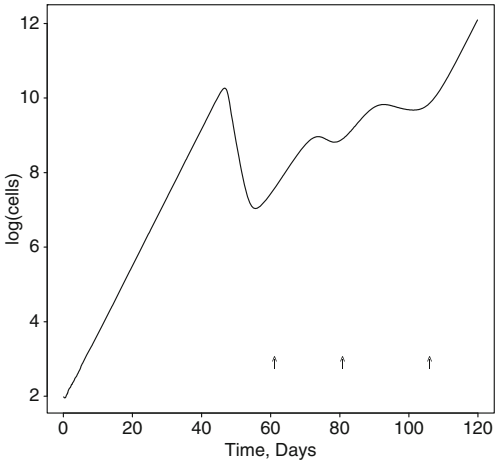

Figure 8.7: Viral load followed through several escapes.

progresses (the arrows denote escapes). This picture is ruled out *a priori* in many versions of traditional population genetics.

8.6 Response Broadening and Escape

The model presented in Chapters 2 and 3 suggests an explanation of the broadening of immune response sometimes observed after the primary stage of infection. If one epitope dominates, other CTL clones may not receive sufficient antigenic stimulation to maintain their populations at detectable sizes, as we saw in Figures 8.1 and 8.2. However, after the virus escapes from the dominant clone (if possible), the VL may increase and the remaining subdominant CTLs may expand and hold the virus at a new steady-state. Figure 8.8 illustrates a case with three epitopes. The curves show the CD8 responses and the arrow the time of replacement of wild-type by mutant. Note how the dominant response drops off and remains subdominant after the escape; then the three responses fluctuate but are often comparable. (The parameters were: $\alpha_1 = 1.0 \times 10^{-9}$; $\alpha_2 = 5.0 \times 10^{-10}$; $\alpha_3 = 1.0 \times 10^{-10}$; same for the κ's; $\mathrm{LOF}_1 = 0.1$; $\mathrm{LOF}_2 = 0.9$; and $\mathrm{LOF}_3 = 0.9$; others as in the tables.) Figure 8.9 shows the wild-type and escaping virus in the same simulation. Note that this figure also demonstrates co-existence of variants (wild-type and 1-mutant), a possibility discussed in Section 8.3.

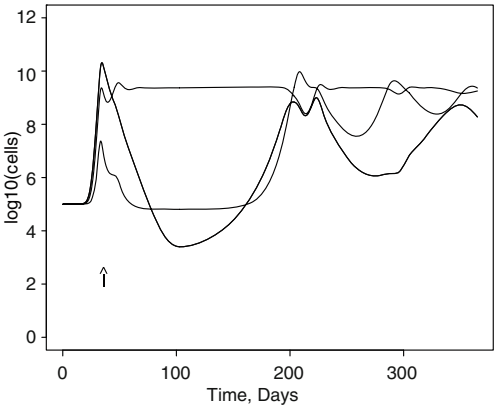

Figure 8.8: Response broadening after escaping the dominant clone.

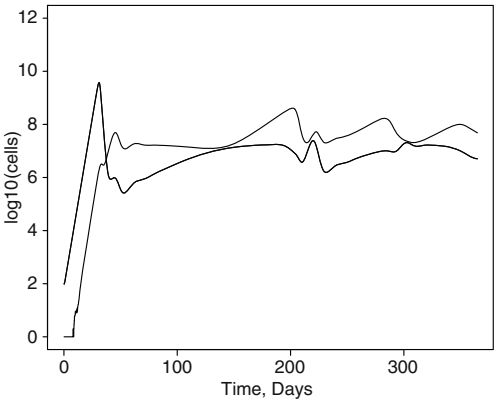

Figure 8.9: Wild-type and mutant virus for the previous figure.

8.7 Host Benefit from Escape

Could an escape be beneficial to the host? Conceivably, a mutation that deleted recognition of one epitope might produce another to which the host makes a stronger response. It has been conjectured that this kind of event might lower viral load or even produce "epitope oscillations".

Let the escape mutation be labeled "m", and assume that the ESA of this mutation is positive, i.e., $\text{REA}_m > \text{LOF}_m$. After the escape variant replaces the wild-type, suppose that a new CTL clone expands and contributes to immune pressure on the virus, resulting in a new steady-state. The clone might have arisen from precursor cells that existed at low frequency, or might have been generated by ongoing CD8 thymopoiesis. (In children, thymopoiesis—generation of new T cells by bone-marrow and thymus—is a torrent, but it slows to a trickle in adults.) Now consider the reverse mutation, that restores the original epitope; let us label it m'. If the new response is indeed superior to the original one, it must be that escaping it is even more valuable to the virus. That is, if no other escapes have occurred, in the new steady-state conditions $\text{REA}_{m'}$ must be greater than the old REA_m. Also, by definition of intrinsic fitness, if LOF_m was positive, $\text{LOF}_{m'}$ is actually negative. Hence the ESA of the reverse mutation is even larger then for the forward one. If the back-mutation rate is identical with the forward rate, the virus will quickly revert to the previous form. Although this situation might produce short-period oscillations, we have seen no convincing evidence that this phenomenon exists, and even if it does it is unimportant for long-term stability.

If, however, the forward mutation had a higher rate than the reverse, a lasting benefit might persist. (Although neither epitope would be stable, if the discrepancy in rates was large enough the wild-type could feed forward faster than the mutant epitope reverted.) There are several genetic mechanisms by which an amino-acid replacement and its restoration might have different rates, independent of selection. Due to the redundancy in the genetic code, some amino-acids have more codon triplets than others. The size of the pools of amino-acids available to synthesize proteins in the host can also differ. But at this point we have concatenated too many speculative hypotheses and have to conclude that a host benefit from escape appears improbable.

8.8 Antibodies and Escape

As we noted in the introduction, antibodies that bind HIV appear late, sometimes not for a half-year, after infection. Moreover, at the point when they do appear (called "seroconversion"), they fail to neutralize the virus or apparently provide any other benefit to the patient. However, the story of

antibodies and HIV *in vivo* represents another evolutionary arms race, in addition to the one between T-cells and the virus we have emphasized in this book.

In a small fraction (about 10 %) of patients, broadly-neutralizing antibodies (that neutralize a spectrum of circulating stains) do finally develop—but only after many years, and again fail to provide a clear benefit to the patient (e.g., by lowering viral load). Immunologists call the process by which the immune system improves antibody over time "affinity maturation". It is due to an amazing genetic mechanism called "somatic hypermutation", affecting the immunoglobulin gene in B-cells. Aided by CD4+ "helper" T lymphocytes, B-cells can recharge a recombination mechanism that introduces point mutations into the gene, at a rate (per-"generation", or division-cycle, as B-cells proliferate) comparable to that of HIV itself! One doubts that this is accidental. (Recall that retroviruses are ancient, their detritus littering the human genome; thus, there has been plenty of time for primates and retroviruses to adapt to each other.)

Why then do not these ten-percent of patients that literally evolve better antibodies in their lifetimes gain a benefit, or even clear the virus? The likely answer is that by the time these novel neutralizing antibodies appear, HIV in that patient has already escaped their clutch. (Recall that the envelope protein, "Env", the one recognized by antibodies, is also the most variable HIV protein. Needless to say, appearance of a potently-neutralizing antibody, that instantly eliminates infectivity of virions, would produce an extreme selection-pressure on HIV to escape it.) Vaccine designers with a predilection for the traditional naturally hope to elicit such broadly-neutralizing antibodies by vaccination. But, even if this proves possible (no one has succeeded at designing such a vaccine at this writing), the ease with which HIV swaps coats suggests that any traditional antibody-directed vaccine that is less than 100 % effective at eliminating HIV from the target population would quickly become irrelevant.

8.9 AIDS and Escape

Whether AIDS is the result of a sequence of escapes leading eventually to collapse of immune control remains controversial. Escapes preceding, and probably associated with, AIDS have been observed in vaccinated animals and in some patients. There are other competing theories, each supported

by evidence but none which can be considered proven as we write. Another evolutionary process runs in parallel with escaping: a gradual increase in what we have called "intrinsic fitness" in previous sections, probably accompanied by increased pathogenicity. In about half of patients, the infecting strain, which usually uses the CCR5 co-receptor in addition to CD4 to gain access to target cells (CCR5 appears on macrophages and a minority of T-cells), evolves a new co-receptor usage: CXCR4, which is found on most T-cells. The founder strain is sometimes said to be "macrophage-tropic" and the mutant "T-cell tropic"; this "tropism switch", if it occurs, provides the virus with a larger population of targets. Several other theories still in the ring include immune exhaustion (chronic exposure to antigen may render immune cells non-functional or unable to proliferate); "tap-and-drain" (the "tap"—the body's attempt at constancy or homeostasis by replacing lost T-cells—cannot keep up with the "drain"—the loss due to CD4s killed by the virus or other causes), and hyperactivation (uninfected "bystander" CD4s are progressively lost through apoptosis, due to the generalized increase in immune activation observed in HIV patients.)

Besides the potential of escape to cause AIDS, what about the converse? AIDS is defined, in addition to immunodeficiency, by a CD4 count dropping below 200. The Escape Formula remains valid at all times, although the two quantities in the definition of ESA: REA–LOF, may change. Assuming as usual that immune-escape mutations do not enhance fitness, the effect of declining target-cells is to increase the LOF associated with the mutation. (See Part II, Chapter 25, for the calculation.) The same question can be asked about the impact of immune exhaustion on escape. REA stands for "relative escape advantage" and would not be altered by waning immune pressure if it affects all epitopes equally; if it impacts solely on the mutation at issue, REA would decrease. Hence, we predict a declining rate of escapes in late disease.

8.10 Notes

For reviews of immunodominance: [103, 132, 336]. Concerning immunodominance in virus-specific CTLs: it is usually computed from a marker such as secretion of the viral-defense molecule, interferon-γ, when exposed to viral peptides, or from CD8 clone size *in vivo*, detected by epitope-specific reagents called tetramers. Whether these assays actually identify the CTLs

or a more general class of T-cells (functional but not cytotoxic), is not known at this time. Our definition of immunodominance includes a functionality factor (κ_e).

Re quasi-species: see [82]. For the expanding cloud of variants after infection, see [220]. For the debate over population genetics *vs.* quasi-species in the context of RNA viruses, see [76, 131] and references therein.

For Muller's ratchet: see [218, 219].

Widespread positive epistasis in HIV mutations (but not necessarily in escape mutants): [32].

For broadening after primary infection, see [89, 158]. But in one case of SIV escape, no new responses appeared to the new epitope, even when the escape strain was injected into other monkeys: [101].

"Epitope oscillations": see [225]. The data shown in this article appears compatible with an escape in one epitope followed by expansion of response to another epitope [4]. It is not surprising that oscillations can be found in high-dimensional ODEs, which the authors exhibited as a theoretical explanation. But, as we argued in Section 1.11, ODEs cannot represent genetic events at multiple loci.

Evolution of virulence *in vivo*: [102, 148, 250]. Some investigators speculate that, on the population level, HIV is becoming less virulent: [14]. Co-receptor switching: [233].

AIDS and escape: in addition to examples in the NHP model, there are observations in humans, especially from studies of patients who made an immunodominant response to an epitope restricted by HLA-B-27, which is associated with slow progression [22, 89, 112, 163].

Chapter 9

HIV's Cloaking Device: The Fascinating Story of Nef

Most pathogens have evolved some mechanism for partially or wholly evading host immune responses. In the case of HIV, the prominent deceiver is a virally-encoded protein called Nef.

Nef was originally thought to be a "Negative factor" that helped the virus establish a form of cellular latency, and this misnomer has stuck. Later, doctors in Australia discovered three persons who had become infected, from a blood-bank donor, with a strain of HIV with a defect in the Nef gene. These patients exhibited low viral loads, vigorous cellular immunity against the virus, and slow progression to disease. Next, investigators infected macaques with Nef-deleted strains of SIV and observed similar outcomes. In one animal, the virus evolved a new Nef gene—which, astonishingly, more closely resembled the HIV version than that of SIV!

In the laboratory, Nef-defective strains grow readily in cell-lines, meaning that it is dispensable for viral replication. Wild-type strains growing *in vitro* frequently develop mutations in the Nef gene (particularly deletions and frame-shifts) that abolish the protein's function. But *in vivo*, these strains never replace the Nef-competent variety, except sometimes in late-stage disease. Nef is highly-immunogenic in both animals and humans; indeed, many of the earliest CTL responses are to epitopes in Nef. One reason is that Nef itself is "early": the protein is expressed several hours before such "late" proteins as Gag. If other aspects of immune recognition are assumed equal

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(they need not be), early proteins should be highly immunogenic relative to late. (See Figures 8.3 and 8.4.) Consequently, Nef should be under strong pressure to escape by mutating its recognized epitopes, if it can do so without loss of function. See Figure 9.1. (Note that this figure again illustrates the co-existence of single mutant escape strains with the wild-type, as discussed in Chapter 8.)

Although escapes in Nef *in vivo* are frequently observed, loss-of-function mutations are not; apparently, the virus has to maintain Nef. But what is the essential function of Nef that cannot be lost? A candidate was soon discovered: cells infected with Nef-positive strains of HIV had lower than normal numbers of HLA molecules on their surface membranes. Nef was suppressing HLA trafficking, partially preventing the infected cell from advertising its distress. Perhaps Nef was the principal immune-defense gene—and losing Nef was akin to dropping one's shield.

The situation became more complicated when other functions of Nef were demonstrated. Cells infected with a Nef-competent strain pull many CD4 molecules off their surface. It was immediately suggested that this function existed to prevent newly-budded virions from reacting with CD4 on the PIT's membrane, facilitating release. Another consequence, perhaps favorable to the virus, is to prevent co-infection by a strain that could compete with it. (Both suggestions are conjectural at this time.) Finally, it was shown that Nef expression had the reverse impact from the original reports: in fact it increased replication, by either the CD4-downregulation mechanism or by improving the efficiency of forming the provirus. Hence, Nef may increase viral fitness as well as conceal the PIT from killers.

HIV shares its anti-immune-system trick of downregulating HLA with many other viruses. As a predictable result, humans have evolved a defense to this strategy: another kind of lymphocyte, called the natural-killer (NK) cell. The NK cell is not part of the adaptive immune response, but rather of the innate; that is, it recognizes special patterns in pathogen proteins not found in human cells (but encoded in human DNA). However, HLA expression depresses NK activity. An NK cell is an effective cell killer when it recognizes a pathogen pattern and the cell displays little HLA. The significance of NK action in HIV disease is unclear as we write.

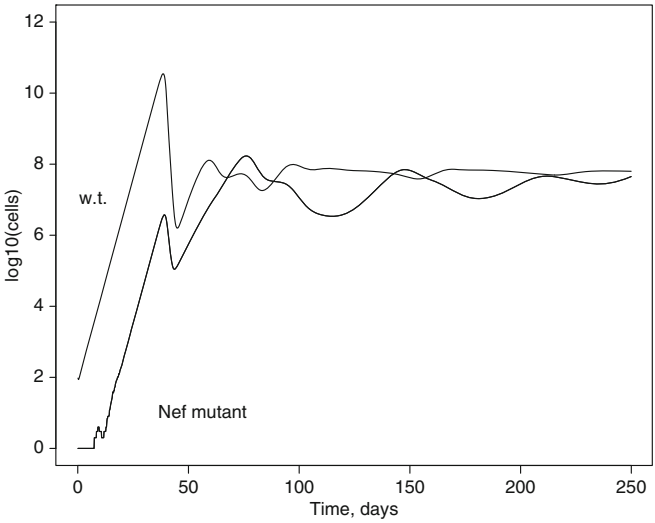

Figure 9.1: Escape in Nef epitopes, without loss-of-function.

9.1 When Invisibility Is a Necessity

Can we gain a theoretical understanding of the stability of Nef function *in vivo*? We next show that theory, combined with new estimates of enhanced CTL killing due to loss of Nef function can indeed explain the necessity of maintaining Nef. Let "CIF" stand for "CTL enhancement factor", meaning the proportional increase in CTL killing that would accrue if HIV lost the HLA-downregulation function. As in Chapter 5, let LOF_m denote the loss-of-fitness associated with a mutation in Nef that simultaneously deletes recognition of a Nef epitope and abolishes Nef action on HLA. Let "FOE" stand for "fraction of immune pressure due to other epitopes". Then we can express the condition preventing escape in the Nef epitope (derived from the Escape Formula, see Part II, Chapter 25):

Nef will be maintained provided:

$$1 - \mathrm{LOF}_m \;<\; \mathrm{CIF} \times \mathrm{FOE}.$$

To exploit this result, the primary unknown we need is, of course, CIF. Although the depletion of HLA is readily measured by flow cytometry,[1] and can be 90 %, the impact on CTL activation is not immediate. Even with active Nef, there may remain 10,000 HLA molecules on the cell's membrane, and it takes only a few to trigger a passing CTL. We estimated CIF from measurements made in the Yang laboratory on the growth-rate of wild-type or Nef-defective HIV in cell-lines, with or without addition of CTLs recognizing a non-Nef epitope. Since Nef downregulates HLA-I types A or B, but not type C, all the epitopes recognized were restricted by the former. The upshot is that CIF varied in the range 2–10, depending on epitope, with a mean of 5.6. In other words, CTL killing is diminished by about 82 % from what it would be, if HIV had never evolved Nef.

If we now imagine a situation *in vivo* in which the wild-type-virus is recognized by at least two CTL clones, one recognizing a Nef epitope, and assume CIF ≥ 3, then escape by mutating the Nef epitope while losing the HLA-downregulation function is impossible. Note that FOE = 1 (meaning the mutation did not delete a CTL epitope, only lost the downregulation function) makes this mutation impossible, period. Our simulations agree with this prediction.

9.2 Blocking Nef

Because of its importance as the HIV defense-molecule, Nef is a natural target for a therapeutic drug. Such a drug, if one can be found, should lower viral load in infected patients. Figure 9.2 shows the VL and total HIV-specific

[1] Flow cytometry is an essential tool of modern cell biology. Designed in analogy to an ink-jet printer, a flow cytometer separates cells on the basis of cellular markers or labels, such as HLA, CD4, or a reagent that reacts with a cell molecule.

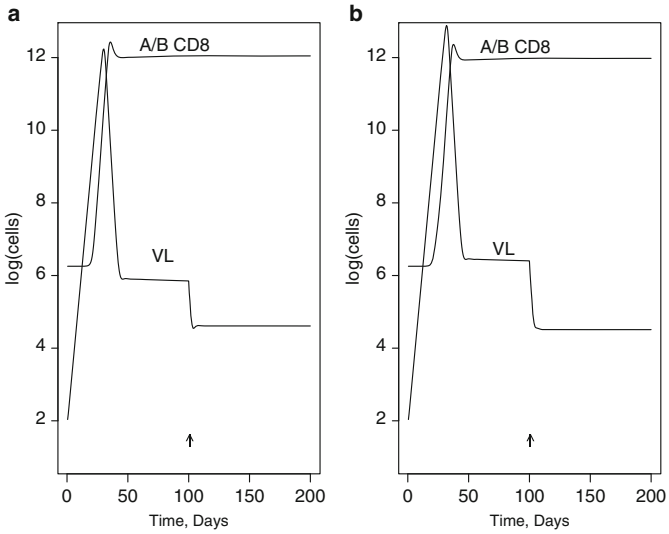

Figure 9.2: Effect of drug-blocking Nef downregulation of HLA and enhancement; DK + NIM (*left panel*) or + SIM (*right panel*). From [317].

CD8s (recognizing HIV epitopes that are HLA type A-or-B restricted) assuming 19 recognized A- or B-restricted epitopes and one C-restricted[2] and a 100 % efficient drug at blocking Nef downregulation of HLA types A and B and replicative enhancement, administered at 100 days after infection. The predicted effect—almost 2.4 logs decrease in viremia in the right panel—would make a substantial impact on disease, delaying the moment at which a patient has to go on antiretroviral drugs (which are toxic). The figure includes two assumptions about whether Nef makes an impact on resting memory CD8 activation: no impact (NIM) and the same impact as on killing (SIM), reflecting our uncertainty about whether memory cells require contact with an antigen-bearing APC or not. (Nef is not active in APCs such as dendritic cells.) The figure was also made under the defective-killing (DK)

[2]One study found a range for number of recognized HIV epitopes of 2–42, with a mean of 18.5. About 7 % of HIV epitopes in the Los Alamos data base with known HLA restriction are type-C restricted.

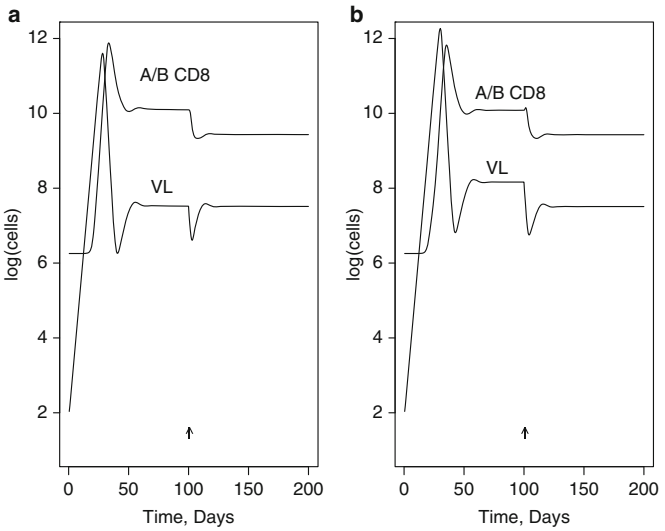

Figure 9.3: Effect of drug blocking Nef downregulation of HLA; DM + NIM (*left panel*) or + SIM (*right panel*). From [317].

hypothesis. Unfortunately for the predictive capacity of present-day models, there is also Figure 9.3, made assuming the defective-memory scenario and no Nef influence on replication *in vivo*. In this figure, in the left panel (DM + NIM) viremia undergoes a brief dip, then returns to the previous level. Although this may seem improbable, given the fivefold increased killing after the drug takes effect, it results from the non-linearity in the steady-state arrived at by virus and immune system.

Perturbations of steady-states are notoriously surprising. A steady-state should be regarded, not as a three-legged stool which, given a mild blow, returns to its previous position and stays there forever; but rather like two sumo wrestlers locked in an embrace and, for the moment, unmoving. If one contestant should relax the pressure for an instant, something will happen—either one wrestler will be thrown out of the ring, or a new stand-off will arise. And it can be very difficult to predict the outcome; that is one reason why computers are valuable in biology and medicine.

Having said that, we admit that the lack of definiteness in our predictions about the effect of blocking Nef is disheartening. But it simply reflects

a level of uncertainty about mechanisms and rates typical in biology and medicine at this time. Here, of course, resides the intellectual justification for clinical trials; they will not be replaced by modeling any time soon. On the other hand, mechanistic models at least have the virtue that the experiments needed to distinguish true from false scenarios should be evident by comparison. With additional work in the lab, biological theory may yet become rigid enough to make definite causal inferences possible—as for the genuinely predictive sciences, chemistry and physics.

9.3 Dropping the Cloak

In this section we indulge in a bit of speculation about a role for target-cell depletion in the Nef story. Although we have remarked that Nef escape mutations with loss-of-function are rarely seen *in vivo*, and proposed a theory to explain this observation, there is one situation where it may be favorable for the virus to abandon its defenses. This scenario requires two stipulations: a trade-off of HLA downregulation for enhanced replication, and target-cell depletion, probably to the AIDS boundary. We next establish a theoretical formula for the threshold at which the virus can drop its cloak of partial invisibility.

The HLA-downregulation and replication-enhancement functions of Nef are separate and either could be altered by mutation without effecting the other. So we are free to speculate that some mutation might abolish the former while amplifying the latter—that is, enhances the replicative enhancement. We do not take a position on the reason that target-cells are lost in late infection (some theories were reviewed in Chapter 8). We simply postulate it at some point in time. For simplicity, we ignore changes in epitopes and consider a mutation (or a set of mutations, perhaps in linkage disequilibrium) that has precisely two effects: it upregulates HLA to the normal expression level and increases production or infectivity of virus (equivalently, R_0). Let the ratio of productivity (ROP) of the mutant relative to the wild-type be defined in the obvious way:

$$\text{ROP} \;=\; \frac{R_0(\text{mut.;m})}{R_0(\text{w.t.})}. \tag{9.1}$$

In Part II, Chapter 25, we demonstrate that there exists a threshold of replacement provided:

$$\text{CIF} \;>\; \text{ROP}. \tag{9.2}$$

Let U denote uninfected target-cells, with a subscript 0 for the initial (pre-infection) level. The threshold is given, in terms of these quantities, by:

Nef can be lost if:

$$U \;<\; U_c,$$

where:

$$U_c \;=\; U_0\,(\,1/R_0(\text{w.t.})\,)\,(\,\text{CIF}-1\,)\,/\,(\,\text{CIF}-\text{ROP}\,).$$

U_c denotes a threshold density of target-cells below which replacement of wild-type by mutant is possible. As we have remarked in Chapter 2 and 8, all R_0's have a concealed factor of U, which has not figured prominently in this book for reasons explained in the Introduction. The R_0 appearing on the right in this formula should be interpreted as the initial reproductive number of the virus. ROP is independent of U.

We can put some numbers into this expression using the estimate of CIF cited above. With CIF = 4.6, ROP = 2.0 (100 % enhancement in productivity or infectivity), and R_0(w.t.) = 6 (yields peak viremia in the HIV–CTL model in 20 days), the formula yields

$$\frac{U_c}{U_0} \;=\; \frac{1}{6}\,\frac{5.6-1}{5.6-2} \;=\; 0.213. \tag{9.3}$$

If we adopt CD4 count as a surrogate for targets, with 1,000 per μl as normal, the predicted threshold is 213. The official AIDS boundary has been 200 since 1993. As usual, there are caveats. This assignment of targets to CD4 ignores cellular activation and the fact that CD4 counts are performed on peripheral blood while 98 % of T-cells live in tissues. However, this prediction illustrates that TCD can play a role in HIV genetics, at least late in infection.

9.4 Notes

For the patients with the Nef-defective virus in Australia, see [258].

HLA class-I, A and B but not class C, downregulated by Nef: [2].

For NK cells in HIV disease, see [5, 88]. In [6], the investigators reported that in primary HIV infection NK cells display kinetics similar to CD8 T-cells but peak earlier. In [30], the ability of NKs to kill PITs was demonstrated *in vitro*; the killing was dependent on the ability of the virus to downregulate HLA. Thus we have another addition to the complex role of Nef—potentiating NKs, as it helps evade CD8s. (The situation is complicated by the different impact of Nef on variant HLAs. Nef effectively downregulates HLA types A and B, but not C and E. NKs that recognize C or E could not kill PITs in the [30] study. Thus, as we write, the relative roles of the two types of killer lymphocytes in controlling HIV is unclear.)

Investigators in the early 1990s reported the enhancement effect of Nef on viral replication *in vitro*. Chowers *et al.* [58] found increased replication, relative to a Nef-deleted mutant, by a factor of 2 after 48 h, in a single-cycle system; assuming a generation time of 2 days *in vivo*, we can conjecture that wild-type Nef has a 50 % impact on R_0. Other investigators reported growth-curves in PBMCs or cell-lines. From these curves we can estimate a difference R_0mut. $- R_0$(w.t.) (approximately using the simplest model with growth rate: $(R_0 - 1)/g$, g = generation time); e.g., from Chowers *et al.* [57] (single-cycle p24 curve at 24 h) the difference is 2.2; from Miller *et al.* [211] (resting PBMC infected and activated, at 8 days), 0.79; Spina *et al.* [281] (similar), 0.74. Assuming R_0(w.t.) *in vivo* is greater than 4.0, these results imply a somewhat smaller impact than 50 %. The influence of Nef on infectivity in these studies could be differentiated from CD4 downregulation and was more likely due to provirus maturation than virion production. However, the effect was most pronounced in infected, resting PBMCs that were immediately activated with mitogen; it was less pronounced in proliferating cell-lines.

In vivo, the impact on infectivity enhancement might be limited to a small fraction of CD4+ target cells that become infected shortly before they are activated by cognate antigen.

For our model of the impact of a drug directed against Nef, see [317].

For Nef gain- and loss-of-function mutations in AIDS, see [49].

Our modeling of escape in Nef epitopes with or without loss-of-function is distinguishable from other work from before 2000 (e.g., [7]) for reasons besides the stochastic-*vs.*-deterministic issues discussed in Section 1.11. At that time, the programmed-proliferation scenario was unknown. Co-existence is generic in ODE models of evolution without a replacement mechanism such as "overkill", described in Section 6.5, or explicit (usually *ad hoc*) competition terms. Hence our model makes very different predictions about persistence of Nef activity and escape.

Chapter 10

Retroviral Sex and Escape

In the late 1960s, virologists made the surprising discovery that retroviruses exhibit a form of sexual reproduction. Humans are genetically "diploid", which means that, except in germline cells, they maintain two sets of genetic information (in DNA, on paired chromosomes), and of course they reproduce sexually. Retroviruses are "pseudodiploid": they package two complete genomes (in the form of RNAs) in each virion, and they reproduce in infected host cells. Simultaneous infection of a cell by multiple HIV strains is common and genes from several variants can contribute to forming a new virion, just as genes from mother and father contribute to a human child.

Beginning with Darwin, evolutionary theorists have propounded many views on the advantage of sexual reproduction. The simplest idea is that sex can bring together variant genes to create new, beneficial combinations. In this chapter, we show that modeling can be used to estimate the rate at which recombination of HIV variants can generate a double-mutant strain, a question of interest both for escape from the immune system and from antiretroviral drugs. We limit ourselves to two loci, for reasons that will become apparent. The goal is to discover whether stepwise mutation or recombination is more likely to generate escapes. Several predictions of the model are testable, including that recombination may rival stepwise escape during the primary-viremic stage but the rate should fall afterward; and, that the distribution of "proviruses" (integrated viral DNA) should differ between resting, latently-infected and activated, productively-infected CD4+ T-cells.

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10.1 Biology of Recombination in HIV

In each infection cycle, a virion first latches onto a CD4 and another cell-surface receptor, then enters the cell and unpackages the RNAs. The RNAs are then transcribed into a single DNA molecule by the viral reverse-transcriptase and other enzymes. The DNA then transits to the nucleus and is inserted into the host genome, at which point it is referred to as a "provirus". Although, as we learned in Chapter 9, after infection a viral protein called Nef downregulates surface CD4 expression, re-infection is frequent. Indeed, infected CD4+ T-cells in the spleen contain an average of 3.6 proviruses and as many as 15.

After integration, the viral DNA is translated back into RNAs, viral proteins are synthesized, and viral and host proteins combine to package two RNAs in a virion. A "heterozygous" virion—one packaging different genomes—sets the stage for recombination in the next round of infection. Recombination is due primarily to homologous crossover events during DNA synthesis; there are an average of 9 crossovers in CD4+ T-cells and may be as many as 30 in macrophages. The affect of these crossovers is that linkage disequilibrium (the tendency of genes to segregate together) is entirely overcome at a distance of one kilobase. Phylogenetic studies have revealed that genomic changes due to recombination are frequent *in vivo*, possibly more frequent than by point-mutations. At the level of human populations, re-infection of an already-infected patient by an HIV strain of a different subtype (sometimes called "superinfection") has been observed; in one population, re-infection was almost as common as first infection. Indeed, many or most circulating strains are recombinants. The conclusion that recombination is a significant force in HIV evolution is unavoidable.

Recombination introduces new twists into the escape story. Although a virion might package an escape-mutant genome, continuing representation of wild-type epitopes in the other genome could mean that killing by the relevant CTLs persists; the escape advantage could be nullified. But another phenomenon, known to virologists as "phenotypic mixing", acts in the reverse direction. Virions formed from a mix of peptides transcribed from different proviruses might have infectivity intermediate between wild-type and mutant, mollifying intrinsic loss-of-fitness.

Recombination is known to play a role in the development of drug-resistance in HIV patients. Potent antiretroviral drugs discovered in the 1990s are able when given in combination to reduce HIV load to almost

unmeasurable levels. An earlier drug, zidovudine, also called AZT, failed when given by itself, often in a year as the virus acquired multiple resistance mutations. The cascade of mutations usually begins with a pair in the drug's target, the RT protein, which appear simultaneously; afterward, additional mutations generate a completely-resistant phenotype. The initial pair of amino-acid changes can be a consequence of recombination. Linked mutations generated by recombination can confer resistance to multiple drugs.

10.2 Waiting for the Double Mutant

The model we developed describes populations through the single-mutant stage by compartmental, deterministic laws (ODEs). As the reader is aware, we cannot assume determinism beyond that stage; the time until the succ essful double-mutant strain appears is random. (It's a waiting-to-win-the-lottery, not a who-wins-a-footrace, problem.) But we do not need to simulate a stochastic process, which as we will see would require a very fast computer. The waiting-time distribution of the double-mutant can be computed using rates (intensities) as well; hence, we call this approximation "semi-deterministic". (See the Notes for discussion of what would be involved in extending to a fully-stochastic model.)

We divided the life-cycle of a provirus in an infected target cell into S stages. The first $EC < S$ stages are "eclipse" (before proviral proteins are expressed) and the remainder are productive ("PIT") stages. We assumed that CD4 downregulation coincided with the transition of a provirus to PIT status (at stage $EC+1$), closing the window for reinfection. We had to adopt a cut-off on the number of wild-type proviruses in a cell, call it PVMAX, in order to avoid an explosion in the number of compartments. With, e.g., $S = 3$ and PVMAX $= 6$, there are 1,889 compartments (by computer enumeration).

Figure 10.1 shows the infection and immune response with the basic reproductive number, R_0, set to 4.0 (yields peak viremia in 40 days), demonstrating compatibility of this large system with the simpler version of Chapters 2–4. Figure 10.2 shows the peak in the average number of proviruses per PIT, at around the same time as peak viremia, followed by a decrease to barely above one as multiply-infected PITs die off. But in the pool of latently-infected cells (LICs), a much larger pool of cells which archive the earlier infections, the average ratio of proviruses/LIC stabilizes above 5, Figure 10.3. (The observed ratio in splenic CD4+ T-cells, by way of *in situ* hybridization

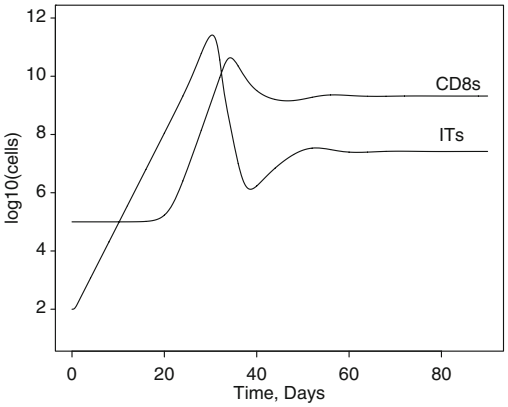

Figure 10.1: The infection process and immune response; $R_0 = 3.0$.

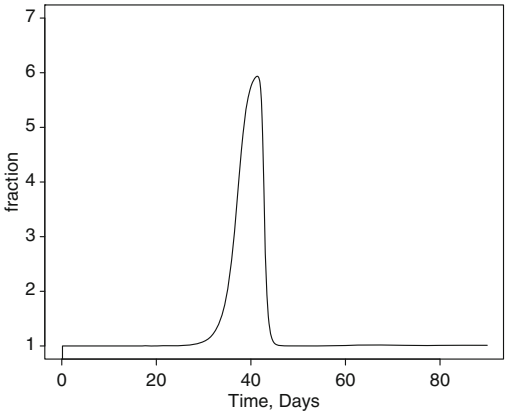

Figure 10.2: Proviruses per PIT; $R_0 = 4.0$.

techniques, is about 3.6.) In this figure and the similar one below (with $R_0 = 6.0$), the distribution of proviruses is influenced by the assumed cut-off at seven per cell (six wild-type and one mutant), so the true distribution in a model without cut-off would presumably be shifted upward. Figure 10.4 shows the computed, cumulative probability distributions for generating the double mutant by stepwise mutation alone, or by recombination alone, with $R_0 = 4$. For the latter, the recombination parameter, R, was set at 0.5,

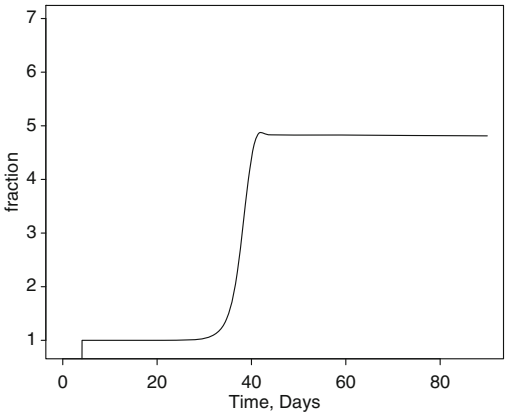

Figure 10.3: Proviruses per LIC; $R_0 = 4.0$.

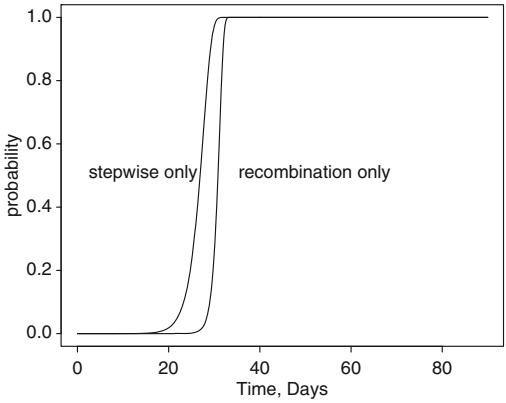

Figure 10.4: Making the double-mutant, stepwise or recombination; $R_0 = 4.0$.

implying independent segregation. The LOF in single mutants in these figures was 15 %, similar to that for several of the AZT resistance mutations. Escape by generating the double-mutant by recombination is almost as fast as by stepwise evolution.

Figures 10.5–10.8 show similar probability distributions with $R_0 = 6.0$ (yielding peak viremia in about 25 days).

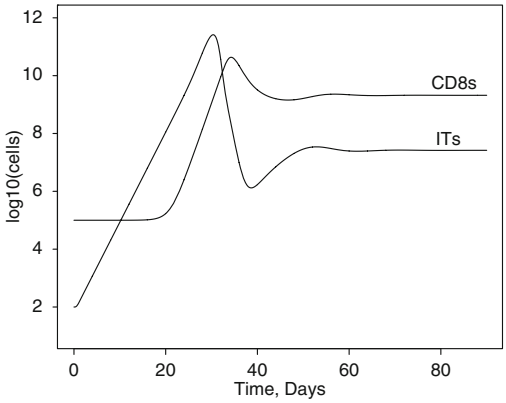

Figure 10.5: The infection process and immune response; $R_0 = 6.0$.

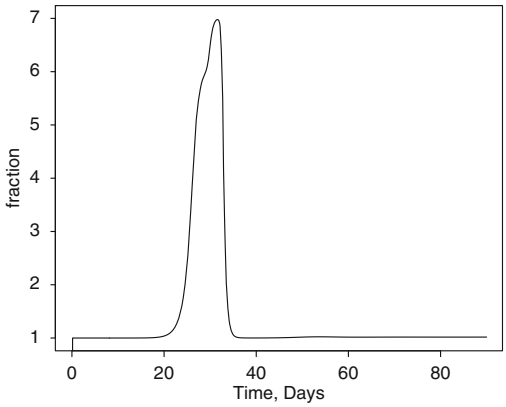

Figure 10.6: Proviruses per PIT; $R_0 = 6.0$.

Figure 10.9 might apply to a treated patient; at 90 days R_0 was lowered by half, producing a one-log drop in viral load (not shown), as for a partially-effective drug; also, the waiting-time distributions were accumulated starting at 90 days. In this situation, stepwise escape clearly dominates recombination. These figures report the distribution of times that the "successful escaper" (that avoids extinction) appears; the time-to-replacement will be longer, by a few months (as we have seen in previous chapters).

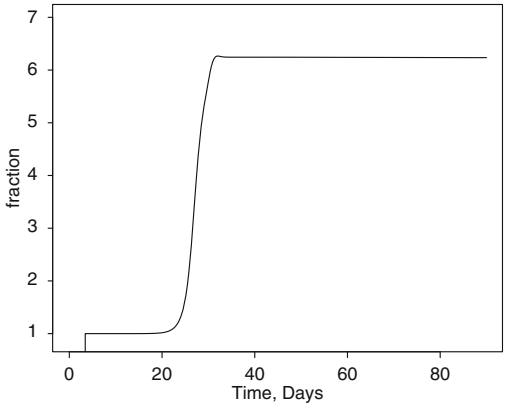

Figure 10.7: Proviruses per LIC; $R_0 = 6.0$.

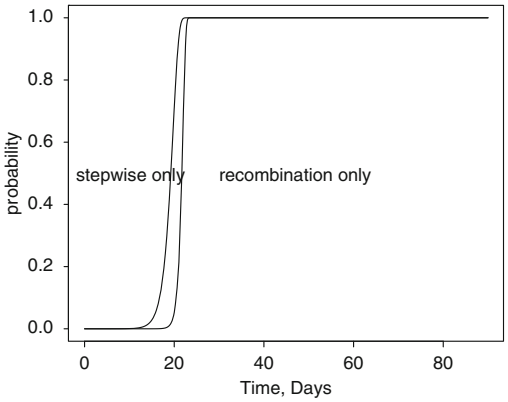

Figure 10.8: Making the double-mutant, stepwise or recombination; $R_0 = 6.0$.

10.3 Explanations and Implications

From the results presented here it can be concluded that production of a double-mutant escape-strain during primary viremia should be about as rapid by recombination as by stepwise-mutation, but the latter mechanism will dominate during the chronic period, except perhaps at the terminal phase of AIDS. Another prediction of the model concerns the proviral distribution

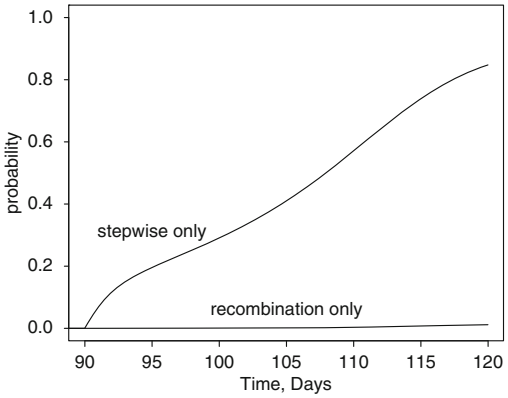

Figure 10.9: Escape in a treated patient.

in CD4+ T-cells, which in the chronic stage is predicted to differ between latently-infected and productively-infected cells. Can we gain an heuristic understanding of these results?

For the semi-deterministic approximation to retroviral evolution, simple estimates can be derived from the intensities of each process and general considerations of the numbers of variants. (See Part II, Chapter 26, for the details.) Let U denote the initial number of uninfected target cells and N the number of infected cells at a later time. Then, we have the order-of-magnitude-estimated intensities for each pathway:

$$\begin{aligned} \text{stepwise}: \quad & [\,\text{1-mutants}\,].[\,\text{infectible}\,].[\,\text{infectivity}\,].[\,\text{mut. rate}\,] \\ = \; & [\,N\,\mu\,].[\,U+N\,].[\,1/U\,].[\,\mu\,] \\ = \; & N\,\mu^2; \end{aligned} \tag{10.1}$$

$$\begin{aligned} \text{recombination}: \quad & [\,\text{first mutants}\,].[\,\text{sec. mutants}\,].[\,\text{infectivity}\,].[\,\text{rec. rate}\,] \\ = \; & [\,N\,\mu\,].[\,N\,\mu\,].[\,1/U\,].[\,R\,] \\ = \; & [\,N\,\mu^2\,].[N/U\,]\,R. \end{aligned} \tag{10.2}$$

The factors of $1/U$ reflect the density-dependence of infection at fixed R_0. The average time-to-escape in each case is proportional to the inverse of the expression (up to a harmless factor of order one).

We assumed that the target-cell population consists of activated CD4+ T-cells and remains fixed, for reasons discussed in the Introduction. It may be possible, for example at peak viremia, for N to grow to the same magnitude as initial U; indeed, this may explain why the stepwise and recombination paths are roughly equivalent in Figures 10.4 and 10.8. In the steady-state period, or during HAART, probably $N << U$; in that case, stepwise will presumably dominate recombination, as in Figure 10.9.

Can the recombination path to the double-mutant ever exceed stepwise, in the escape context? We have identified five hypothetical scenarios where it may be possible. First, there is the hyperactivation picture. As both infected and uninfected target-cell populations rise, the former may actually exceed the initial value of the latter, i.e. at some times perhaps $N(t) > U$. A second possibility is reproduction in macrophages, where, for some cellular reason unknown at this writing, recombination rates are ten times higher than in T-cells. A third mechanism may be important during highly-active antiretroviral therapy (HAART), which can lower the PIT pool by several logs. In these conditions, reactivation of latently-infected, CD4+ memory T-cells may drive the persistent replication in these patients; as shown in Figures 10.3 and 10.7, the model predicts a substantially higher average number of proviruses in these cells. Fourth, there is the N_e affair. If N_e is in fact on the low side, the mechanism may be "bio-graphic" isolation of different strains in different tissues, EPV at the cellular level, or frequent selective sweeps. If the first explanation is the correct one, and HIV reproduces in isolated sites, with high neighborhood infection but lower "distant transmission" rates, local target cells may be exhaustively infected (again leading to $N \approx U$). Finally, in late-stage disease replication rates may rise, leading to the same situation. Given the biological uncertainties, firm conclusions about the evolutionary advantage conferred by recombination in the escape problem, if it does confer an advantage, are not obvious at this time.

We note the continuing popularity of phylogenetic trees for analyzing sets of HIV sequences. No evolutionary trees have been displayed in this book; one reason is that the prevalence of recombination in retroviral reproduction renders these analyzes doubtful as reflecting real events. Trees can only represent things breaking apart; recombination can bring entities together. Moreover, Darwin's metaphor of the "tree of evolution" was meant to describe speciation. By definition, members of distinct species no longer produce offspring in the wild. By contrast, any pair of HIV genomes that exist in a person apparently may be recombined by the mechanisms discussed

in this chapter (although we cannot predict whether the result would still be capable of infecting or reproducing). With respect to HIV *in vivo*, Darwin's tree of life may have to be replaced by some form of network.

Finally, it should be possible to test several predictions of the model described here. That recombination should rival stepwise escape early, during primary infection, but the rate should fall before rising again at AIDS may already have been observed. That proviral distributions should differ between latently-infected cells and PITs in chronically-infected patients should also be observable.

10.4 Notes

For the discovery that retroviruses may exhibit a form of sex, see [79, 137, 291]. Jung *et al.* reported that infected CD4+ T-cells in the spleen contain an average of 3.6 proviruses, [155]. For the mechanisms of crossover, see [222, 291]. For macrophages, see [180]; the rate is an order of magnitude higher than recognized before 2004. (Studies before that year did not assess HIV recombination in its natural target cells; they were usually carried out in fibroblastic cell lines.) Phylogenetic studies revealed that recombination events are frequent: [149, 259], and possibly more frequent than due to point-mutations [274]. Overcoming linkage disequilibrium: [62, 259, 260]. For super-infection, see [15, 48, 54, 72, 87, 135, 241, 251, 325, 330]; in one population, re-infection was almost as common as first infection [54]. Recombination is a significant force in HIV evolution [45]; indeed, many or most circulating strains are recombinants [137].

Continuing expression of wild-type epitopes on heterogeneous virions means that killing by CTLs persists: [155]. "Phenotypic mixing": [223]

The first AZT mutation can be a consequence of recombination:[162]. Curiously, several common RT mutations have the side-effect of increasing the rate of RNA template-switching and possibly recombination [166]. Later, many investigators reported observations of linked mutations generated by recombination that conferred resistance to multiple drugs [71, 118, 176, 206, 217, 338].

Levy *et al.*, [180], noted that, in *in vitro*, single-round assays, recombination occurred proportionally to the square of the infection fraction. This result can be readily explained by the theory developed here. The intensity of recombination is a function of the product of the densities of single-mutant

populations, which in turn (at least in steady-state conditions) exist at fixed percentages of the infected-cell population (the $N\mu$ of previous discussions).

In 2002, Jung *et al.* announced the discovery of multiply-infected spleen cells in HIV patients. They also noted a peculiar proviral distribution in CD4+ T cells, with a drop between one and three proviruses[155, 207]. Two mathematicians considered some infection scenarios, including cell-to-cell contact, to explain this distribution but their best-fitting model yielded only a small non-concavity [75]. Another possibility is HIV replication in a subset of target cells other than CD4+ T-cells. In macrophages, virions bud into endosomes before being released, possibly in large pools, into the extra-cellular medium [172]. Moreover, these virions may be ten times more infectious than those replicating in T-cells, due to increased expression of envelope protein [192]. A similar story has recently been reported for dendritic cells, which, although not supporting HIV replication, can capture and transmit virus particles [320]. The suggestion is that these cells, which can act as antigen-presenting cells, may generate extremal provirus distributions (e.g. with a large mean and a normal distribution), when transmitting a pulse of virions to a CD4+ T-cell. However, we have noted that a mixture of a normal distribution with mean greater than three and a point mass at one does not yield the observed drop at two proviruses, so the situation remains mysterious.

Mathematical biologists have frequently modeled viral evolution in the host, including recombination [25, 28, 29, 33, 41, 63, 82, 98, 130, 287, 314]. Bretscher *et al.* [41] discussed some of the more interesting aspects of retroviral biology, such as phenotypic mixing, in the context of drug-resistance using a deterministic, classical model. (They concluded that "contrary to the commonly held belief . . . recombination is expected to slow down the rate of evolution of multi-drug-resistant virus during therapy.") Bocharov *et al.* [28] carried out stochastic simulations of HIV replication, including mutation and recombination, in a small population of cells, and demonstrated the importance of recombination in increasing the effective mutation rate as well as the stochastic nature of HIV evolution in the host. In general, in past discussions, even if the genetic events were granted a stochastic law, the population dynamics of infected cells were assumed to be of compartmental deterministic type. Replacement is defined as the moment when the double-mutant population grows past the wild-type. As we discussed in Section 1.11, this definition can produce mathematical artifacts.

Concerning going beyond the deterministic approximation, see Part II, Chapter 26.

That the recombination rate should fall after primary before rising again in AIDS may already have been observed: [175, 188]. It should be detectable using the methods described in [274]. That proviral distributions should differ between latently-infected cells and PITs in chronically-infected patients should also be observable, by measuring cellular-activation markers together with *in situ* hybridization for viral DNA, as in [155].

Chapter 11

The N_e Business, Again, and a Proposed Experiment

In 1997, A. Leigh-Brown proposed that the effective population size, traditionally denoted N_e, of HIV *in vivo* is surprisingly small. Leigh-Brown based this conclusion on the analysis of certain nucleotide sequences coding for a part of the envelope protein ("Env"); on the basis of established genetic tests (assuming neutrality), he concluded that N_e was closer to 100 than to the population size of virions (10^{10}–10^{12}), or of PITs (10^7–10^8) in chronic infection. (We will write the latter as N_d, and call it the demographic population size, as usual.) If correct, this demonstration has implications for the timing of appearance of drug-resistance and immune-escape mutations and perhaps can even explain the remarkably low probability of HIV infection given exposure.

Two years later, Leigh-Brown's conclusion was disputed by Rouzine and Coffin, who argued that the neutral theory did not apply to the Env sequences employed. Rather, they proposed, the variation in a (separate) set of Env sequences was due to selection after a transmission bottleneck. Shriner *et al.* in 2004 responded to this argument by suggesting that Rouzine's and Coffin's conclusion was confounded by their choice of sequences from an unusually variable part of the Env protein. As this chapter is being written, it is reasonable to regard the issue as unsettled.

The founders of population genetics introduced the concept of effective population size in the 1920s to capture the effect of differential reproduction on genetic variation in the population. A dual-sex, randomly-mating population with an N_e of 100 is expected to exhibit the genetic variation

W.D. Wick and O.O. Yang, *War in the Body: The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines*,
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of a "canonical" (Fisher-Wright) population of that size—even if its N_d is, say, 10,000. Many scenarios have been proposed that can generate a low N_e; they all incorporate some form of reproductive bottleneck. These include frequent selective sweeps; large population size-fluctuations, due to environmental change or migration-extinction events; and heterogeneity at the level of the individual—which we have called, in previous chapters, "extra-Poisson variation" (EPV). As we have seen, retroviruses are mutation-machines, the host applies ample selective pressure, and replacement events are frequent. The selective-sweep mechanism might therefore be responsible for a low N_e, as might the migration-extinction scenario: the infection in one tissue might be extinguished by an immune response, but not before seeding another. In this chapter we shall be interested in a third proposal: that the bottleneck occurs at the level of the individual PIT.

This proposal is suggested by the following observations. First, the viral reproductive-cycle contains many steps, each prone to error or failure. The links in the chain include reverse-transcription of the viral RNA into DNA, transposition of the DNA provirus into the nucleus, successful integration into the host genome, host activation and transcription of viral proteins, and interactions with host proteins. All of these steps are prone to interference by host or viral factors or chance events. As a result, the distribution of virion production in PITs is highly variable; it ranges from 50–5,000 virions. Moreover, at least in infected cell-lines, a very large fraction of virions successfully budded from the host cell nevertheless prove non-infectious—perhaps 99.9 %. These facts suggest that HIV reproduction may resemble assembly lines operated by workers who may be drunk or incompetent, and whose output widgets rarely pass inspection. If you imagine trying to start production at the factory with a single assembly-line, you can even grasp why the infection probability given exposure for HIV is so small; probably some dunderhead is on the line you assembled.[1]

In mathematical language, we are proposing that the number of virions or offspring produced by a PIT exhibits "extra-Poisson variation". This phrase has been used many times in this book; beyond dart-throwing analogies, let us now explain its origin more fully. It derives from the simplest mathematical assumption about an offspring distribution: that it is Poisson. A Poisson

[1] At this time, the EPV-hypothesis about infection probabilities is still conjectural; there are many alternative explanations, e.g., a rare mucosal target cell, or a stochastic barrier that must be penetrated.

random variable arises from an accrual process with a rate-of-production constant over a fixed period. Let the average number of offspring PITs be denoted, as always, by "R_0". If the offspring distribution is Poisson and, e.g., $R_0 = 3$, the chance of observing 100 offspring is infinitesimal. (The tail of the Poisson distribution falls off super-exponentially; see Part II, Chapter 20, Exercise 4.) However, this probability may be much higher, even with the same mean. For example, we might postulate three types of PITs; type 1 has no offspring; type 2 has one; and type 3 has 100, with probabilities

$$p_1 = 0.673; \quad p_2 = 0.3; \quad p_3 = 0.027. \tag{11.1}$$

A Poisson random variable is characterized by a variance-to-mean ratio (VMR) of one; this distribution has $R_0 = 3$ but a VMR = 87.0.

In this book we have called the occurrence that the offspring distribution is closer to the above example than to a Poisson random variable the "extra-Poisson variation" (EPV) scenario. (In the HIV infection model of Chapter 2, we incorporated EPV slightly differently: into virion production, and then arranged for a selected offspring R_0.) We adopt the VMR of this distribution as quantifying the extra-Poisson variation and, somewhat loosely, replace it by EPV (so that we can refer to variance in other contexts; e.g., viral strains in the chronic period). EPV in reproductive dynamics leads to a low N_e; as we saw in Chapter 6, the relation is approximately

$$N_e = \frac{N_d}{EPV}. \tag{11.2}$$

Thus the example above and an $N_d = 10^7$ would produce an N_e of $\approx 10^5$, on the high side in the controversy. By increasing the high offspring number, to, e.g., 10,000, we can produce examples with much lower N_e.

We next describe some experiments that could elucidate whether the effective population size for retroviruses is in fact much smaller than the demographic size, and if so, whether the cause is EPV at the cellular level. We also display some simulations and power computations, to demonstrate that the experiment is not impractical. The experiments might be carried out *in vitro*, with HIV propagated in cell lines, or *in vivo*, with strains of SIV inoculated into monkeys (or another retrovirus in a different non-human host). Although the idea of detecting EPV in cell-lines is perhaps unorthodox, no observations appear to preclude it. If performed *in vivo*, the experiment has the advantage of not relying on mutations; it can get directly

at the underlying population dynamics, entirely evading the issue of neutral evolution vs. selection. It thus has the potential to resolve the N_e controversy. A disadvantage of the *in vivo* experiment is that, if it should support a large EPV and correspondingly low N_e, the underlying dynamical mechanism will not be immediately evident. By contrast, since a laboratory viral strain, well-adapted to a cell-line, should not exhibit selective sweeps, and the cells can be maintained in a uniform environment, the only explanation for observed EPV would be variation at the cellular level.

11.1 Barcoding HIV

The key idea is to introduce extra codons, with several variations, into the retroviral genome, yielding new strains with the following properties:

1. Each strain is still viable, and if possible, has the same fitness as the wild-type-strain (as measured in competitive-growth assays in cell-lines), or if not, with a fitness that does not depend on the particular additional nucleotides;

2. The added sequence is stable under replication, except for a small frequency of mutation or deletion;

3. The supernumerary codons can be easily detected, either by a specific assay for each variation, or by sequencing.

Concerning item 1, molecular virologists have added entire sequences of proteins to the HIV genome, either in the hope that the protein will be expressed in the PIT and act as a reporter of infection, or that the resultant virus will be attenuated (and potentially useful as a vaccine). Sequences longer than 300 bp have a tendency to be deleted during passages, but smaller sequences are stable for a week or more *in vitro*. For item 2, only a few variations are required, e.g., 2–10, depending on the statistical power required; so as few as two supernumerary nucleotides could work in principle. (More would be preferable, because of the possibility of deletion of neutral sequences.) On the other hand, considering item 3, if sequencing is required it would be preferable to keep the supplementary sequences as short as possible.

11.2 The *In Vitro* Experiment

The model has a number 'B', (in range 2–10), of "barcoded" strains, in addition to the compartments described in Chapter 2. Because target-cell limitation may develop in a well, a number U of uninfected target cells was included; every infection drops U by one. For initial conditions, we assumed $U = 2 \times 10^5$ cells in each well and an infusion of enough virions, sufficient to create about 10^3 infected cells of each barcoded variety. The initial population sizes are therefore random (for each genome, Poisson with mean 1,000, distributed over the types according to the selected probabilities); but the initial fluctuation in each species is small (on order $\sqrt{1000}$). For this *in vitro* experiment, the immune system is not included.

Figures 11.1–11.3 show some simulated trajectories of the infection with four barcoded strains. The numbers in the figures refer to the strains of highest or lowest frequency at the end of the experiment. As the four strains had identical fitness, the lucky winner will be different on different simulation runs. The stochastic nature of the population dynamics in the third figure is evident. However, there is some randomness even in the first figure, which was produced assuming only one type of PIT (see next section).

If specific assays are available to detect the density of each barcode in the wells, distinguishing Figure 11.3 from Figures 11.1 or 11.2 will obviously not

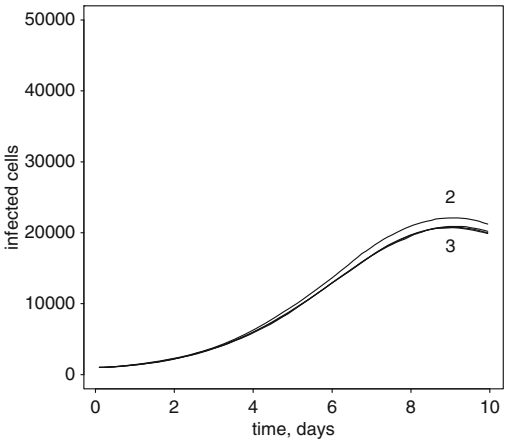

Figure 11.1: *In vitro* infection, with four barcodes and no EPV.

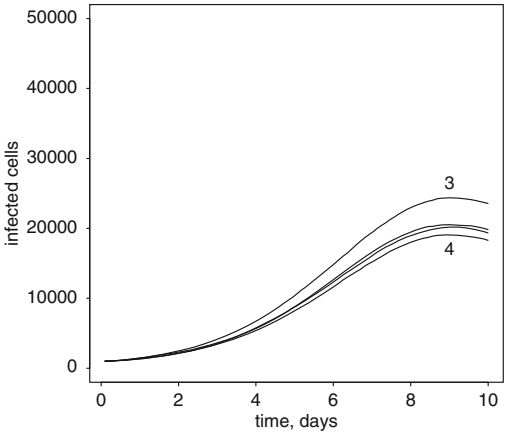

Figure 11.2: *In vitro* infection, with EPV = 192.

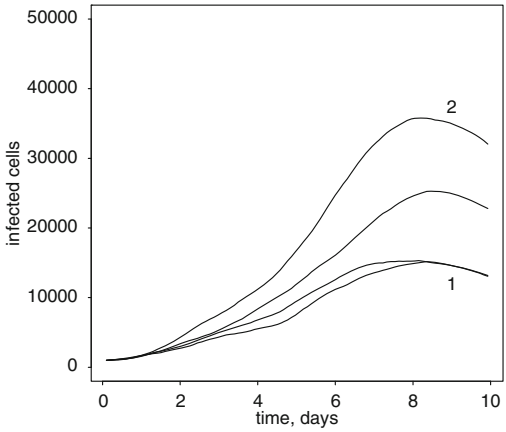

Figure 11.3: *In vitro* infection, with EPV = 897.

require statistical tests. The experiment need only be repeated in a sufficient number of wells to assure that no barcoded variant has a selective advantage. As sequencing may be required, we report some power results in Table 11.1, below. The statistic employed was the variance of the strain-frequency vector, call it 'VSF', at the final time (10 days). For these experiments we set $B = 10$ and assumed a variable number of clones are derived from the well

Table 11.1: Power in the in vitro experiment

Clones:	20	30	50
"null" model EPV:			
None[a]	0.62	0.73	0.90
192	0.69	0.78	0.83

[a]Only one PIT type; but see Section 11.4

and sequenced (sequencing is assumed error-free); the empirical frequencies were then used to estimate VSF. (Multiple wells are needed only to verify the neutrality of the barcodes and the stochasticity of the fluctuations i.e. that a particular barcoded strain is not the inevitable winner.) We chose two values for EPV. The null hypothesis, H_0, was that EPV was the lower number, and the alternate hypothesis, H_1, that it was the higher number. The type-one error rate was set to the standard .05 and the upper one-sided critical point determined by simulating the experiment, with the lower EPV, 200 times. The table reports the estimated statistical power when sequencing the indicated number of clones: namely, the fraction of 200 additional runs, with EPV = 897 (corresponds to the third production level, called K in Chapter 2, of 1,000; used for Figure 11.3), which rejected H_0 (EPV in the first column; the second entry corresponds to $K = 10$ and was used for Figure 11.2). The 200 runs are required solely for this computation; in principle only one experiment is required, just as one observation in one patient can refute one hypothesis at a given type-one error rate, if the mean and variance are known *a priori*.

11.3 The *In Vivo* Experiment

Next, let us add the CTLs. Figure 11.4 illustrates primary viremia brought down by HIV-specific CTLs (the upper curve) and the formation of the chronically-infected steady-state. The lower curve actually represents four variant, bar-coded viruses, without EPV; with the log-axis and pixel resolution used, the curves overlap. Figure 11.5 shows a typical experiment with four barcodes and upper production $K = 1,000$, resulting in $EPV = 897$; the ratio of highest:lowest strains at the end of this simulation was about 1.5 logs when none went extinct. Remarkably, in 27 % of the runs one strain did in fact disappear, a higher percentage then observed in the *in vitro* case

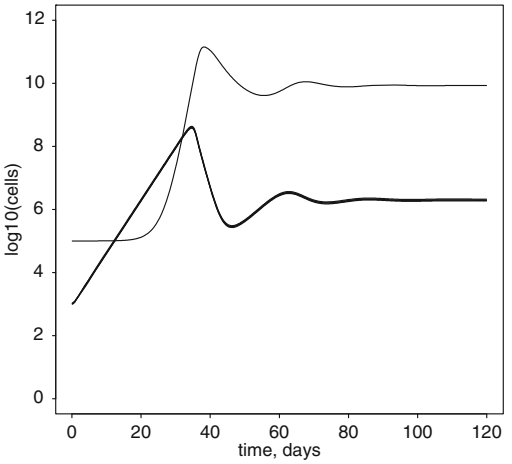

Figure 11.4: *In vivo* infection, with four barcodes and no EPV.

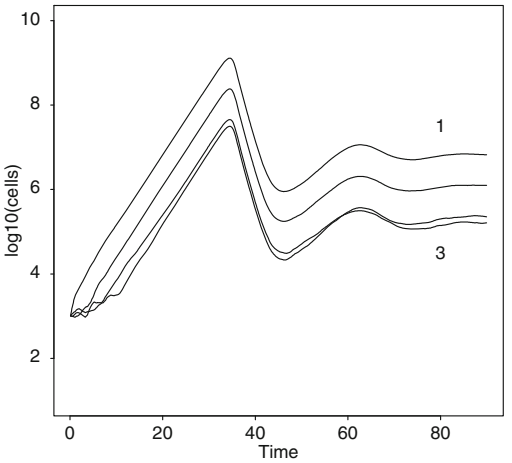

Figure 11.5: *In vivo* infection, with four barcodes and EPV = 897.

with identical initial conditions, implying a role of the immune component in the disappearances. In repeated trials, with 4 strains the average log-ratio was about one log, with variance 0.57; with 10 genomes, the average was 1.7, and the variance, .66; in 56/100 runs at least one extinction occurred.

As differences at these levels are easily detected, these simulations indicate that statistical tests will not be required to detect EPV *in vivo*, if it exists.

11.4 Variations and Caveats

How might a "barcoded" virus be created? One possibility is to reverse-engineer several nucleotides into a non-coding region such as the LTR or a dispensable gene such as Nef. Another is to isolate well-adapted laboratory clones that have accumulated various neutral mutations, for example synonymous nucleotide changes. Will these barcodes be stable? Although any prediction about stability can easily be confirmed or contradicted by experiments, we can at least observe that neutral codon markers *in vitro* will not be confounded to any great degree by pointwise mutation. Assuming at most 10^5 PITs at 10 days, the virus has experienced 5 generations; with a mutation rate of 3×10^{-5} changes per n.t. per round and two n.t. barcodes, only about 30 PITs are expected to have spontaneously altered barcodes. Even with a 10-n.t. barcode, this number is only 150 PITs. In the *in vivo* experiments the number of generations will increase but the frequencies of altered barcodes, by single-site mutations, will still be small. A tendency to shed the supplementary codons, which do not provide fitness advantages, would be more troublesome. The *in vivo* experiment is clearly more vulnerable to deletion and re-emergence of the wild-type, since waiting for the steady-state may require 60 generations. Even a small fitness loss would likely result in the wild-type overgrowing all barcoded forms. Hence the importance of synonymous substitutions for this experiment.

Might Figures 11.3 or 11.5 represent merely dynamical blow-ups of an initial fluctuation in numbers? Indeed, the randomness apparent in these simulations results from both fluctuations in initial conditions and dynamical stochasticity. For example, consider Figure 11.6, which was made, perversely considering the main thrust of this chapter, entirely with deterministic updating.[2] Evidently, the randomness in this figure accrued from initial conditions amplified[3] by expanding directions in the deterministic model—not exactly Lorenz-type "chaos" but in that spirit.

[2]In other words, using ODEs for both immune system and HIV infection, updating all compartments by the RK method.

[3]The ratio: smallest to largest genome compartment, went from 5 % at the start to 300 % at the finish, but clearly no Lorenz-like gyrations appear in the figure.

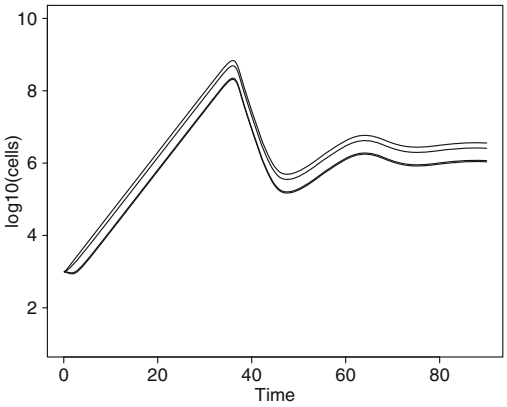

Figure 11.6: *In vivo* infection updated deterministically, with four barcodes and EPV = 897.

Table 11.2: Probability predictions in the in vitro experiment

Prediction:	IPF	I3PF	I3PF3
Initial PITs:			
1,000	.245	.50	.485
10,000	.275	.59	.49

Returning to the *in vitro* model, Table 11.2 reports the probabilities from a simulation study of four barcoded strains, with 200 repetitions, and either 1,000 or 10,000 initial ITs per genome, randomly distributed over types with the selected frequencies. The table entries are: the probability that the initial largest genome compartment correctly predicted the final largest genome compartment (IPF); the initial largest third-type compartment of a genome (the type with largest virion production and smallest probability of formation) correctly predicted the final largest genome compartment (I3PF); and the initial largest third-type genome compartment correctly predicted the final largest third-type genome compartment (I3PF3).

Evidently, the largest initial genome compartment predicted the final one (with $B = 4$) at chance level; the largest type-3 compartment did better, predicting correctly 50–60 % of the time. Thus, in the *in vitro* experiment with MOI = .005–.05, "ordinary" stochasticity—the result of random fluctuations in appearance and replication of PIT types in the different

lineages—contributed at least 40 % of the variation. With deterministic updating, as for Figure 11.6, the largest initial genome compartment of course predicts the winner with certainty. The variances are also diminished relative to the stochastic simulation.[4]

The stochasticity in Figure 11.1, generated with just one PIT-type, may appear surprising. The Poisson law for virion-production would follow if the PITs had fixed lifetimes. But the Markov property in our model with one life-stage means that it has an exponential distribution (with more stages, a Gamma). This law has a thick tail; hence so does the virion distribution (which is geometric rather than Poisson; see Part II, Chapter 20, Exercise 4). In this sense, a Markov model even with a unique PIT-type exhibits some EPV. A more-realistic model of the "no EPV" case would be an agent-based model (mentioned in Chapter 4); we could make the PIT lifetime a constant or give it a Weibull distribution with a thinner tail.

In an *in vivo* experiment with barcodes, a mixture of the labeled strains would be injected into an animal. After primary viremia, clones would be derived from blood draws and sequenced. If "small EPV" is rejected, the low-N_e theory would be vindicated, but the biological interpretation would be less clear than for the *in vitro* experiment. The extra variation might be due to a number of factors: ongoing evolution by positive selection could not be ruled out as a source of the bottlenecking; nor, of course, could EPV at the cellular-level. The *in vitro* experiments could however rule out the latter and we would then wish to know the extra-cellular source of the stochastic variation observed in retroviral replication.

11.5 Notes

Many observations suggest that replication rates of HIV in the body are heterogeneous. Sequence analysis has shown that different tissues are infected by genetically-distinct variants [80]. Qe Dang *et al.* [66], found non-random rates in both direct and cell-mediated re-infection, which they explained by heterogeneity in infectibility of target cells. HIV may reproduce in isolated sites with "distant transmission" rates, [117].

[4] It is also clear from comparing, e.g., Figure 11.5 with 11.6, that the cross-overs appear early and are due to demographic stochasticity rather than to "chaotic" dynamics.

That many virions successfully bud from the host cell yet nevertheless prove non-infectious—perhaps 99.9 %: M. Emerman, personal correspondence.

Large inserts have a tendency to be deleted during passages: [43].

Chapter 12

Can a T-Cell Vaccine Block Escape?

Since Edward Jenner's 1796 experiment, now unacceptable to institutional review boards (he inoculated a boy with pus from a cowpox-infected milkmaid's scab, then with smallpox), scientists have tested a plethora of vaccine concepts. Louis Pasteur discovered, somewhat by accident, the first "live-attenuated" vaccine (it was an aging culture of fowl cholera), a weakened preparation of the disease agent that still replicates in the host and generates immunity. After the realization in the 1930s that particles too small to be filtered out or seen under the light microscope—viruses—cause many diseases, the "whole-inactivated" method was developed. The virus is grown in the laboratory and then denatured with chemicals; the resulting non-infectious particles constitute the immunogen. In the 1950s, Jonas Salk applied the method to create a vaccine against polio, which proved efficacious in a large-scale trial sponsored by the March of Dimes. Later, Albert Sabin and collaborators introduced a live-attenuated polio vaccine that for a time replaced Salk's (but the live formulation caused some cases of vaccine-related polio, leading to the resurrection of the whole-killed preparation.)

More recently, new kinds of vaccines have been developed that do not require injecting whole virions and so cannot cause disease. These formulations include protein subunit, "naked DNA", and "vectored" vaccines. The subunit class are made from viral peptides, such as HIV's gp120 (part of its coat protein), destined to be the immunogen in the first large-scale HIV vaccine trial. DNA vaccines, discovered at a biotech startup in 1990, involve inserting

W.D. Wick and O.O. Yang, *War in the Body: The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines*,
DOI 10.1007/978-1-4614-7294-0_12, © Springer Science+Business Media New York 2013

viral sequences into a small piece of bacterial DNA called a plasmid. To much surprise, when this "naked DNA" was injected into control animals it was taken up by many cell-types, which then produced viral antigens. DNA vaccines for diseases including influenza and HIV have since been shown to be safe and immunogenic (but not efficacious) in people as well. The third concept makes use of a "vector", a replication-competent species engineered to eliminate virulence and transport some part of the target-organism's genome into host cells. A great variety of viral vectors have been proposed, including vaccinia (the contemporary smallpox vaccine strain); canary-pox; adenovirus (a cause of the common cold); vesicular stomatitis virus; poliovirus; alpha viruses; Venezuelan encephalitis virus; Semliki forest virus; and measles virus. Bacteria have also been proposed as vaccine vectors, including Salmonella and Shigella (which infect intestinal cells) and even Bacillus anthracis (the agent causing anthrax). Because foreign antigens must be displayed by host cells to garner the interest of lymphocytes, besides the live-attenuated, only the DNA and vectored approaches could yield a T-cell vaccine.

The march toward a vaccine against AIDS began the moment the virus's discovery was announced, in 1984, along two fronts. Robert Gallo at NIH infected chimps with HIV and launched the non-human primate (NHP) arm of the campaign. But the chimps developed few symptoms and frequently cleared the virus, so Gallo soon switched to macaques. In California, Jonas Salk raised 20 million dollars to develop a traditional attenuated vaccine and almost initiated the human program. Another false start occurred 2 years later, when Daniel Zagury of the Université Pierre é Marie Curie in Paris, in a hallowed tradition among physicians, injected himself with a vaccine made from HIV's coat protein (an extended part called gp160).[1] Genentech scientists in San Francisco prevented infection in two chimps with a gp120 vaccine. In Seattle, Shiu-Lok Hu and Lawrence Corey brought the two arms together. Hu, then at the company Oncogen, had inserted gp160 into vaccinia and published in Nature (in 1987) an experiment demonstrating that his vaccine—called HIVAC—could prevent infection in monkeys. Corey agreed to be the principle investigator in a human trial; approved by the FDA in November 1987, the first volunteers were injected in March 1988. Unfortunately, HIVAC only generated anti-HIV antibodies in vaccinia-naïve persons, the antibodies did not neutralize primary isolates of virus, and evidence for

[1]Zagury later injected his vaccine into 18 children in Zaire, with permission from Zairean, but not European, officials, and was criticised on ethical grounds.

stimulation of CTLs was unclear. Bristol Meyers pulled funding for HIVAC and the investigators abandoned it in 1992, ending the first wave of vaccine development in the HIV field.

In the 1990s, investigators showed that live-attenuated SIV preparations can protect macaques from subsequent infection with that strain or even by other, more virulent varieties. Epidemiologists studied commercial sex workers in Kenya and found evidence that frequent exposure to HIV may have a similar protective effect. But most vaccinologists ruled out the live-attenuated or whole-killed approaches for HIV on safety grounds, because of the inevitably fatal outcome of active infection (before the introduction of HAART) and, for the live-attenuated concept, because of the retrovirus's notorious genetic instability.

Enthusiasm for the gp120, antibody-directed vaccine remained strong among many HIV investigators, however. In 1992, the NIH asked 8 institutions to begin planning for a phase-III trial in 6–25,000 people.[2] In 1993, they asked for bids for the statistical coordinating center, which was eventually awarded to the Fred Hutchinson Cancer Research Center in Seattle and dubbed HIVNET.[3] The Division of AIDS (DAIDS) of the National Institute of Allergy and Infectious Diseases (NIAID) of the NIH issued preliminary guidelines for companies or investigators who wished to propose candidate vaccines for the upcoming trials. Although "it is impossible to develop rigid criteria", the guidelines admitted, a "reasonable expectation" of efficacy must be forthcoming. Genentech and Chiron were the presumed product-developers. But doubts remained about that "reasonable" part and DAIDS established a Vaccine Working Group (VWG), composed of government and non-government scientists and community leaders, to advise about the wisdom of conducting an expensive trial. The director of NIAID, Anthony S. Fauci, would make the final decision.

The VWG met on April 21, 1994, at the Dulles Renaissance Hotel near Washington. The desideratum in many people's minds was that the antibodies generated by Genentech's and Chiron's products should neutralize circulating strains. The vaccines had been developed using laboratory-adapted virus; representatives of both companies presented data that their antibodies

[2]The phases of clinical research are labeled I–III; phase I trials are small and address biological response and safety concerns; phase II are larger, often in the range 1K–3K and generate data about biological action and limited information about efficacy; phase III are large licensure trials, with numbers depending on the "attack rate" of the disease.

[3]An author of this book (W.D.W.) entered the HIV field when HIVNET was founded.

neutralized the lab variety—but not other strains. Moreover, Chiron reported three "breakthrough" infections in a phase-I trial in persons at risk of acquiring HIV. Proponents of going ahead with the big trial pointed to data from the chimp model (but many investigators had abandoned it as unreliable). Vaccinologists remarked that knowledge about mechanisms historically postdated a vaccine's introduction in the clinic. Donald Francis of Genentech warned that the biotech companies would abandon the search for an HIV vaccine if the trial was canceled.

On the question of whether NIH should carry on with an immediate, informative trial of some kind, the vote was 27 yes to 2 no; on the phase-III, it was 15 yeas to 13 nays. NIAID scheduled a meeting of its advisory panels for June 17, after which the final decision would be made.

But before the second meeting, a well-known and influential virologist, Bernard N. Fields of Harvard, published an article in Nature critical of the rush to test vaccines in large clinical trials before the essentials of establishing immunity are known.[4] Other biologists agreed. Ashley Haase (who cast one of the two "nay" votes in April; the other was by Corey) told the New York Times that the VWG made "the worst decision I've ever been involved with", and David Baltimore called it "anti-science". Meanwhile, the Chicago Tribune broke the story of the breakthroughs ("at least five"; in fact there were 13) in the phase-I and II trials. Although the article quoted Jack Killen, the director of DAIDS, to the effect that there is "no statistical basis" for alarm, it generated much anyway, especially among activists and in the popular press.

The climactic meeting was held on June 17th, 1994, at the Hyatt in Bethesda, Maryland, near the NIH campus. DAIDS produced estimates that a phase-III trial would require 9,000 volunteers and last 3 1/2 years (but a scaled down test-of-concept trail would require only 2,500 and 2 years; HIVNET had already enrolled several thousand at various sites). The cost would be 9–18 million dollars per year—but Don Francis parried that medical

[4] "The focus on drugs and vaccines made sense a decade ago, but it is time to acknowledge our best hunches have not paid off... we need to put increased emphasis on basic science..." Field's comments reflected the eternal tension between mechanists and empiricists. Several years later, his colleagues Bruce Weninger and Max Essex, prompted by NIH's creation of AVRC headed by David Baltimore (a Nobelist in biology and medicine but not a vaccinologist) replied, again in Nature: "the theorists leisurely pursue new knowledge to build models to explain and perhaps offer elegant solutions. The empiricists apply existing knowledge to seek pragmatic answers..."

care for AIDS victims in the United States was running ten million per day. Some scientists wanted to wait until a vectored vaccine that generated CTL responses was available for a head-to-head trial. Francis pointed out that the death rate from HIV in the US was now a Vietnam War every year. Three gay men rose to object; Act Up NYC called the trial "not only premature but extremely unethical and dangerous", while an HIV-positive man warned of demonstrations in front of trial sites. The final motion considered by the panel read: "The Institute should continue ongoing programs and current trials... as well as development of other vaccine candidates." It passed, 23 yeas to 4 abstentions. Fauci met the press immediately afterward to announce that the government's support for an efficacy trial of a gp120 protein-subunit vaccine was temporarily at an end.

Genentech dropped its gp120 program and Don Francis[5] left to found a new vaccine start-up in San Francisco, eventually called VaxGen. With private investors providing the capital, in 1998 VaxGen launched a phase-III trial of its second-generation gp120 vaccine in 5,000 volunteers in the US, mostly men who have sex with men, and 2,500 in Thailand, mostly drug injectors and including women. The government changed its mind and decided to collaborate, NIAID issuing a press release arguing that, as the new product was bivalent, it covered more HIV strains than the original, monovalent construct—so the decision did not contradict that of June 1994. The company reported the results of the trial on February 23, 2003. The vaccine provided no protection against infection by HIV in either population, although there were some disputed trends towards efficacy in blacks and Asians.[6]

With the failure of VaxGen's gp120 product in the world's first HIV-vaccine licensure trial, and the continuing inability of vaccinologists to elicit broadly-neutralizing antibodies with any other formulation, interest naturally increased in the T-cell concept. Shiu-Lok Hu, Norman Letvin, Harriet Robinson and others had demonstrated that vector vaccines could lower viral loads in macaques challenged by chimeric SIV-HIV constructs called SHIVs. (However, these highly-pathogenic viruses that caused AIDS in a year were attacked as unreflective of HIV dynamics and too-easily neutralized

[5]Francis was furious about the June 17th decision, saying understandably, if hyperbolically, "They had no idea that what they did was kill vaccines."

[6]Statisticians warn against such *post hoc* subgroup analyses, because the trial was not designed with sufficient power to estimate effects in smaller groups.

by antibodies. The vaccines generally failed to protect monkeys when the challenge virus was derived entirely from SIV.) One of the first T-cell directed HIV vaccines to reach phase-II trials in humans was a canarypox construct produced by Pasteur Mérieux Connaught. (The trials were conducted in collaboration with HIVNET.) Unfortunately, the vaccine generated HIV-specific-CTLs in fewer than 30 % of the volunteers who were injected and this was taken as insufficient justification for further trials. (Nevertheless, the canary-pox and VaxGen vaccines would reappear and be tested in a combination; see Chapter 14.)

What are the problems that must be overcome if we place our bets on CTLs? Designers of T-cell vaccines must surmount four obstacles. First is the bar the French canary-pox construct failed to reach: a vaccine must elicit CTL responses in a large fraction of vaccinees, of adequate magnitude to have an impact on infection. Second is the diversity issue that vexed VaxGen's candidate: the responses generated must cover a high proportion of circulating strains. Third, if the vaccine cannot prevent infection, it must lower viral load in vaccinees who become infected (the "breakthroughs"). Finally, granted that abatement of VL, the vaccine must prevent, or at least delay, the virus escaping immune control through mutation.

The problem of generating an adequate response has produced the concept of the "prime-boost" regime. The subject is first injected with DNA containing HIV sequences, then after some weeks or months, given a second injection of a vector also loaded with HIV sequences. The thinking behind this protocol is that DNA, although it can elicit HIV proteins from cells, does not produce the concomitant inflammatory events of a real infection. Inflammation stimulates other immune-system players to signal "danger!" to T-cells and may be essential to getting them to mature and differentiate into functional memory cells. For this reason, vaccine formulations often include an "adjuvant" whose purpose is to produce inflammation at the site of the injection. Consequently, DNA alone is not a potent immunogen; but it "primes" the system to respond to vectored delivery of antigens—the "boost". Unfortunately, the vector will also be recognized as foreign, and if the subject has pre-existing immunity, CTLs might eliminate the infected cells before they can display HIV antigens.

Overcoming genetic diversity in virus and host is a most difficult problem. Since the founding moment of the epidemic, probably mid-20th century, HIV has radiated into a constellation of circulating strains. We have discussed in previous chapters the astonishing rate of accumulation of changes in the

HIV genome, even in a single patient (HIV sequences taken at different time points typically differ by 10 %; by contrast, the change of a single amino-acid in the envelope protein of the influenza virus raises fears of a new pandemic). Introductions and re-introductions of HIV strains in different populations has probably produced what geneticists call "founder effects", as well as allopatric progression (evolutionary change due to a new environment). Multiple main groups, and subgroups called "clades", of HIV have been identified and different groups and clades are prevalent in different continents. For instance, in the main group "M", subgroups A and C predominate in central Africa; A,C, and D in eastern Africa; B in Europe, Australia and the Americas; and C in sub-Saharan Africa and India. The clades themselves are by no means homogeneous; intra-clade diversity can reach 25 % in some proteins. Strains that have resulted from super-infection and recombination ("retroviral sex"; discussed in Chapter 10) called "circulating recombinant forms" (CRFs) exist in many areas. For instance, AE is common in Thailand, AG in western Africa, and BC in China. But the virus is not the only source of diversity. People exhibit a huge variation in HLA types and immune responses to a pathogen.

Many proposals have been put forward as to how to formulate HIV vaccines that can act despite all this pathogen and host diversity. Artificial sequences that aim at consensus or ancestral prototypes have been proposed. A more recent suggestion is to try to focus the immune response on relatively conserved epitopes, in the hope that these sequences will co-exist in many variant viruses. (The conservation issue is also important for escape, discussed below.) One possibility under development is to optimize prime and boost immunogens, in such a way that only these relatively-stable epitopes will be shared between them, otherwise leaving natural sequence features intact. (Simply arranging selected epitopes like beads on a string fails to generate much of a response.)[7]

For the third hurdle, lowering viral load, evidently the vaccine must cure a "defect" in CTLs that explains their inability to suppress natural infection. Here our models can make predictions, albeit with considerable uncertainty as to the nature of the "defect". Let us first make a distinction between repairing a functional or "inherent" defect *vs.* fixing a "cell-population frequency" defect. By an inherent repair, we mean (in model terms) altering a rate parameter, for example the T-cell activation parameter, α_e; the killing

[7] W.D.W. thanks Fusheng Li of the FHCRC for relating these ideas.

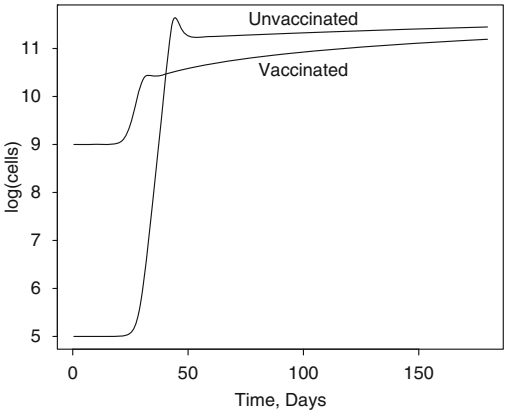

Figure 12.1: Vaccine effect on HIV-specific CD8s at the cell-population level is transient.

parameter, κ_e; or the memory-cell lifetime. By a frequency adjustment, we mean, for example, increasing the number of resting, memory, HIV-specific CD8s. The effect of a frequency shift without altering kinetic parameters is transient, typically disappearing in 6 months. See Figure 12.1, which shows simulated HIV-specific CD8 responses assuming 10^9 memory cells in vaccinees (about 1 % of total CD8s), *vs.* 10^5 naïve precursors in unvaccinated. ("Normal memory" scenario.) Figure 12.2 shows the viral loads in the two cases; not only do the curves coalesce, they cross-over after primary viremia—with the vaccinated actually having slightly higher VL at later times! Note that the vaccine lowers peak viremia.[8] Although seemingly paradoxical, the explanation is apparent from the pair of figures: natural infection allows the virus to reach higher levels, which means more immune stimulation, which in turn generates more viral suppression than in vaccinees.

For one scenario—the "memory-defect" of Chapter 3, in which a pool of short-lived CTLs control HIV—we assumed that a vaccine generated proper, long-lived, HIV-specific memory T-cells and simulated from the model. The steady-state viral load dropped two logs relative to unvaccinated subjects; see Figure 12.3. Other assumptions about the purported CTL-defect and

[8]If the "primary viremia drives the epidemic" theory is true, such a vaccine might provide a benefit, but it would certainly be hard to convince the FDA to license it based on data resembling Figure 12.2.

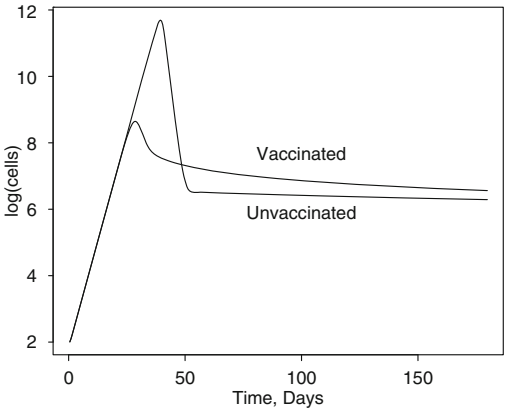

Figure 12.2: Vaccine effect on viral load at the cell-population level is transient. ("Normal memory" scenario.)

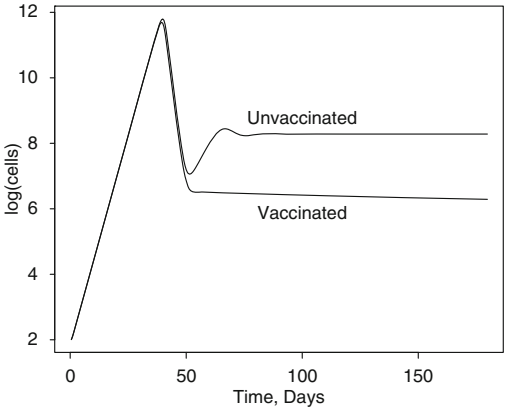

Figure 12.3: "Defective memory" cured: a vaccine drops the VL several logs.

vaccine action can generate similar figures. In one theory of the origin of CTL memory, pathogen-specific CD4+ "helper" cells are required to induce CD8 activation and maturation; we can therefore speculate that a successful T-cell vaccine will have to induce CD4 responses as well.

Concerning the last obstacle—overcoming escape—we can make some definite predictions. First, if only one or two epitopes are recognized and

any viable escape mutations exist, immune control will quickly be lost. For example, if $E = 1$, even if $\text{LOF}_m = 0.9$, meaning 90 % loss of fitness due to the mutation, in our simulations without EPV the virus typically escapes in 3 months. (As we saw in Chapter 5, extra-Poisson variation in replication can delay escape.) The analysis of Chapter 7 suggests that three or more epitopes recognized after initial escapes, and negative ESAs in the Escape Formula, is the proper design goal. Thus the situation is similar to prior experience with drugs: three is the critical number; any fewer and the virus quickly escapes.

Immunodominance, discussed in Section 8.1, and "original antigenic sin" can limit the CTL response to a few epitopes. (The latter refers to a tendency of the immune system to respond to previously-recognized antigens which cross-react with those delivered in a vaccine.) As we noted in Section 7.1, broad is better, and any good ideas for broadening response should be pursued. But the issue is not solely one of numbers.

Perhaps we should try to maximize something like immunogenicity-times-breadth? As we learned in Chapters 6 and 7, even with a broad response the virus will quickly lose epitopes with small LOF in the corresponding mutation, the more-immunogenic going first. Nevertheless, the remaining ones might establish a persistent state of immune control. As noted in Section 7.2, whether a stable situation will eventually be established or not is a complex question. Thus, one danger in vaccine discovery is that a product might be judged promising because it generates many, highly-immunogenic, responses—but, unknown to the designers, the control is vulnerable to a cascade of escapes. One approach to avoiding this defeat is to develop laboratory routines for measuring parameters that appear in the Escape Formula. With these parameters in hand, we can exploit the iterative algorithm of Section 7.2 or simulation from the model to make a prediction about stability. This research program would require considerable investment in laboratory time and resources. CTL responses will have to be assessed in volunteers; CTL clones derived; virus propagated in the presence of CTLs; and fitness screens conducted on any escape-variants that emerge. The effort is large, but it should be compared to the time-span and cost of carrying out a definitive vaccine trial (perhaps 4 years and 200 million dollars, plus the investment by the trial's participating subjects).

Why not inject whole HIV proteins, or a "shotgun" mix of epitopes, and let nature select the "best" ones? Here the likely pitfall involves immunogenicity *vs.* stability. Unfortunately, there is little evidence, and no

biologically-plausible theory, that suggests they are associated. One might even speculate that evolution in the retroviridae to evade host immunity might have resulted in a negative correlation: highly-immunogenic epitopes might be less stable than weakly-immunogenic. Indeed, from the virus's perspective, ideal would be if each epitope the host recognizes as foreign can be replaced by another at little cost. Thus the risk is that, due to prior evolution and immunodominance, the CTL response might concentrate on a few epitopes which are too unstable to be of any value for establishing control.

Perhaps vaccine-developers should target only relatively-conserved, dominant epitopes? But, besides the question of whether any exist, as we saw in Section 8.1 the subdominant epitopes should not be slighted—because it is the combined pressure of dominant and subdominant CTLs that restrains escape. The subdominant need not be as conserved as the dominant, but without them the immunodominant epitope would have to be literally immutable to prevent escape. What about ignoring the question of the dominance hierarchy altogether and aiming solely for stability? But ignoring functionality may lead to a vaccine response too weak to lower viral load or ameliorate disease.

Can we hope to "cure" immunodominance? The inherent-*vs.*-frequency distinction in "repairing" CTLs has negative implications for some schemes to reduce immunodominance. As we write, the only proposal that has been tested is to deliver HIV genes in separate plasmids. This approach is based on the intracellular-competition hypothesis, which states that immunodominance is due to competitive exclusion within antigen-presenting cells (APCs); the idea is to get different epitopes into different APCs and thus overcome the competition. Although this technique has proven ability to increase response to the vaccine, it falls into the cell-frequency-enhancement category. If a vaccine cannot prevent infection, when a subject "breaks through" and becomes infected, APCs will absorb whole viral genomes and the competitive-exclusion mechanism will presumably be at work again.

By contrast, a method to selectively increase immunogenicities in certain epitopes, say by injecting them separately together with adjuvants, would fall in the inherent category if the change was stable (i.e., due to maturation effects in the T-cells). In summary, our modeling experience suggests a plausible alternative to maximizing breadth-times-response, injecting whole proteins, or shotgunning: identify the critical responses that can establish immune control and selectively enhance at least three of them, by manipulating

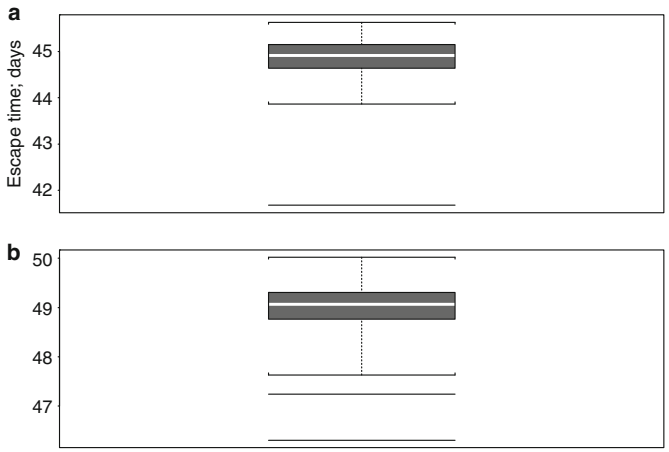

Figure 12.4: Vaccine failure due to escape.

timing and delivery of antigens. This program of whittling-down the list of candidate epitopes will require the same investment in research described above.

Figures 12.4 and 12.5 show four vaccine scenarios with different assumptions about immune parameters and the number of epitopes recognized. The vaccine was assumed to cure a "defective memory", but other hypotheses that dropped the viral load two logs would yield similar figures. Figure 12.4 shows boxplots of escape times made assuming two or three epitopes and parameters just outside the no-replacement domain; these figures demonstrate vaccine failure through escape. In Figure 12.5, made with three epitopes and parameters just inside the stability domain, the scenarios might represent success, depending on the criteria employed. (Left panel: equivalent epitopes, parameters just inside the replacement boundary, as discussed in Section 7.3: $\mathrm{LOF}(1:0) = 0.34$ and $\mathrm{LOF}(2:0) = 0.67$. There were 15 escapes out of 100 runs, in 4 years. Left panel: "Gag immunodominance scenario" of Section 8.1, parameters as in Figure 6.5. There were 3 escapes/100 in 4 years.)

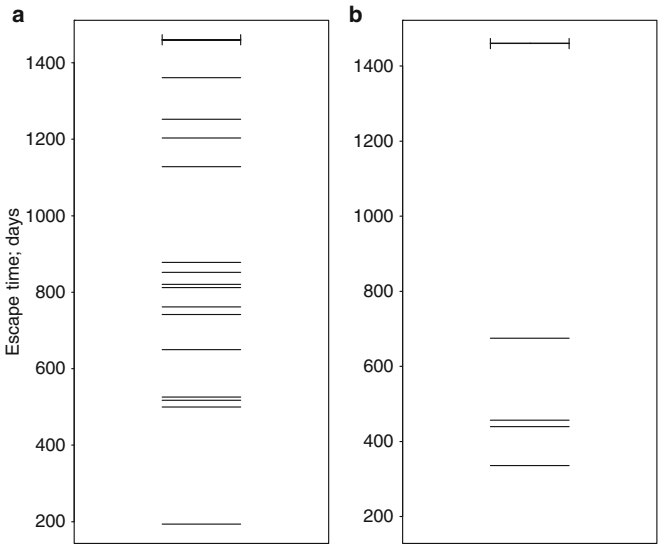

Figure 12.5: A successful vaccine?

12.1 Notes

On polio vaccines: [27].

Patricia Thomas wrote an exciting, if controversial, popular account of the search for an HIV vaccine before 2001, [293]. Academic reviews include: [204].

Gp120 antibodies fail to neutralize primary strains: [195, 214].

The concept of the DNA vaccine was discovered in 1990 at Vical, Inc., and the University of Wisconsin; subsequently Merck Inc. developed a DNA vaccine for influenza in mice. See [292].

Commercial sex workers in Kenya may have been protected by frequent exposure to HIV: [160].

Re HIV vaccine design: valuable opinion, theory, or review articles include [78, 113, 151, 327, 328]. Interesting papers on the NHP model as it relates to vaccines include [10, 17, 123, 136, 284, 295, 322].

Overcoming the antigen diversity problem: [181]. Optimizing prime-boost to focus the response on conserved epitopes: [182].

The peculiar result shown in Figure 12.2 (control of the primary peak but not the chronic VL, independent of escape) may have been observed in experiments with SIVmac239, a virulent form of SIV, in rhesus macaques vaccinated with a prime-boost regime [201, 302].

The role of subdominant epitopes in controlling HIV has been observed in one case [96].

"Original antigenic sin": [170].

The prediction that injecting epitopes at different anatomic sites, different times, or by different modalities might ameliorate immunodominance has been observed in one experiment (in mice, with two SIV epitopes) [186].

Chapter 13

The STEP Trial, Monkey Trials, and the Future of T-Cell Vaccines

On September 21, 2007, the world's first large clinical trial[1] of a T-cell vaccine for HIV/AIDS was terminated prematurely by the Data Safety and Monitoring Board (DSMB). A pre-planned interim analysis had revealed that the vaccine was unlikely to have any efficacy at preventing infection or disease. More startlingly, the DSMB found a hint that the vaccine might have increased the number of infections, at least in a subgroup. The announcement, by HIV Vaccine Trials Network (HVTN) head Lawrence Corey, of the failure of the second vaccine for HIV/AIDS in a clinical trial made news worldwide.

The STEP trial had begun in December 2004 with the enrollment of 1,500 men and women, at high risk of acquiring HIV, in the US, Peru, Brazil, the Caribbean, and Australia. The proposed vaccine, a product of the pharmaceutical company, Merck, was trivalent: an admixture of three vectors each containing inserts from one of three HIV proteins (Gag, Pol, and Nef).[2] The vector was an engineered form of a common human cold virus called "rAd5," short for "recombinant adenovirus, serotype five". The discovery that many trial participants had prior exposure to the serotype-5 strain of adenovirus lead to the decision, in July 2005, to enroll an additional 1,500 subjects

[1] "Phase-IIb": a trial design intended to quickly detect lack of efficacy, rather than ensure licensure.

[2] The sequences chosen were near-consensus clade B and codon-optimized.

W.D. Wick and O.O. Yang, *War in the Body: The Evolutionary Arms Race Between HIV and the Human Immune System and the Implications for Vaccines*,
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with low titers of Ad5 antibody, on the worry that vaccine efficacy would be limited by pre-existing immunity to the vector. The scientific justification for testing this construct came from one experiment showing modest success at lowering viral load (VL) in vaccinated monkeys challenged by one strain of SIV, and preliminary (phase I) studies showing that it generated some T-cell responses in humans. Although protection from infection by T-cells was controversial, such an impact (or *via* some other immunological mechanism) could not be ruled out. Therefore, the trial would have an unusual dual-endpoint design: efficacy might be demonstrated either by the vaccine preventing some infections, or by decreased VL in those vaccinees that became infected ("breakthroughs"). The trial was to be conducted double-blinded, with subjects randomized to receive the vaccine (three injections) or a placebo (ditto, of a saline solution), with data about who received what and infection status available only to the DSMB before the trial's end. Statisticians calculated that reasonable efficacy could be demonstrated at the point that 100 infections had occurred among vaccine and placebo recipients.

Consistent with the goal of this kind of trial (quick assessment of futility), the interim analysis was set at an accumulation of 30 events. Two cohorts were analyzed, differing in their adherence to trial requirements: PP ("per protocol": meaning those who accepted all shots and attended all blood-draws at clinics) and MITT ("modified intent-to-treat", meaning all who received at least one injection and attended some follow-ups). The results were as follows ("VE" stands for "vaccine efficacy," see Notes):

Infections at the Interim Analysis[3]

Group	Assignment	No. infections	P-value (test)
MITT	V	24	0.74 ($VE_{inf.} > 0$)
	P	21	
PP	V	19	0.94 ($VE_{inf.} > 0$)
	P	11	

[3]Among 1,850 men and 1,150 women enrolled meeting either protocol requirements.

In the scientific community, a comparison is regarded as significant only if the probability it might have occurred by chance (assuming the "null hypothesis;" i.e., that the vaccine was the equivalent of sterile water) is less than .05 (the famous "five percent rule").[4] Thus significance would follow only if the last column contained an entry $< .05$; there was no efficacy to prevent infection.

The viral load results were:

Viral loads at the Interim Analysis

Group	P-value (test)
MITT	$0.66(\mathrm{VE}_{\mathrm{VL}} > 0\ )$
PP	$0.53(\mathrm{VE}_{\mathrm{VL}} > 0\ )$

there was no indication of efficacy here either. The DSMB also calculated that the probability an efficacy boundary for either endpoint would be crossed when 100 events had accumulated was low. No doubt the increased number of infections in the vaccine groups (not statistically significant; statisticians use the language "indication of a trend" in this situation) was also on their minds as they voted to halt the trial.

Later, SCHARP[5] statisticians performed secondary analyses which did find statistically significant increases in infection rate in two subgroups. One analysis compared infection rates (in vaccinees vs. placebos) between groups with low *vs.* high Ad5 titers; another analysis compared rates for men who were circumcised *vs.* those who were not. The results, expressed as relative-risks with 95-percent confidence intervals, are as follows:

[4]It is due to an historical accident dating to the 1920s: two premier statisticians fought over publication of statistical tables, which then were prepared by "computers" (usually retired algebra teachers) and consequently very expensive. R. A. Fisher, inventor of the p-value, could only acquire the .1 and .05 tables; hence the origin of the "5-percent rule" that permits publication in journals.

[5]The Statistical Center for HIV/AIDS Research and Prevention, located at the Fred Hutchinson Cancer Research Center in Seattle; the statistical analysis arm of the HVTN, at which an author worked from 1996–2011.

Subgroup Analyses

Ad5 titer ≤ 18	Ad5 titer > 18	Circum.	Uncircum.
1.0	2.4	1.0	3.8
(0.5,1.8)	(1.2,4.7)	(0.6,1.7)	(1.5,9.3)

Many theories have been elaborated to explain these unexpected findings. Investigators have searched for evidence of increased T-cell activation in the high-Ad5 group that might explain the increased infection rates, without success. That circumcision is protective against HIV infection in men had been established by several epidemiological studies in Africa, but why it should interact with vaccine status to alter infections rates is mysterious. Nor can we rule out the possibility that all these subgroup results are spurious, due to the low numbers of events and chance—despite the formal confidence intervals excluding one. (The statistical methodology assumes that people's outcomes are like independent coin-flips, which may be violated in some unknown way.)

The failure of the Merck vaccine, and the suggestion that the vaccine might actually have caused harm, had immediate and severe impact on other ongoing or planned trials. Enrollment in a trial in South Africa called "Phambili," of a prime-boost, DNA/AD5 vaccine designed by the Vaccine Research Center (VRC) of the NIH was halted and the subjects unblinded, so that all the participants could be told of a possible risk from the vaccine and take suitable precautions if desired. (Follow-up of these participants is being pursued.) Besides the prime-boost vaccine, the trial would have tested the T-cell concept where a different clade of HIV predominates (clade C primarily circulates in Southern Africa). A large trial (PAVE 100; 8,500 planned participants) of a VRC, rAd5 multiclade vaccine was canceled after an NIH "summit meeting" in March 2008, where Anthony Fauci (director of NIAID) called for proof that an rAd5 vaccine could actually lower viral loads before committing further funds to an efficacy trial.

Reminiscent of the recriminations after the failure of VaxGen's gp-120, antibody-based vaccine, a chorus of laboratory scientists lambasted the empirically-minded vaccinologists for conducting the trial. At the March

meeting, a well-known virologist, Ron Desrosiers, accused NIH of "trying to do what Parma is supposed to do" (i.e., design vaccines), while pharmaceutical companies are not pursuing it "because they don't think it is feasible". Other were more blunt; e.g., Robert Gallo called STEP the "Challenger disaster" of vaccine research.

After STEP, how should we regard the T-cell vaccine concept? Is it now entirely implausible? However, there are several reasons to doubt such a conclusion. First, at the outset the design goal of STEP was flawed. Vaccine evaluation was based on a single assay: the interferon gamma enzyme-linked immuno-spot (ELISpot) using excess small peptides to label target cells for CTL screening. (See Figure 13.1. Levels of epitope copies per cell surface are higher for both vaccine-transduced and exogenously peptide-loaded cells for ELISpot, compared to HIV-infected cells. The horizontal dotted lines represent hypothetical avidity thresholds of epitope presentation for killing of infected cells. The red dotted line represents a CTL response raised against HIV infection, which by definition has sufficient avidity to recognize an infected cell. The blue dotted line represents a hypothetical CTL response raised against the rAd5 vaccine, which has sufficient avidity for ELISpot but insufficient avidity to recognize an HIV-infected cell.) It was assumed that because ELISpot measures CTLs that help suppress viremia in HIV-infected persons, that CTLs raised by a vaccine detected by this assay would help suppress viremia—or, put into logical terms, that A implies B is equivalent to B implies A. The caveat is that the assay did not detect the ability of CTLs to recognize epitopes processed and presented on HIV-infected cells, which are found at lower levels. The rAd5 vaccine was designed to maximize ELISpot responses by maximizing expression of HIV proteins to elicit CTLs (using codon-optimized versions of HIV genes driven by a high-expressing promoter). Thus rAd5 expression of HIV proteins would be expected to exceed that of HIV-infected cells, and CTL responses raised against rAd5 could have insufficient affinity to recognize infected cells while giving detectable ELISpot responses (Figure 13.1).

A second and perhaps more important caveat is that the vaccine design did not address the failure of CTLs in natural infection, and therefore mimicked a failing process. As we have emphasized in previous chapters, a central problem for CTL containment of HIV is epitope sequence mutation and escape from immune control. The rAd5 vaccine contained genetic sequences coding for near-consensus HIV proteins, which does not cope with the genetic diversity of HIV either inter- or intra-host. An analysis of HIV sequences in

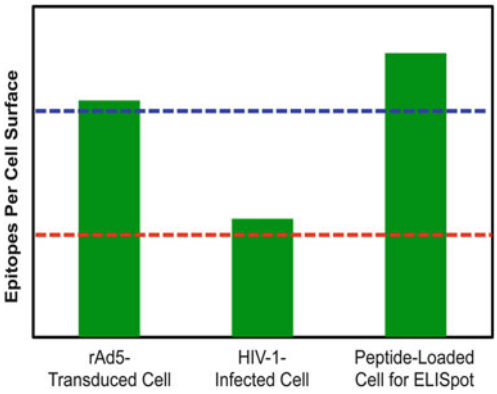

Figure 13.1: Misinterpretation of vaccine-induced CTL responses by exogenous peptide-based assays.

vaccinees who later got infected with HIV ("breakthroughs") demonstrated evidence of selection due to vaccine-induced CTLs. That the infected persons had no discernable impact of vaccination on immune control of HIV indicates that the virus easily side-stepped vaccine-induced CTLs with no consequence.

From STEP data, an HVTN scientist calculated an additional, and unfortunate, statistic about the Merck vaccine in humans: the average number of epitopes recognized by CTLs in vaccinees. It was one. Even more unfortunate was that the epitopes recognized were highly variable, as indicated by a study of HIV sequence data bases.[6] The reader will recall that, where escape from CTLs is concerned, three is the minimum number that gives us any confidence of successful containment of the virus (although it would be prudent to generate more, say 8–12, because of early escapes, see Chapter 7). From the point of view of the theory elaborated in this book, the Merck T-cell vaccine never stood a chance.

On November 20, 2008, Nancy A. Wilson gave a fascinating and important presentation at the semiannual HVTN full-group meeting in Seattle. Dr. Wilson presented data from a study in the laboratory of David Watkins, in Madison, Wisconsin, of an rAd5 vaccine against SIV in macaques. The monkeys were vaccinated with all SIV proteins, except envelope, from one

[6]The authors thank Fusheng Li of the HVTN for these observations.

strain in rAd5 vectors, then challenged with a "swarm virus."[7] This heterologous challenge is meant to imitate the situation in human populations, where diverse strains circulate; as an added dose of realism, the challenge route was mucosal and low-dose. All control monkeys became infected, with high peak and set-point viral loads (more than four million copies per ml). But the vaccinated macaques had lower loads—12,600 on average—and, in two of five vaccinated monkeys, the virus peaked at less than 400 copies per ml and then went extinct. Because the investigators omitted Env, no antibodies against SIV were presumably generated by vaccination, so the CTLs must have done the job.[8] It may only be one experiment, in 11 monkeys, but without doubt it validates the theory of T-cell vaccines against retroviruses.

By contrast to STEP, the number of epitopes recognized by the macaques in Wilson's study was in the range 11–34. At the conclusion of the meeting, Corey lauded it as a "wonderful experiment" that raised the crucial question: why did a product equivalent to Merck's generate all those responses in Asian monkeys, but not in humans? A common response is to remark that macaques have more HLA class-I genes and so can display more epitopes. While it is true that macaques have ten HLA-I type B genes to our two, does it automatically follow that a macaque makes five times the immune response as a human? Gene duplication is common in mammalian evolution; indeed, it is considered a primary mechanism of genetic variation.[9] Perhaps the responses made by the monkeys in the Wilson study had nothing to do with genome size, and the larger number was due to design issues (such as the length of protein sequences inserted in the vector).

A more recent study (2012) from Watkin's lab showed, somewhat contrary to the breadth thesis, that CTL responses to a few, carefully selected, SIV epitopes could sustain control in macaques. A similar phenomenon is known with the Epstein-Barr Virus: a single CTL clone is capable of suppressing it, which at least provides a precedent for control by a one-epitope response. On the other hand, EBV is a huge, double-stranded DNA virus with 85 genes and probably less susceptible to sequence variation and escape than HIV.

[7] Strain E660.

[8] This may be regarded as fulfilling a model-based prediction by an author and colleagues: namely, that T-cells alone may be able to halt a retrovirus infection in its tracks. See [316].

[9] The theory is that, while it may be difficult to vary an essential gene without losing viability, once the gene is duplicated the spare one is free for evolutionary experiments.

(Viruses with DNA genomes are generally stabler than retroviruses, as they do not rely on a sloppy RT to reproduce.) So the issue is whether HIV can be targeted in such a way that it has no escape options—but it would be a risky strategy.

Another question should be investigated: should we have used a human cold virus at all, as the vector in STEP? Humans and adenoviruses may have been co-evolving for millennia. Perhaps humans have evolved restriction factors, other than antibody from prior exposure, affecting the ability of Ad5 to elicit antigen display from human cells. Such restriction factors, often intracellular mechanisms that degrade viral genomes or diminish protein expression, have been discovered for many other viruses (including HIV). Another vector—perhaps derived from a monkey cold virus?—might be more antigenic if it appears more "foreign" to the human immune system.

Recall Robert Gallo invoking the Challenger disaster. If one desires an astronautical analogy, perhaps a better one might be the early attempts to launch the Atlas rocket that later propelled John Glenn into orbit. The first four exploded above the launching pad.[10] But the United States did not give up on the concept of space travel. In fact, von Braun and his team were just one extra flange, or one retightened bolt, from putting a man into space. Might we be one extra adjuvant, or one rerouted injection, or one tweak of a vector, from a successful T-cell vaccine against HIV/AIDS?

13.1 Notes

For the influence of the vaccine on breakthrough strains: [264]. Pre-trial criticisms (and negative predictions) about STEP: [327, 328]. On August 22th, 2007 (1 month before STEP reported), an author gave an invited hour talk to the AIDS Vaccine 2007 meeting in Seattle [329], during which he discussed the problem with ELISpot and included this remark on a final slide:

> If current vaccine trials fail to demonstrate clinical benefit despite "immunogenicity," it may be premature to discount a CTL-based vaccine approach based on lack of protection in vaccine "responders."

[10] The spectacular footage was included in the movie "The Right Stuff"; the actors, playing would-be astronauts hoping to ride the rocket into space, provide the appropriate reactions.

Chapter 14

Afterword: The RV144 Trial and the Current Status of HIV Vaccine Research

The long history of vaccine development, starting with observations about cowpox and smallpox, has been mainly empirical. The two biggest pioneers of modern vaccines, Salk and Sabin, were proponents of two competing strategies: kill or weaken the pathogen, and mimic infection without disease. These approaches, with modifications such as recombinant subunits in place of whole killed virus, have carried our research for decades and given us dozens of successful vaccines, without impetus to understand the mechanisms of protection.

HIV vaccine development using this empirical philosophy, however, has been stymied. It should have been obvious that conventional approaches would fail. To date, all successful vaccines have targeted infections where protective immunity is commonly generated in response to actual infection among survivors. The criterion for success has been to mimic infection and allow the typical immune response to develop. Applying this bar to HIV infection, it is obvious that the vast majority of infected persons never control infection. Thus using conventional approaches, whether whole killed HIV or recombinant viruses expressing HIV proteins to mimic HIV infection, the process being mimicked is one that fails. (See Figure 14.1. Top: For other infectious organisms, upon initial infection the pathogen (red) initially rises rapidly unopposed by adaptive immunity (blue), but is

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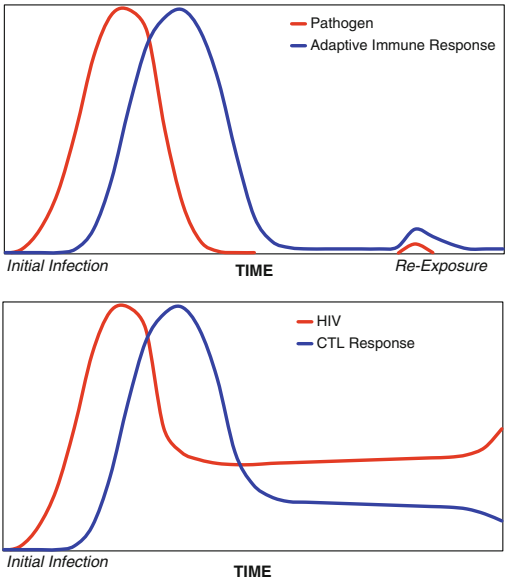

Figure 14.1: Schematic of protective adaptive immunity versus immune responses against HIV.

eventually eliminated. The adaptive immune response develops "memory," and subsequent re-challenge with the same pathogen is rapidly controlled. Bottom: In contrast, HIV infection is marked by failure to control, with the development of a chronic persisting infection that is only partially suppressed by CTLs, which eventually fail.)

How then should a vaccine work? All vaccines work through immunologic "memory," and thus involve either or both of the two major arms of adaptive immunity: B cells and T cells. Although antibody responses (generated by B cells) are excellent markers of efficacy for most existing vaccines and may be the dominant effector of protection for some, it is highly likely that T cells have a key role in many as well. The CD4+ ("helper") T-cell arm is crucial for the support of B cell responses as well as CD8+ CTL responses. Because several vaccines are efficacious in live-attenuated form (e.g. vaccines for Varicella-Zoster, Measles Virus, and others), this suggests that viral replication is crucial for protective immunity, which in turn suggests that the HLA class I pathway and CTLs are involved. Additional circumstantial evidence

is that vaccines available as both killed and live attenuated forms tend to show somewhat better protection for the latter (e.g. Influenza Virus and Poliovirus vaccines).

Given that immunologic memory is mediated by the B and T cells that comprise adaptive immunity, which is more important for HIV infection? Virtually all infected persons develop both types of responses. However, it is clear that antibodies are ineffective at preventing or containing infection. Highly exposed yet uninfected persons tend not to have antibody responses against HIV, indicating that B cells are not protecting them from infection. In chronic infection, most antibodies are rapidly escaped by mutation of envelope (the most variable of all HIV proteins), and neutralizing antibodies are rare and tend to arise late in infection (and without apparent benefit to the patient).

By contrast, plentiful evidence suggests that CTLs play a key role in partial immune control of HIV-1 infection. Several studies have shown HIV-specific CTLs in highly exposed yet uninfected persons, although this remains controversial. What is more clear is that CTLs bring down viremia from its peak during acute infection, and keep it partially suppressed for months to decades during the asymptomatic phase of infection. The single most powerful host genetic factor determining disease progression rate is the HLA class I locus, and HIV sequences in each infected person show that CTLs apply heavy selective pressure, forcing viral adaptation. It thus seems likely that a successful HIV vaccine to prevent infection or disease will require CTLs. Unfortunately, after the failure of the rAd5 vaccine in the STEP trial, many researchers interpreted the result to indicate that CTLs have no useful role for a vaccine.

Most recently, the HIV vaccine research field has redirected its focus after the RV144 trial in Thailand that purported to show 31% efficacy for protection against HIV infection (announced September 24, 2009). This $105,000,000 study, designed and conducted by the United States military collaborating with the Thai Ministry of Health, has been touted as proof-of-concept that a vaccine can be effective and became a major focus for many vaccine research efforts, including that of the Center for HIV/AIDS Vaccine Immunology (CHAVI), a consortium that has received well over $300,000,000 since 2006. Notably, this vaccine was poorly immunogenic for neutralizing antibodies and for CTLs (the latter even if measured by ELISpot, a doubtful criterion we discussed in the last chapter). The putative success shifted the focus of researchers away from these “conventional” immune responses

towards non-neutralizing antibodies, which almost by default (because this was the only HIV-specific vaccine response consistently detectable) has been assumed to mediate protection.

But does this trial really reveal a new "outside the box" mechanism of immune protection that yields insights for HIV vaccine design? On the surface, the abstract of the report (which was first disseminated by press release before formal publication) seems convincing: a 31% protection rate in a study that included 16,402 participants evenly divided into vaccine versus placebo recipients. The abstract specifies that protection is supported by p-value of 0.04 for one of three pre-determined analyses, the other two yielding p values of 0.08 and 0.16. Missing from the abstract are the raw numbers for the three analyses: 50/6,002 versus 36/5,874 (placebo versus vaccine infection rates, "per protocol"), 76/7,325 versus 56/7,747 "intent to treat"), and 50/6,002 versus 36/5,874 ("modified intent to treat"). The 0.04 p-value was for the modified intent to treat analysis, and the 31% reduction of infection (95% confidence level of 1–51%; in fact, vaccine trials are typically powered so that a 30% efficacy means that the lower confidence interval touches or goes below zero) was calculated from infection rates of 1.01% versus 0.69%; these numbers are less than confidence-inspiring.

A crucial consideration neglected in discussions about these results is Bayes Theorem, which describes the influence of pre-test probability that a hypothesis is correct on the probability that a result of an imperfect test is meaningful if positive. In essence, the less likely the hypothesis being tested to be true, the less likely that a positive test result is correct. (Indeed, in an important article from 2005 [141], J.P.A. Ioannidis showed that, in a field that tests too many false hypotheses, a kind of scientific meltdown can occur in which the probability that a reported correlation is true drops below 0.5. In other words, you would learn as much from reading scientific reports as you would by flipping a coin for each hypothesis: "Is it an AIDS vaccine? Heads says it is ....") What then was the pre-test probability that the vaccine tested in RV144 would be protective, in relationship to the "positive" result of 31% protection by borderline statistics? The vaccine was a combination of two other previously tested vaccines, a recombinant canarypox vector and a recombinant HIV envelope subunit (as in VaxGen's gp-120 vaccine). These two vaccines were designed to elicit CTL and neutralizing antibody responses respectively. When tested individually, each failed unambiguously to accomplish its design goal. As the RV144 study was being planned, a

group of leading scientists published a letter in Science [46] questioning the rationale for testing a combination of these two vaccines, writing:

> For a phase-III trial to be justifiable, there should be a reasonable prospect that the vaccine will benefit the study population, i.e., that it will protect at least some of the participants from HIV infection or its consequences.

Clearly these scientists considered the pretest probability of the hypothesis to be dismal. An apt analogy would be that of designing airplane wings: if two wing designs failed to show any lift when tested individually, it would make little sense to combine these two wings on an airplane and test its capability to fly.

Unfortunately, a combination of failure of conventional vaccine approaches, and a lack of consideration of caveats about the rAd5 vaccines "successful" generation of HIV-specific CTL responses that failed to protect, has provoked a sense of desperation. This has been further compounded by the results of the RV144 trial, channeling research efforts to non-neutralizing antibodies (regarded as "outside the box" thinking). However, it is clear that there has been insufficient attention to the tools inside the box. To date, not a single vaccine tested in humans was designed with a strategy to cope with HIV escape from CTLs. Given the large body of evidence that CTLs mediate protection against other viral infections and the success of CTLs in containing HIV as a chronically suppressed asymptomatic infection in rare persons, it is not so much of whether a CTL-based vaccine could have efficacy, but more a question of how to achieve this goal through manipulation of the CTL response.

Part II

The Mathematics

Chapter 15

Short Tutorial on Dynamical Models

A *dynamical model* is a mathematical description of a number of *dependent variables*, which in biology are non-negative quantities describing populations of molecules, viruses, cells, or organisms, and a single *independent variable*, namely time. A model is *deterministic* if the dependent variables are fixed by initial conditions at all later times, and otherwise is *stochastic*. In some models, time is assumed discrete; these models are sometimes called "cellular automatons". If time is assumed continuous, deterministic models are defined by ODEs; the other kind, called "stochastic processes", have a more complicated description.

A *(time-homogeneous) ordinary-differential equation (ODE) system* is a family of rate-equations of form:

$$dx_i/dt \;=\; f_i(x_1, x_2, \ldots, x_n); \qquad i = 1, \ldots n. \tag{15.1}$$

In vector notation, the same system is often denoted more simply by:

$$dX/dt \;=\; F(X), \tag{15.2}$$

where capital letters denote n-vectors; e.g., $X = (x_1, x_2, \ldots, x_n)$. The x_i are the dependent (aka, in biology, compartmental) variables and t, for time, is the independent variable. The phrase "time-homogeneous" refers to the exclusion of time as a separate variable in the function $F(\,)$. This function is often described as defining a "vector field" (usage as in "poppy field" for

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a field of poppies), because it can be thought of as defining a vector, with a direction and length establishing a speed, at each point in the "phase space" or "state space" (allowed configurations of the x_i, usually in biology the non-negative n-tuples of real numbers). An "initial value problem" (IVP) for an ODE system is set by prescribing an initial condition at some time, usually time 0:

$$X(0) \;=\; X_0. \tag{15.3}$$

"Solving the IVP" means finding a smooth curve $X = G(t)$ such that $G(0) = X_0$ and

$$dG(t)/dt \;=\; F(G(t)) \tag{15.4}$$

for all times t.

A *(time-homogeneous) continuous-time, Markov jump process* is a random process $X(t) = (x_1(t), x_2(t), \ldots, x_n(t))$ with piecewise constant sample paths (often assumed right-continuous in order to have them defined for all times) satisfying the Markov property and

$$P\,[\,X(t+h) = X + Y_j \,|\, X(t) = X\,] \;=\; r_j(X)\,h + \mathrm{o}(h), \qquad j = 1, \ldots, J, \tag{15.5}$$

where Y_j denotes an allowed jump-increment and $r_j(X)$ is the corresponding rate, given that the process is at X at time t. The expression "$P\,[\,A \,|\, B\,]$" is read "the probability of event A, given (conditional on) event B", and the symbol "$\mathrm{o}(h)$" means a quantity of higher order in h (i.e., $\lim_{h\to 0} \mathrm{o}(h)/h = 0$). The Markov property means that

$$\begin{aligned} &P\,[\,X(t+s) = X_f \,|\, X(t) = X_p,\, X(t_1) = X_1,\, X(t_2) = X_2, \ldots,\,] \;= \\ &P\,[\,X(t+s) = X_f \,|\, X(t) = X_p\,], \end{aligned} \tag{15.6}$$

for any vectors X_f (future value), X_p (present value), X_k, $k = 1, 2, \ldots$ (past values), and times $t > t_1 > t_2 > \ldots$ and $s > 0$. Again, the phrase "time-homogeneous" refers to the non-appearance of time as a separate argument in the rate functions. An IVP for a jump-process means finding a probability law on the space of trajectories (usually taken to be piecewise constant, right-continuous, with only finitely may jumps in any interval of time) satisfying the Markov property, (15.5) for all times, and

$$P\,[\,X(0) = X_0\,] = 1. \tag{15.7}$$

Kolmogorov's, aka Feller's "forward", equation reads:

$$d\mathcal{E}\, H(X(t))/dt \;=\; \sum_{j=1}^{J} \mathcal{E}\, \{\, r_j(X(t))\, [\, H(X(t)+Y_j) - H(X(t))\,]\,\}, \quad (15.8)$$

where $\mathcal{E}$ denotes mathematical expectation and $H(\,)$ is any bounded, continuous function of n variables. It is a direct consequence of (15.5).

If the state-space is countable, say equals $\{\, X_k \,\}$ for $k = 1, 2, \ldots$, the so-called *master equation* reads:

$$\frac{dp_k}{dt} \;=\; \sum_j r_{j,k}\, p_j \;-\; \left(\sum_j r_{k,j} \right) p_k, \quad (15.9)$$

where $p_k(t) = P[\, X(t) = X_k\,]$ and $r_{j,k}$ is the rate of jumping from X_j to X_k, when the state is the former. It also follows directly from the definition of a jump process.

15.1 Exercises

1. Assume that all first partial derivatives of $F(X)$ are bounded and prove that the IVP for the ODE system has a unique solution.

2. Solve explicitly ("in the 19-th century sense"; i.e., by a formula) the *logistic growth model*:

$$dx/dt \;=\; a\,x - b\,x^2. \quad (15.10)$$

Show that this model with $a > 0$ and $b > 0$ has a *stable steady-state*: the solution for all positive initial conditions tends to $x_{\text{S.S.}} = a/b$. (Hint: separate variables and integrate. Recall, or look up in a calculus text, the method of "partial fractions".) Thus any perturbation of the steady-state will generate a trajectory that returns to it; hence the modifier "stable". By contrast, the origin in this model is an example of an *unstable steady-state*. This exercise is continued by Exercise 20 of Chapter 19.

3. In biology, compartment variables usually describe populations of entities (animals, cells, viruses,...) and so are restricted to non-negative real numbers. Hence, only certain ODE systems can be entertained. These systems are called "Lotka-Volterra type", after the founders of the field. Derive a general form of a Lotka-Volterra ODE model with two compartments that might describe a predator species consuming a prey species. Assume the predator population grows only if the predators eat prey, and the prey population grows except for being eaten. Solve it by any method (numerical or analytical) and show that the solution can display oscillations. (The 2-D Lotka-Volterra model has been reduced to a quadrature; see Notes.)

4. Again in biology, jump-process models describe discrete populations and so only certain models can be entertained. Derive suitable restrictions on the jumps and rate functions for use in biology. Consider a 1-compartment linear model describing births and deaths in some population:

Jump	*Rate*
$X \longrightarrow X + 1$	$\gamma\, X$;
$X \longrightarrow X - 1$	$\delta\, X\, 1[X > 0]$,

(1[ ] denotes indicator function; 1 if the condition is satisfied; otherwise 0.) Solve the model in the sense of determining the probability distribution of population size at each time. One approach to this problem is to employ the *moment-generating function* (MGF), aka *Laplace transform*:

$$\phi(\xi, t) \equiv \mathcal{E}\, e^{-\xi\, X(t)} \tag{15.11}$$

where ξ is a positive variable. How does the MGF generate the *moments* $\mathcal{E}\, X^k(t)$ of the time-dependent random variable? Using Kolmogorov's equation, derive a linear *partial-differential equation* (PDE) for ϕ in the two variables ξ and t, and solve it. (Do NOT attempt to solve a non-linear, 2-compartment system in this sense, as the resulting PDE is intractable. Thus, rather surprisingly, the stochastic analog of the Lotka-Volterra model is unsolvable, except by simulation.)

5. Assume that the rates in a jump process are linear in the compartmental variables, i.e., $r_j(X) = \sum_k \alpha_{j,k}\, x_k$. Using Kolmogorov's formula, derive an ODE system governing the means: $\text{mean}_i \equiv \mathcal{E}\, x_i$ and variances: $\text{var}_i \equiv \mathcal{E}\, x_i^2 - (\text{mean}_i)^2$. Suppose some rate is quadratic (e.g., $r_1 = \beta_1\, x_1\, x_2$, representing an interaction); does such a (finite) ODE system still exist?

6. Continuing from Exercise 5, investigate the question of how large the *fluctuations* are compared to the means. What are the root-mean-square variations, aka standard deviations, of the $x_i(t)$ relative to the magnitudes? This comparison implies a *Law of Large Numbers*, justifying use of the ODE approximation if all compartment variables are large. The simplest case of the *Central Limit Theorem* for stochastic processes also appears in this example. The LLN and the CLT hold more generally, e.g., for non-linear models, depending on the issue of orbital stability; one does not expect the CLT to hold for stochastic versions of so-called "chaotic" models with bounded but unstable orbits, except for short times. (In these models, the dynamics will likely expand the *demographic noise* exponentially. See also Exercise 3, Chapter 19.)

7. Demonstrate from the definition of the jump process that we can strengthen (15.5) to read:

$$P\,[\,X(t+h) = X + Y_j \,|\, X(t) = X\,] \;=\; \left\{1 - \exp\left(-\sum_{k=1}^{J} r_k(X)\, h\right)\right\} \times \frac{r_j(X)}{\sum_{k=1}^{J} r_k(X)} \;+\; \mathrm{o}(h). \tag{15.12}$$

This exercise is continued in Exercise 23 of Chapter 19.

Chapter 16

Deterministic Modeling: A Cautionary Example

In this chapter we consider the simplest deterministic (ODE) model of population dynamics with mutation at several loci. We are interested in the scenario discussed in Chapter 1, Section 1.11, in which single-mutant variants have a fitness loss but double-mutants a gain (due to "escape"). Let $N = X_{\mathrm{AB}}$ be the constant number of wild-type viruses, X_{aB} the variants with mutation at the first locus, and X_{Ab} the variants with mutation at the second locus. Let X_{ab} denote the double-mutant. With time measured in generations, the rate equations are:

$$\begin{aligned} dX_{\mathrm{aB}}/dt &= \mu N - \gamma X_{\mathrm{aB}}; \\ dX_{\mathrm{Ab}}/dt &= \mu N - \gamma X_{\mathrm{Ab}}; \\ dX_{\mathrm{ab}}/dt &= \mu \left(X_{\mathrm{Ab}} + X_{\mathrm{aB}} \right) + \beta X_{\mathrm{ab}}. \end{aligned} \tag{16.1}$$

Here γ is the decay rate of 1-mutants, β is the growth rate of the double-mutant, and μ is the mutation rate, assumed the same for both loci. The solution is:

$$\begin{aligned} X_{\mathrm{aB}} &= \left(\frac{\mu N}{\gamma} \right) \left\{ 1 - e^{-\gamma t} \right\}; \\ X_{\mathrm{Ab}} &= \text{same}; \\ X_{\mathrm{ab}} &= \left(\frac{2\mu^2 N}{\gamma} \right) \left\{ \left(\frac{\gamma}{\beta\,(\beta+\gamma)} \right) e^{\beta t} + \left(\frac{1}{\beta+\gamma} \right) e^{-\gamma t} - \frac{1}{\beta} \right\}. \end{aligned} \tag{16.2}$$

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Let us define time-to-replacement, $T_{\text{d.m.}}$, to be when $X_{\text{ab}} = N$. Since μ^2 is very small, the first term in the last equation must do the deed; hence, $T_{\text{d.m.}}$ is well-approximated by:

$$T_{\text{d.m.}} \quad = \quad \frac{1}{\beta} \ln \left\{ \frac{\beta\,(\,\beta + \gamma\,)}{2\,\mu^2} \right\}. \tag{16.3}$$

With $\beta = 5$, $\gamma = 1$, and $\mu = 10^{-5}$, formula (16.3) produces the nonsensical result that replacement takes about five generations. If $N = 10^8$, since the 1-mutants achieve steady-state at about $2\,\mu\,N = 2 \times 10^3$, the correct answer is about 60 (a few for the 1-mutants to reach steady-state, 50 for the double-mutant to appear, and about $\ln(10^8)/\beta \approx 3.7$ generations to outgrow the wild-type). Of course, these conditions are chosen to magnify the error.

Note how the deterministic result (16.3) for the replacement time depends heavily on β, but not at all on N, which is the tip-off that it cannot be right in a "waiting to win the lottery" context. (If you buy more tickets, you will win quicker.) This example argues against deterministic modeling for any genetic problem with multiple loci, except perhaps when all selection coefficients are negative.

16.1 Exercises

1. Derive (16.2). (Hint: if you have trouble, look up "integrating factors" in any ODE text.)

2. Make a stochastic version of this scenario and explore the waiting-time until the double-mutant outgrows the wild-type. For simulation, you can use the "direct" method of Chapter 4. Assume that the double-mutant has a birth rate: $\mu \left(X_{\text{Ab}} + X_{\text{aB}} \right) + \alpha\, X_{\text{ab}}$, and a death rate: $\delta\, X_{\text{ab}}$. Set $\delta = 1$ and $\beta = \alpha \;-\; \delta$. To keep the run time reasonable, change the numbers to: $N = 10^4$, $\mu = 10^{-3}$, so that $2\,N\,\mu^2 = .02$, as in the text. Compare with formula (16.3).

3. Continuing from Exercise 2, change the double-mutant growth-rate to $\beta = 0.1$. The formula now yields 109 time-units (generations), while $\ln(10^8)/\beta \approx 92$, which plus 50 is 142. Thus the formula seems more reasonable in this case—but is there something missing in the analysis?

Compare with the answer to Exercise 2 obtained from simulation (for this case). Discuss. (Hint: show that, for small growth rate, $R_0(\text{d.m.}) - 1 \approx \beta$, and consider Exercise 1 of Chapter 20.2. What factor has been overlooked?)

4. Continuing Exercises 2 and 3, use the answer to Exercise 4, Chapter 15, to prove that the expected compartment sizes in the stochastic model are correctly predicted by (16.1). Does this observation support the conclusion that formula (16.3) ought to work, or not? Discuss.

Chapter 17

The Infection Model Defined

The number of epitopes recognizable by CTLs will be denoted by E (not to be confused with mathematical expectation, which will be denoted $\mathcal{E}$ if required). Genomes are indicated by a subscript g, which is short-hand for the sequence of 0's and 1's describing wild-type and mutated epitopes. Different types of ITs and PITs are indicated with the index "k". The infected compartments acquire two indices: $X_{g,k}$ and $Y_{g,k}$. For the allowed transitions and rates in the basic model we have:

Detailed Infection Process Schema

Type	*Jump*	*Rate*
Births:	$X_{g,k} \longrightarrow X_{g,k} + 1$	[Inf](g,k);
Progression:	$X_{g,k} \longrightarrow X_{g,k} - 1$ and $Y_{g,k} \longrightarrow Y_{g,k} + 1$	$\eta\, X_{g,k}$.
Deaths:	$X_{g,k} \longrightarrow X_{g,k} - 1$	$\delta_{\mathrm{IT}}\, X_{g,k}\, 1[X_{g,k} > 0]$;
	$Y_{g,k} \longrightarrow Y_{g,k} - 1$	$\delta_{\mathrm{PIT}}\, Y_{g,k}\, 1[Y_{g,k} > 0]$;

The function Inf defines the birth rate in the (g, k)-eclipse compartment and is given by

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$$\begin{aligned}\mathrm{Inf}(g,k) \quad &= \quad \iota\, p_k \sum_{g',k'} v_{k'} \times \\ &\qquad \left\{ (1-\mu)\, 1[\, g' = g\,] + \mu\, 1[\, \sum_j |\, g_j - g'_j\,| = 1\,] \right\} Y_{g',k'}.\end{aligned} \tag{17.1}$$

Here μ is the mutation-rate, regarded here as fixed. (It may in fact vary with the locus, as discussed in Chapter 5.) The array of parameters, p_k and v_k, provides for reproductive variation; a PIT of type k produces virus at rate v_k and a virion generates an IT of type k with probability p_k. For EPV, we often use the simplest non-trivial model, which has three types. In order to implement a requirement (discussed in Chapter 22) that EPV contributes to mean production, we often chose: $v_1 = 0$; $v_2 = 1$, $v_3 = K$ and

$$\begin{aligned} p_3 \quad &= \quad \frac{3\, F_{\mathrm{mean}}}{K}; \\ p_2 \quad &= \quad 3\, (\, 1 - F_{\mathrm{mean}}\,); \\ p_1 \quad &= \quad 1 - p_2 - p_3. \end{aligned} \tag{17.2}$$

We derive the infection rate ι from the formula:

$$R_0(\mathrm{w.t.}) \quad = \quad \frac{\iota\, (\sum_k\, p_k\, v_k\,)\, \eta}{\delta_{\mathrm{PIT}}\, \left(\, \delta_{\mathrm{IT}} + \eta \right)}. \tag{17.3}$$

This formula is most easily derived from the ODE description of the infection process, which is a good approximation once the infected-cell population becomes large. The matrix form of this system of equations, for one viral genome but with two PIT types, is:

$$\frac{d}{dt} \begin{pmatrix} X_1 \\ Y_1 \\ X_2 \\ Y_2 \end{pmatrix} \quad = \quad \begin{pmatrix} -\left(\delta_{\mathrm{IT}} + \eta\right), & \iota\, p_1\, v_1, & 0, & \iota\, p_1\, v_2 \\ \eta, & -\delta_{\mathrm{PIT}}, & 0, & 0 \\ 0, & \iota\, p_2\, v_1, & -\left(\delta_{\mathrm{IT}} + \eta\right), & \iota\, p_2\, v_2 \\ 0, & 0, & \eta, & -\delta_{\mathrm{PIT}} \end{pmatrix}$$

$$\times \begin{pmatrix} X_1 \\ Y_1 \\ X_2 \\ Y_2 \end{pmatrix}. \tag{17.4}$$

The condition that the process exhibits growth is that the matrix on the right-hand side has a positive eigenvalue, which in this case is equivalent to it having a negative determinant:

$$\delta_{\mathrm{PIT}} \left(\delta_{\mathrm{IT}} + \eta \right) \quad - \quad \eta\, \iota \, \left(p_1\, v_1 + p_2\, v_2 \right) \quad < \quad 0, \tag{17.5}$$

which yields (17.3). This formula holds generally; the proof is left to the interested reader. We usually specify R_0 and then, given the other parameters, solve (17.3) to find ι.

We must also specify the basic reproductive number for the mutant strains. For the "null-epistasis" model:

$$R_0(\mathrm{mut.;g}) \quad = \quad 1 + \left(R_0(\mathrm{w.t.}) - 1 \right) \prod (1 - f_m), \tag{17.6}$$

where the f_m represent fitness-loss from each locus and the product is over the mutations m in genome g. A second choice might have an additive structure:

$$R_0(\mathrm{mut.;g}) \quad = \quad R_0(\mathrm{w.t.}) \left(\frac{1}{1 + [\sum f_m]} \right). \tag{17.7}$$

Since in this case the factors are not multiplied, the intrinsic fitness landscape displays "epistasis". Other specific choices are described in various chapters. For these strains, $\iota(\mathrm{mut.;g})$ is obtained from $R_0(\mathrm{mut.;g})$ using (17.3) as before.

In order to incorporate the CTL response, we put in place of δ_{PIT}

$$\delta_{\mathrm{total}} \quad = \quad \delta_{\mathrm{PIT}} \quad + \quad \sum_{e=1}^{E} \kappa_e \, 1[\text{ epitope } e \text{ is expressed}]\, C_e, \tag{17.8}$$

where κ_e is a another positive parameter (kill-rate) and C_e is the density of the CTL clone recognizing epitope "e", introduced in the next chapter. The antigen expression that activates CTLs recognizing epitope e is defined as

$$\mathrm{Ag}_e \quad = \quad \sum_{g,k} 1[\text{genome } g \text{ expresses wild-type epitope } e]\, Y_{g,k}. \tag{17.9}$$

17.1 Exercises

1. Formulate an ODE model of Phillips's 1996 theory we called target-cell depletion in the Introduction. That is, add another compartment representing uninfected target cells which are transformed by infection into ITs and then killed by the virus. (Continued by Exercise 17 of Chapter 19.)

2. Formulate an ODE model of the hyperactivation theory also mentioned in the Introduction. That is, add compartments representing uninfected, resting and activated target cells and additional parameters describing generation, "normal" and "HIV-infection-driven bystander" activation, infection, and death of these cells. (For simplicity, limit the uninfected-cell compartments to resting *vs.* activated status, neglecting the cell-cycle of the latter.) (Continued by Exercise 18 of Chapter 19.)

Chapter 18

The CTL Model Defined

Let $Z_{i,e}$ denote the count of cells in a particular compartment. The index i ranges over NR (naïve resting compartment) to $NR + n_d$ and from MR (memory resting compartment) to $MR + n_d$. An index equal to $NR + j$, or $MR + j$, with $j = 1, \ldots, n_d$, describes cells which appear after j divisions. The index e, $e = 1, \ldots, E$, records which epitope the CTL recognizes. Since cells of different specificities have the same kinetics, except for the activation rate, we can drop the index "e" when listing the jumps and rates:

Detailed CD8 Process Schema

Type	*Jump*	*Range*	*Rate*
Birth:	$Z_i \longrightarrow Z_i + 1$	MR	β;
Mitosis:	$Z_i \longrightarrow Z_i - 1$ and $Z_{i+1} \longrightarrow Z_{i+1} + 2$	$NR, \ldots, NR + n_d - 1$ and $MR, \ldots, MR + n_d - 1$	$[\mathrm{Act}]_i \, Z_i$;
Deaths:	$Z_i \longrightarrow Z_i - 1$	NR, MR, $NR + n_d$, $MR + n_d$	δ_{NR}, $\delta_{MR} \, Z_i \, [Z_i > 0]$; $\delta_{\mathrm{CTL}} \, Z_i \, 1[Z_i > 0]$;
Reversion:	$Z_i \longrightarrow Z_i - 1$ and $Z_{MR} \longrightarrow Z_{MR} + 1$	$NR + n_d$ and $MR + n_d$	$\rho \, Z_i$.

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The function $[\text{Act}]_i$ yields the per-cell activation or meiosis rates. Activation into the cell-cycle of resting, naïve-or-memory CD8s that recognize epitope "e" occurs at rates:

$$\begin{aligned} [\text{Act}]_{NR} &= \alpha\, \text{Ag}_e; \\ [\text{Act}]_{MR} &= \alpha\, [\,\text{memory-factor}\,]\, \text{Ag}_e. \end{aligned}$$

Here "Ag_e" is the total expression of (wild-type, or unmutated) epitope e on PITs, defined in the previous chapter. Otherwise, $[\text{Act}]_i = 1/$ (cell-cycle time). The δ_i are cell death-rates (by apoptosis); CTLs promote rather than die except for the finally-differentiated states ($i = NR + n_d$ or $i = MR + n_d$); at these terminal states they can die, at rate δ_{CTL}, or revert to resting in the memory compartment at rate $\rho = (\text{Revert}\,\delta_{CTL})/(1 - \text{Revert})$. Memory cells react faster, have a shortened cell-cycle, and kill better than naïve cells.

We set β ("birth" of resting, naïve HIV-specific CD8s) $= Z_{NR}$ (at time zero) $\times\, \delta_{NR}$, implying a steady-state immune system, absent activation. The CTLs recognizing epitope e, denoted C_e, are the sum of $Z_{i,e}$ for $i = NR + 4, \ldots NR + n_d$ (naïve CD8s promote to effector status after 4 divisions) or $i = MR + 1, \ldots, MR + n_d$ (memory promote after one).

18.1 Exercise

1. Formulate a model of an alternative theory of T-cell dynamics, in which "effectors" and "memory cells" are generated in parallel immediately after activation, presumably by some stochastic choice mechanism. (Continued by Exercise 19 of Chapter 19.)

2. Another plausible modification of the basic CTL model involves the activation functions called $[\text{Act}]_i$ in the schema. Activation is dependent on ligand-receptor-type interactions, and they may *saturate* or reach an asymptote at high ligand densities (represented, for CTL

activation, by HIV antigen expression). The mathematical expression of saturation occurs in the Hill function[1]

$$f(x) = \frac{a\,x}{1 + b\,x} \tag{18.1}$$

Formulate an alternative CD8 model with Hill-type activation rates. Ditto for the infection model and killing rates (the variable in the Hill function is CTL density). This exercise is continued by Exercise 24 of Chapter 19.

[1]Believe it or not, it is named after early 20th-century American mathematician George Hill, who was known for his work in astronomy. When the great French mathematician Henri Poincaré got off the boat, visiting America around 1900, he reportedly asked: "Ou est Monsieur Hill?"

Chapter 19

On Simulation Techniques

Granted a set of rate functions, the corresponding ordinary-differential equation system is derived from

$$\begin{aligned} dC_i/dt &= \sum_{\text{jumps}} (\text{total rate})\,\triangle(i;\text{jump}); \quad i \neq \text{NR}; \\ dC_{\text{NR}}/dt &= \beta, \quad i = \text{NR}. \end{aligned} \tag{19.1}$$

Here C_i stands for a compartment of either the cellular immune-system or infected-cell population, "total rate" for each jump is taken from one of our tables, and $\triangle(i;\text{jump})$ denotes $+2$, $+1$, -1, or 0, depending on the effect of a jump on the compartment. The left-hand sides denote the rates-of-change of the compartmental variables. Solving this system means expressing all the C_i's as functions of time, such that they have the required rates. In the 19th century, mathematicians showed that, given initial conditions for all compartments, these functions of time are unique. Specifying rate-equations became the standard paradigm for determining the future with certainty and mathematicians became adept at solving ODEs with one or two compartments by explicit formulas.

As explained in the simulation chapter, we cannot solve higher-order equations exactly, but they can be solved approximately (if on a computer, mathematicians say "numerically"), by standard methods. These techniques are improvements over the simplest routine, called in the ODE trade the "Euler method", which works as follows:

$$C_i(t+h) = C_i + (\text{ right-side of equation 19.1 }) \times h. \tag{19.2}$$

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This method is said to have first-order error control, meaning that the magnitude of the error per time-step could be $O(h)$. Because there are typically of order $1/h$ time-steps in a simulation, the overall numerical error may be unacceptable. The most popular improvement is called "4-th order Runge-Kutta", abbreviated "RK4". This simple and clever scheme improves the order per time-step to $O(h^4)$, without requiring the programmer or the computer to do much additional work (e.g, taking derivatives of the right sides). The routine takes a full step by way of two half-steps (time-increment $h/2$), and makes three more calls to the functions that calculate the right-hand sides in (19.1).

When updating the immune-system side of the process, we sometimes used a simpler method, "RK3" (see Exercise 2), which has lower order but makes only two calls to the right-hand sides. The motivation is speed, justified by the conjecture that the CTL system is orbitally-stable. An ODE system is called "orbitally-stable" if a small perturbation in the compartment variables is actually diminished by the dynamics over time (it is the opposite of "chaos"). We do not go into the difficult question of whether stability or instability prevails for the combined model in general, but note only that in simulations of the CTL side with a few epitopes, stability appears the winner.

Concerning the "direct" method of simulating a jump process, let τ denote the projected time-interval before a particular jump occurs. Then assuming the Markov property, the law is:

$$P[\,\tau > s\,] \;=\; \exp\,(\,-s \,\times\, \text{rate}\,)\,. \tag{19.3}$$

which is easily implemented by the formula (let U denote a uniform random variable in [0,1] delivered by the RNG):

$$\tau = -\frac{\log\,(\,U\,)}{\text{rate}}. \tag{19.4}$$

Concerning the "switch method", we update continuous compartments in discrete time, with time-step denoted h as usual. The update formula is:

$$\log\, C_i(t+h) \;\;=\;\; \log\, C_i(t) \;+\; \left(\sum_{j=1}^{n} Q_{i,j}^{(1/2)}\,\xi_j\right) h^{1/2} \;+\; \left(q_i^{(1)} + \sum_{j=1}^{n} Q_{i,j}^{(1)}\;[\xi_j]^2\right) h \;+$$

$$\left(\sum_{j=1}^{n} Q_{i,j}^{(3/2)} \, \xi_j \right) h^{3/2} \quad + \quad q_i^{(2)} \, h^2. \tag{19.5}$$

In (19.5), ξ is a vector of independent, N(0,1) random variables drawn at each time-step and the quantities denoted $q^{(.)}$ and $Q^{(.)}$ are vectors and matrices, respectively, of coefficients depending on the state at that time and parameters. These are selected, by explicit formulas, to match the moment-generating functions of the one-step distributions of the direct routine. The term of order $h^{1/2}$ is derived essentially from the central limit theorem; note that it is centered (has mean zero). The term of order h is an Euler-type "drift", and the higher-order terms are corrections. If h is sufficiently small, the term $\mathrm{O}(h^{1/2})$ will be the largest, so the routine genuinely implements a stochastic process. The numerical work scales as $N_c^3/2$ (about the same amount of work as inverting an $N_c \times N_c$ matrix), where N_c is the number of continuous, Milshtein-updated compartments. To validate the technique, we proved that the method can operate with an error of order $\mathrm{O}(h^3)$ per time-step, provided the compartmental variables remain bounded, and also carried out simulation studies comparing the direct and Milshtein methods for relatively small populations.

One very important caution about RK or the switch-method must be emphasized. Both methods are based on an expansion in the time-step (see exercises). Power-series expansions are not a particularly good approach to solving ODEs, or Kolmogorov's equations for a stochastic process, because they may have a small radius of convergence. Indeed, the very idea is usually omitted from textbooks, unless devoted to simulation. Trying to sum a power series beyond its radius of convergence guarantees numerical catastrophe. For RK, a useful method for finding a "safe" time-step (perhaps ensuring 4-th order error-control) has been invented (see the section on "adaptive solvers" in any text on computer solution of ODEs). We have not discovered an equivalent procedure for the switch-method, although we sometimes use a cheap version we call tip-toeing. The primary danger in our simulations occurs around the time of peak viremia, when the CTLs begin to function and the viral-load suddenly changes direction. Hence, we temporarily divide 'h' by 10 in a window around the peak—i.e., tip-toe past the trouble spot. Even for RK with adaptive-error-control, the wisest course is to repeat the simulation with a smaller time-step—as well as reflect on whether the solution appears reasonable. Beware of deducing "chaos" in your model without mathematical justification, as it may be the method of solution that is chaotic.

19.1 Exercises

1. Consider the initial-value problem for the one-dimensional ODE:

$$\begin{aligned} dx/dt &= f(x); \\ f(0) &= x. \end{aligned} \tag{19.6}$$

Consider the power-series approach to updating the solution by the time-step 'h':

$$x(h) = x + a_1\, h + a_2\, h^2 + a_3\, h^3 + \ldots \tag{19.7}$$

Show that the coefficients a_k can be determined from knowledge of $f'(x)$, $f''(x)$, ... i.e., from the derivatives of $f(x)$ with respect to x. Hence, given routines that calculate these derivatives, we could simulate the solution of an ODE in this way to any desired degree of error-control per time-step.

2. Following from Exercise 1, consider Runge's and Kutta's brilliant trick for updating without bothering to compute derivatives:

$$x(h) = x + h\, f(\, x + 0.5\, h\, f(x)\,). \tag{19.8}$$

Show using the power-series approximation that (19.8) yields $\mathrm{O}(h^3)$ error-control (implementing "RK3"; for the celebrated, and generally-employed, RK4, see any numerical analysis text).

3. Consider the *Vance-Gilpin* model:

$$dx_i/dt = x_i \left(r_i - \sum_{i=1}^{3} A_{i,j}\, x_j \right), \tag{19.9}$$

with $i = 1, 2, 3$ and the parameters

$$r = \begin{pmatrix} 1 \\ 1 \\ -1 \end{pmatrix}; \quad A = \begin{pmatrix} .001 & .001 & .01 \\ .0015 & .001 & .001 \\ -.004 & -.0005 & 0 \end{pmatrix}. \tag{19.10}$$

Simulate trajectories in this model (by any higher-order method) for various values of parameter $A_{3,1}$, which is called a "bifurcation parameter" because the qualitative orbit structure changes at critical values. At $-.002$, the system has a stable fixed-point; at $-.003$, it has a periodic orbit; and at $A_{3,1} = -.004$, the system exhibits so-called "spiral chaos" (see Notes). Can this model represent real biological populations?

4. For use in Exercise 6, derive the formula for the general, one-dimensional Gaussian integral: let Z be $N(0,1)$, b any number, and B a number less than $1/2$ and show

$$\mathcal{E}\ \exp(\,b\,Z + B\,Z^2\,) \quad = \quad \left(\frac{2\,\pi}{1-2\,B}\right)^{1/2} \times \exp[\,(1/2)\,(1-2\,B\,)^{-1}\,b^2\,]. \quad (19.11)$$

(Hint: complete the square.)

5. Again for use in Exercise 6, Let $\triangle$ be any *difference operator*: $[\triangle\, f](X) = f(X+X') - f(X)$, for some vector X'. Note that Kolmogov's formula has the form:

$$d/dt\,\mathcal{E}\,f(X(t)) \quad = \quad \sum_j \mathcal{E}\,\{\,r_j(X)\,(\triangle_j\,f)(X)\}\,. \quad (19.12)$$

Prove the (modified) "Leibniz formula" for a difference operator:

$$\triangle[\,f\,g\,] \quad = \quad [\triangle\,f\,]\,g \quad + \quad f\,[\triangle\,g\,] \quad + \quad [\triangle\,f\,]\,[\triangle\,g\,]. \quad (19.13)$$

6. Consider the one-dimensional, stochastic, birth-and-death process with jumps:

Jump	*Rate*
$X \longrightarrow X+1$	$\gamma(X)$;
$X \longrightarrow X-1$	$\delta(X)\,1[X>0]$,

where $\gamma(.)$ and $\delta(.)$ are any non-negative functions bounded by a constant times X (to avoid "explosion", i.e., the process reaching infinity in a finite time). Consider the Milshtein scheme for updating, given by:

$$x(h) \quad = \quad x + b_1\,Z\,h^{1/2} + \left\{\,b_2\,+ b_3\,Z^2\,\right\}\,h + b_4\,Z\,h^{3/2} + b_5\,h^2, \quad (19.14)$$

where Z denotes a normal, mean-zero, variance-one, random variable and the b_k are variable coefficients. Show by expanding the moment-generating function (MGF; defined in Exercise 3 of Chapter 15), $\phi(\xi, h)$,

in ξ and h that the Milshtein scheme can yield $O(h^3)$ error-control for simulating this stochastic process when X is moderately large. (Hint: compare the MGF's for the exact and approximate processes. Use Kolmogorov's equation for differentiating ϕ with respect to h for the jump process, Exercise 4, and the Gaussian integral formula in Exercise 3 for the approximating process. You may assume that X remains bounded by some constant. What is the justification for including the term $b_3 Z^2$?)

7. Continuing from Exercise 6, let $\gamma(X) = \gamma X$ and $\delta(X) = \delta X$ and implement the direct and Milshtein methods for simulating this branching-process in your favorite computer language. Make 1,000 runs of each and compare. What are useful statistics for comparing two stochastic processes? (That last is really a research question.)

8. Using the recipe (19.1), show that the ODE approximation to the basic infection process of Chapter 17, with $\delta_{\mathrm{IT}} = \delta_{\mathrm{PIT}} = \delta$, one PIT type, one viral genome, and no CTLs has the form:

$$dX/dt = \iota Y - (\eta + \delta) X; \tag{19.15}$$
$$dY/dt = \eta X - \delta Y. \tag{19.16}$$

9. Following from Exercise 8, solve the ODE system in the 19th century sense, i.e., by explicit formulas. (Hint: re-write the system in matrix form, and find the eigenvalues and eigenvectors of the matrix.)

10. Formulate the condition that the system of Exercise 8 exhibits growth of the infection (X + Y). I.e., discover the analog of formula (17.3) and show that growth occurs if and only if $R_0 > 1$.

11. Either by implementing RK3 (or RK4) or by using a commercial ODE package, check that the simulated trajectories approximate the exact formula from Exercise 9. Observe what happens if h is carelessly taken too large. Is there a relationship between the eigenvalues of the matrix and how large is too large?

12. Implement the direct simulation routine for the jump-process version of the model in Exercise 8. Assume 100 initial ITs and compare with the

solutions of the ODEs while the infection remains below 10,000 (ITs + PITs).

13. Continuing from Exercise 12, assume now one initial IT, repeat the stochastic simulation 1,000 times and calculate the fraction of runs in which the infection went extinct. What is the relationship to R_0?

14. Consider a simplified cellular immune-system model obtained by ignoring the naïve- and memory-cell distinction. Show that suitable ODEs for one clone recognizing one epitope might take the form:

$$\begin{aligned} dZ_{\mathrm{MR}}/dt &= \beta - (\,\delta_{MR} + \alpha\, Y\,)\; Z_{\mathrm{MR}} + \rho\, Z_8; \\ dZ_1/dt &= \alpha\, Y\, Z_{\mathrm{MR}} - \zeta\, Z_1; \\ dZ_2/dt &= 2\,\zeta\, Z_1 - \zeta\, Z_2; \\ \ldots &= \ldots; \\ dZ_8/dt &= 2\,\zeta\, Z_7 - \rho\, Z_8. \end{aligned} \tag{19.17}$$

Assume that $C = Z_1 + Z_2 + \ldots + Z_8$, add a term $\kappa\, C$ to δ in equation (19.16) of Exercise 8, and generate a figure similar to Figure 4.2.

15. (Warning: this is a research project!) Prove that (a) the combined system of equations, (19.16) and (19.17), has a unique steady-state; (b) the system will approach this steady-state from any non-zero initial conditions; and (c) small perturbations of a trajectory tend to zero asymptotically (i.e., the system is "orbitally stable"). (Hint for part c: derive the first variation equations and examine the eigenvalues.) Are these claims valid also with multiple epitopes and mutant viruses? If not, prove that the extended system possesses a biologically-relevant instability, derive an interesting prediction and publish in a major journal.

16. Again for the combined system, with a deterministic implementation (RK something), vary the eclipse period (inverse of η) and the basic reproductive number(R_0) and search for cases in which the CTLs actually drive the infection to extinction. Such cases might describe other viral diseases that are "cured" by CTLs (e.g., influenza, without prior exposure to the strain).

17. This exercise continues Exercise 1 of Chapter 17. Simulate from your model of Phillips's 1996 theory (target-cell depletion). Vary parameters

and investigate which one is responsible for the 2–3 log drop in viral load after the peak.

18. This exercise continues Exercise 2 of Chapter 17. Simulate from your model of the hyperactivation theory and plot VL, targets (activated CD4), and total CD4. How much bystander activation would result in target cells remaining roughly constant over the primary stage of infection?

19. This exercise continues Exercise 1 of Chapter 18. Simulate from your model of the alternative theory of T-cell dynamics. Plot the effector, memory, and total CD8 response over the course of primary infection. Are there substantial differences from the model of Chapters 3 and 18 that might be observed?

20. This exercise continues Exercise 2 of Chapter 15. An interesting variation on an ODE such as the logistic growth model improves it to the *"Itô process" or stochastic differential equation*:

$$dx \;=\; \left\{ a\,x \;-\; b\,x^2 \right\} dt \;+\; \sigma\, dw(t), \tag{19.18}$$

where $w(t)$ denotes the *Wiener process*: a mean-zero, Gaussian stochastic process with continuous sample paths representing "noise". The size of the noise is controlled by positive parameter σ and an assumption about its root-mean-square deviation:

$$\left(\mathcal{E} \left\{ |\, dw(t) \,|^2 \right\} \right)^{1/2} \;=\; \sqrt{dt}, \tag{19.19}$$

which is based on analogy with the (discrete-time) *drunkard's walk* from probability theory. Such a model incorporates *active or "process" noise*, due to compartments omitted from the model or external factors. In an HIV infection model, active noise could represent the impact on HIV replication due to fluctuating T-cell activation. (CD4 T-cells do not exist merely to provide HIV with targets; they respond to all infections, including colds or the flu.) Simulate from this model as follows: start with any method to solve the ODE (Euler or RK), and add a mean-zero, standard normal random variable with prefactor $\sigma \sqrt{h}$ to the deterministic update at each time-step. (Be careful about descending below zero!) The resulting process yields a more

biologically-realistic description of a *stochastic steady-state* that fluctuates around the ODE value. Is it possible that, in this stochastic model, the population might eventually go extinct?

21. Continuing from Exercise 20, consider another kind of SDE related to the logistic model:

$$dx \;=\; \left\{ a\,x \;-\; b\,x^2 \right\} dt \;+\; \sqrt{|\,a\,x \;-\; b\,x^2\,|}\,dw(t). \qquad (19.20)$$

That is, "σ" in model (19.18) is replaced by the square-root of the magnitude of the "drift" in this model. Simulate from this model. While the model of the previous exercise can represent external, active noise, this model attempts to approximate a jump process by incorporating *demographic stochasticity* (a random part of the incremental change, due to fluctuations in the number of jumps).

22. Show that the only waiting-time distribution for a jump that has no memory:

$$P[\,\tau > s+t\,|\,\tau > t\,] \;=\; P[\,\tau > s\,], \qquad (19.21)$$

and can therefore implement a Markov process, is the exponential law (19.3). The NTD absurdity of Chapter 4 is usually embodied in probability texts in the *bus-waiting paradox*: suppose buses appear with inter-arrival times independent and with a fixed exponential distribution with mean, say, of 20 min. If, as you arrive at the stop (and there is only one bus route that serves it), you see a person sitting, ask how long she has been waiting, and receive the answer: "15 min", should this information improve your mood?

23. This exercise continues Exercise 7 of Chapter 15. Show that the result from that exercise provides an alternative to the "direct" simulation routine described in Chapter 4 and here. Prove that the two routines implement the same process. Does the alternative method save FLOPS (floating-point operations; numerical effort) relative to the one described there?

24. This exercise continues Exercise 2 of Chapter 18. Simulate from a modification of the combined model in Exercise 13 in which either activation or killing rates (or both) have Hill-function form. Investigate whether the effect of a saturated killing rate means that, for sufficiently large R_0, immune-control breaks down.

19.2 Notes

For RK4, adaptive, and other methods for simulating from ODEs on the computer, see [247] or any text on numerical analysis.

Concerning Exercise 3: for more discussion of "spiral chaos" and whether this model can truly be said to represent biological populations, see [108, 199, 266, 276, 298]. It would be interesting to invent a discrete, stochastic analog of this model and investigate whether its large-population limit is described by the ODEs, or not.

The motivating idea for the "switch-method" came from G. N. Milshtein's 1976 paper on simulating solutions of stochastic differential equations [212], although in [312, 313] the authors skipped this intermediate approximation and passed directly from discrete- to continuous-state processes. The justification for including the term $b_2 Z^2$ is explained in [313].

Concerning Exercise 16, and the related problem of extinction in the combined stochastic model, see [316].

Concerning Exercises 17 and 18, at this time it has still not been proven (to our knowledge) that bystander-activated CD4s support HIV replication, so the crucial parameters in this theory are still unknown. The discussion of putative production by resting cells (essential for Phillips's theory) also continues.

Concerning Exercises 20 and 21: SDEs were introduced by the Japanese mathematician K. Itô during World War II [143, 144], who built on earlier work by Bachelier (Poincaré's student), Einstein and Wiener.[1] SDEs, which are still very popular in finance and economics (for modeling prices of stocks or bonds over time), are also known as "Itô processes". For a discussion of what "noise modeling" is trying to capture, and the various alternatives, see [294]. Because "active noise" is ubiquitous in biology, SDEs should play a greater role in modeling in the future (perhaps even supplanting ODEs).

Concerning Exercise 24, a Hill function was entertained in [319] for killing and estimated from *in vitro* data; but the saturation observed in the glass well is probably not observed *in vivo*, where the killer-to-target ratio is lower.

[1] Itô died in 2008; for his New York Times obituary, see[142].

Chapter 20

Derivation of the Escape Formula

We will consider a variety of mutations in the HIV genome, denoted in formulas by the index "m". Let $\alpha_{e;m}$ measure the extent that the mutation "m" abrogates killing by CTL clone C_e, where e denotes epitope (running over $1, \ldots, E$), with $\alpha_{e;m} = 1$ implying total abrogation (no killing) and $\alpha_{e;m} = 0$ killing at the same rate as wild-type PITs. Let "REA_m" be the "relative escape advantage" accrued through mutation m by partially or wholly escaping immune pressure from CTLs:

$$\mathrm{REA}_m \;=\; \frac{\sum_{e=1}^{E} \kappa_e \, \alpha_{e;m} \, C_e}{\sum_{e'=1}^{E} \kappa_{e'} \, C_{e'}} \tag{20.1}$$

REA_m depends on current CTL densities, which in turn depend on the current composition of the viral population; the "ecological" interactions mentioned in the Introduction enters here.

Define the intrinsic loss-of-fitness denoted "LOF_m", of a genome bearing mutation m alone (we abbreviate it by "1-mutant" below), to be a number between zero and one, with $\mathrm{LOF}_m = 1$ meaning unviable and $\mathrm{LOF}_m = 0$ meaning growth at the same rate as the wild-type. In terms of basic reproductive numbers of wild-type and mutant, ignoring immune response, (define $[x]^+ = x$ if $x > 0$ and zero otherwise):

$$\mathrm{LOF}_m \;=\; 1 - \left[\frac{R_0(\text{mut.;m}) - 1}{R_0(\text{w.t.}) - 1} \right]^+ . \tag{20.2}$$

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20.1 The Escape Formula Without EPV

In order to illustrate the ideas, we begin by deriving the formula for the infection model without "extra-Poisson variation"; in other words, with only one type of PIT. The following formula will be derived for the expected number, per generation, of PITs infected with an escape mutant whose lineage is destined to avoid extinction.

The Escape Formula (no EPV):

$$P\,[\,\text{Esc.}\,] \;=\; N\,\textstyle\sum_{m=1}^{M} B_m\,\mu_m\,[\,\text{REA}_m - \text{LOF}_m\,]^+.$$

In this formula, N is the number of PITs, μ_m is the mutation rate at this locus per cycle, and the B_m are unimportant constants of order one.

To begin the derivation, consider the simplest branching-process model of infection, with one compartment (i.e., ignoring the eclipse period as well as the PIT types). The basic reproductive number of the infection by the wild-type strain takes the form

$$R_0(\text{w.t.}) \;=\; \frac{\iota}{\delta}. \tag{20.3}$$

Here ι is a constant combining virion production, infectiousness, and other transmission parameters, and δ is the inverse lifetime of a PIT, neglecting immune killing. Let us assume that ι varies from one viral strain to the next but δ does not. In the presence of the cellular immune response we have to add a killing term to δ in (20.3), which yields an effective reproductive number:

$$R_{\text{eff.}}(\text{w.t.}) \;=\; \frac{\iota}{\delta + \sum_{e=1}^{E} \kappa_e\, C_e}$$

$$= \frac{R_0(\text{w.t.})}{1+\sum_{e=1}^{E}\kappa_e\, C_e/\delta}. \tag{20.4}$$

The formula for 1-mutant strain is an obvious modification:

$$R_{\text{eff.}}(\text{mut.;m}) = \frac{R_0(\text{mut.;m})}{1+\sum_{e=1}^{E}\kappa_e\,(1-\alpha_{e;m})\,C_e/\delta}. \tag{20.5}$$

The probability of avoiding extinction in this case is simply

$$\text{P}[\,\text{Esc.}\,]_m = \left[1 - \frac{1}{R_{\text{eff.}}(\text{mut.;m})}\right]^+; \tag{20.6}$$

hence, ignoring the unlikely event of two or more distinct mutant lineages appearing in one generation and surviving, the probability of any one new mutant lineage growing out is

$$P\,[\,\text{Esc.}\,] = N\sum_{m=1}^{M}\mu_m\,\text{P}[\,\text{Esc.}\,]_m. \tag{20.7}$$

Defining

$$B_m = \frac{1}{\xi - \text{LOF}_m}; \qquad \xi = R_0(\text{w.t.})/(R_0(\text{w.t.})-1), \tag{20.8}$$

the basic formula now follows from $R_{\text{eff.}}(\text{w.t.}) = 1$ (definition of steady-state), (20.5)–(20.8), and simple algebra.

For the two-compartment branching model with the eclipse period, the only modification required is in the quantities called B_m, which acquire an additional factor of $\eta/(\delta_{\text{IT}}+\eta)$. Note that, since $R_0(\text{w.t.}) > 1$ and $\text{LOF}_m \le 1$, the B_m are in any case unimportant constants of order one.

20.2 The Escape Formula with EPV

With heterogeneity in production of infectious virions by PITs, or in ability of the virions to create other PITs, the formula is modified by a crucial, and potentially very small, factor. Recalling the definition of the infection process in Chapter 2, let us define:

$$\text{var}(v) \;=\; \sum_k p_k\, v_k^2 - [\sum_k p_k\, v_k\,]^2; \tag{20.9}$$

that is, var(v) is the variance of virion-production in PITs.

The Escape Formula (with EPV):

$$P\,[\,\text{Esc.}\,] \;=\; \left(\frac{N}{\text{var}(v)+1}\right)\,\nu\,\textstyle\sum_{m=1}^{M}\, B_m\,\mu_m\,[\,\text{REA}_m - \text{LOF}_m\,]^+.$$

In the Escape formula with EPV,

$$\begin{aligned} B_m &= \frac{1}{\xi - \text{LOF}_m} \times \frac{1}{\lambda}; \\ \lambda &= \frac{\delta_{\text{IT}} + \eta}{\eta}, \end{aligned} \tag{20.10}$$

and ν is a constant between $1/\lambda$ and $4 \times \lambda$. The reason for including ν is that no exact formula exists in this case; rather, the escape-rate satisfies an equation from which upper and lower bounds can be derived. The explanation for the factor containing var(v) is that replicative heterogeneity increases extinction-rates.

The ingredients we need for the derivation are: formulas for R_0(w.t.) and R_0(mut.;m) (available from Chapter 17) and an expression for the extinction-rate starting with an IT or PIT of each type. For the mutant genome we need only substitute the mutant infection-parameter for that of the wild-type:

$$R_0(\text{mut.;m}) \;=\; \frac{\iota_{\text{mut.}}\,(\,\sum_k\, p_k\, v_k\,)}{\delta_{\text{PIT}}\,\lambda}. \tag{20.11}$$

Next, we write $\delta_{\text{IT}} = \delta$, $\delta_{\text{PIT}} = \delta + \sum\, \kappa_e\, C_e$ (for the wild-type) and $\delta_{\text{PIT}} = \delta + \sum\, \kappa_e\, (\,1 - \alpha_{e;m}\,)\, C_e$ (for the mutant), to obtain the effective reproductive numbers, and follow the scheme of Section 20.1, assuming a steady-state for the wild-type. The result is the useful equality:

$$\delta_{\mathrm{PIT}} \;=\; \delta\,\{\,1+(\,R_0(\text{w.t.})-1\,)\,(\,1-\mathrm{REA}_m\,)\,\}\,. \tag{20.12}$$

Directly from (20.11) and the definition of LOF, we have also

$$\iota_{\text{mut.}}\sum_k p_k\,v_k \;=\; \lambda\,\delta\,\{\,1+(\,R_0(\text{w.t.})-1\,)\,(\,1-\mathrm{LOF}_m\,)\,\}\,. \tag{20.13}$$

Now let r_k and s_k denote the extinction probability of the lineage generated by a single IT, respectively PIT, of type k, infected by the mutant virus, in the branching-process approximation (fixed immune response). The following equations relate these probabilities to the rate-parameters of the (stochastic) infection process:

$$0 \;=\; \delta_{\mathrm{IT}}\,(\,1-r_k\,) \;+\; \eta\,(\,s_k-r_k\,)\,; \tag{20.14}$$

$$0 \;=\; \delta_{\mathrm{PIT}}\,(\,1-s_k\,) \;+\; \iota_{\text{mut.}}\,v_k\,s_k\left\{\sum_k p_k\,r_k-1\right\}. \tag{20.15}$$

There are several ways to derive these equations. An informal method proceeds by consideration of the first events in the process. Let Ext. stand for the extinction event. Then, reasoning informally:

$$\begin{aligned} r_k \;&=\; P[\,\text{Ext.}\,|\,\text{one initial IT, type k}\,] \\ &=\; P[\,\text{Ext.}\,|\,\text{died as an IT}\,]\times P[\,\text{died as an IT}\,] \;+ \\ &\quad\; P[\,\text{Ext.}\,|\,\text{a PIT, type k}\,]\times P[\,\text{progressed to a PIT}\,] \\ &=\; \left(\frac{\delta_{\mathrm{IT}}}{\delta_{\mathrm{IT}}+\eta}\right) \;+\; s_k\;\times\;\left(\frac{\eta}{\delta_{\mathrm{IT}}+\eta}\right); \end{aligned} \tag{20.16}$$

which yields (20.14). Similarly,

$$\begin{aligned} s_k \;&=\; P[\,\text{Ext.}\,|\,\text{one initial PIT, type k}\,] \\ &=\; P[\,\text{Ext.}\,|\,\text{died before reproducing}\,]\times P[\,\text{died before reproducing}\,] \\ &\quad\; +\sum_n\,\{\,P[\,\text{Ext.}\,|\,\text{an IT, type n}\,]\times P[\,\text{produced an IT, type n}\,]\,\} \\ &=\; \left(\frac{\delta_{\mathrm{PIT}}}{\delta_{\mathrm{PIT}}+\iota_{\text{mut.}}\,v_k}\right) \;+\; s_k\sum_n r_n\left(\frac{\iota\,p_n\,v_k}{\delta_{\mathrm{PIT}}+\iota_{\text{mut.}}\,v_k}\right); \end{aligned} \tag{20.17}$$

which yields (20.15).

The next goal is to compute the escape probability by mutation "m", assuming the new mutant IT is of type k with probability p_k:

$$\mathrm{P}[\,\mathrm{Esc.}\,]_m \;=\; 1 - \sum_k p_k\, r_k. \tag{20.18}$$

Let

$$\begin{aligned} x_k &= 1 - r_k; \\ x &= 1 - \sum_k p_k\, r_k \;=\; \sum_k p_k\, x_k. \end{aligned} \tag{20.19}$$

In terms of these quantities, (20.14)–(20.15) reduces to

$$0 \;=\; \delta_{\mathrm{PIT}} \;+\; (\,1 - \lambda\, x_k\,)\,\left(\iota_{\mathrm{mut.}}\, v_k\, x + \delta_{\mathrm{PIT}}\right). \tag{20.20}$$

Further define, for convenience in the following developments,

$$\alpha \;=\; \frac{R_0(\mathrm{w.t.})}{R_0(\mathrm{w.t.}) - 1}; \tag{20.21}$$

$$\theta \;=\; \frac{\mathrm{REA}_m - \mathrm{LOF}_m}{\alpha - \mathrm{LOF}_m}; \tag{20.22}$$

$$\lambda \;=\; \frac{\delta_{\mathrm{IT}} + \eta}{\eta}; \tag{20.23}$$

$$\overline{v} \;=\; \sum_k p_k\, v_k; \tag{20.24}$$

$$g_k \;=\; \left(\frac{\overline{v}}{\lambda\, v_k}\right)\,(\,1 - \theta\,). \tag{20.25}$$

Solving for x_k in (20.20), multiplying by p_k, and summing yields the following equation for x:

$$F(x) \;\equiv\; \lambda^{-1} \sum_k p_k \left(\frac{1}{x + g_k}\right) \;=\; 1. \tag{20.26}$$

This equation yields a high-order polynomial if cleared of denominators, so we eschew the search for a closed-form solution, even when possible, and instead approximate the solution by finding upper and lower bounds. First, note that (20.26) has a positive solution if and only if

$$\theta \;>\; 1 - \lambda^{-1} \sum_k \frac{p_k\, v_k}{\overline{v}} = 0; \tag{20.27}$$

as logic dictates. One method approximates F by a linear or quadratic function. The linear approximation

$$F(x) \;\approx\; F(0) \;+\; F'(0)\,x \tag{20.28}$$

yields the result:

$$\begin{aligned} x &\approx \frac{\theta}{\lambda\,(1-\theta)}\left(\frac{\overline{v}^2}{\sum_k\, p_k\, v_k^2}\right) \\ &> \frac{\theta}{\lambda\, S}; \\ S &\equiv \frac{\sum_k\, p_k\, v_k^2}{\overline{v}^2}. \end{aligned} \tag{20.29}$$

Since $F''(x) > 0$, the latter expression is actually a lower bound on x.

To find an upper bound we examine the quadratic approximation:

$$F(x) \;\approx\; F(0) \;+\; F'(0)\,x \;+\; (1/2)\,F''(0)\,x^2, \tag{20.30}$$

which yields the approximate root for (20.26):

$$x \;\approx\; \frac{-F'(0) \,\pm\, \sqrt{[\,F'(0)\,]^2 - 2\,F''(0)\,[\,F(0)-1\,]}}{F''(0)}. \tag{20.31}$$

Substituting and simplifying, the smallest root (taking the minus sign) yields

$$\begin{aligned} x &\approx (1/2)\;(1-\theta)\left\{U - \sqrt{U^2 - 4\,\theta/T}\right\}; \\ U &= \frac{S}{\lambda\, T}; \\ T &= \frac{\sum_k p_k v_k^3}{\overline{v}^3}. \end{aligned} \tag{20.32}$$

Because $F'''(0) < 0$, this expression is an upper bound. We can obtain a further simplification of this bound by expanding in the small quantity $4\,\theta/T$, with the result

$$x \;\approx\; \lambda\,(1-\theta)\left(\frac{\theta}{S}\right), \tag{20.33}$$

but this is not an upper bound; it is, however, if multiplied by a convergent power series with sum less than 4 (left to the reader). Also, $(1-\theta)$ is bounded by one, yielding the rigorous upper bound

$$x \;<\; 4\,\lambda\left(\frac{\theta}{S}\right). \tag{20.34}$$

Finally, note that a scale factor in v_k drops out of all expressions (it might have been absorbed at the start in the infection rate parameter, $\iota_{\text{mut.}}$); hence we can set, without loss of generality, $\overline{v}=1$. Defining

$$\text{var}(v) \;=\; \sum_k p_k\,v_k^2 \;-\; 1, \tag{20.35}$$

the formula with EPV then follows from $S=\text{var}(v)+1$, (20.29), and (20.34).

20.3 Exercises

1. Verify formula (20.6). That is, prove that, for the simplest continuous-time branching process (one type and no eclipse phase), defined by

Type	*Jump*	*Rate*
Birth:	$X \longrightarrow X+1$	$\iota\,X$;
Death:	$X \longrightarrow X-1$	$\delta\,X\,1[X>0]$;

   and R defined to be ι/δ, the extinction probability beginning with one ($X(0)=1$) is $1/R$. (Hint: consider the first event.) What is the probability starting with $X(0)=n$?

2. According to the result of Exercise 1, if $R=1$, the branching-process goes extinct with probability one. How then is it possible for a population to exist in a "steady-state" with $R_{\text{eff.}}=1$, but without immigration? Is this a paradox? Discuss.

3. Check the algebra leading to the Escape Formula without EPV.
4. Show that the number of events accrued up to time τ in a one-component Markov jump process, with jumps by $+1$ at constant rate λ, is given by the Poisson law:

$$P[X = n] = e^{-\lambda\tau} \left\{ \frac{(\lambda\tau)^n}{n!} \right\}. \tag{20.36}$$

Compute the mean and the VMR of this distribution. If the mean is 3, what is the probability of 6 jumps in this interval? Of 100? (Hint for the latter question: look up "Stirling's approximation".) Next, assume the time-interval is, rather than fixed, an exponential random variable of mean τ, and show that the Poisson distribution is replaced by a geometric distribution:

$$P[X = n] = (1 - p)\, p^n. \tag{20.37}$$

What is p in terms of λ and τ? Same questions as before for the probabilities of 6 or 100 jumps.

5. Prove the formula:

$$\mathrm{FF} = \frac{\mathrm{IF} - \mathrm{KR}}{1 + \mathrm{KR}}, \tag{20.38}$$

from which the proportionality, (8.2) of Chapter 8, derives.

6. Check the factor of 4 in (20.34).

20.4 Notes

The rigorous method of deriving equations (20.14)–(20.15) proceeds by introducing the moment-generating function; differentiating with respect to time, using the Kolmogorov's equation; solving the resulting PDE by the method of characteristics, obtaining a system of ODEs; finally solving these ODEs yields the extinction probabilities. This rigorous (if rather pedantic) method can be found in texts on stochastic processes, or in an appendix to [309].

Concerning Exercise 2, the Problem of the Names—the alarming rate of disappearance of bourgeois family names, noted by 19th century demographers, despite a stable population was the first conundrum solved in mathematical biology. See [121].

Chapter 21

Statistics of the Mutant Lineage in Classical Genetics

Concerning the classical, deterministic formula for escape, mentioned in Chapter 6, the differential equation for the wild-type frequency, $f(t)$, where t is time in generations, during an escape episode in the high-N_e, strong-selection regime is:

$$\frac{df}{dt} \;=\; -\,s\,f\,(1-f\,) \;-\; \mu\,(2\,f-1\,). \tag{21.1}$$

(Note: $s > 0$ here means that the mutant has the selective advantage.) The solution is (let the initial mutant frequency be negligible):

$$f(s;t) \;=\; \frac{\mu}{s} \;+\; \frac{(\,1-\frac{\mu}{s}\,)\exp(-\,s\,t\,)}{\frac{\mu}{s} \;+\; (\,1-\frac{\mu}{s}\,)\exp(-\,s\,t\,)}. \tag{21.2}$$

The "more sophisticated" argument that deterministic classical genetics cannot explain the "fall off a cliff" escape pattern goes as follows. Given the observations for patient 'B' (10/10 wild-type at 3.35 years and 12/12 mutant at 3.54 years), the Bayesian posterior expected frequencies with flat priors are 11/12 and 1/14, respectively. The largest (in magnitude) slope of a tangent to the graph of (21.2), with s fixed, is $s/4$; it also bounds the slope of the secant to any two points on the graph. If the Bayesian values are taken to be such points, we find the lower bound on the selection coefficient:

$$s \;\geq\; 4\left(\frac{11/12-1/14}{3.54-3.35}\right)\left(\frac{2}{365}\right), \tag{21.3}$$

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(assuming generation time of 2 days), which yields $s \geq .097$. On the other hand, setting $\mu = 3.5 \times 10^{-5}$ and solving the relation $f(s; 3.35 \times 365/2) = 11/12$ for s yields $s = .0039$, which is an upper bound.

Concerning the probability of escape in the classical, low-N_e regime, we can write the probability per generation of the event as:

$$P\,[\,\text{Escape}\,] \quad = \quad N_d\,\mu\,p_{\text{grow}}. \tag{21.4}$$

where p_{grow} denotes the probability of growing (avoiding extinction). Before frequencies change appreciably, the mutant lineage can be treated as a branching-process, with mean number of successful offspring $1 + s$ and variance-to-mean ratio VMR, which can be derived from the offspring distribution and sampling hypothesis (see below). Provided a condition on a third moment holds (it rules out trivial examples where rare events yield a large VMR without contributing to the mean or extinction probability, see Chapter 22),

$$p_{\text{grow}} \quad \approx \quad \frac{s}{VMR}. \tag{21.5}$$

Hence we can improve (21.4) to read:

$$P\,[\,\text{Escape}\,] \quad = \quad \frac{N_d\,\mu\,s}{\text{VMR}}. \tag{21.6}$$

In terms of the inbreeding definition of N_e, we can rewrite this formula as

$$P\,[\,\text{Escape}\,] \quad = \quad N_e\,\mu\,s. \tag{21.7}$$

In order to discuss extinction probabilities before the mutant lineage becomes comparable with N_d, we require the mean and variance of the mutant's offpring after sampling. We consider two classical hypotheses.

Let a mutant individual have offspring distribution p_n, $n = 0, 1, \ldots$, with mean $\sum_n p_n\,n \equiv M_{\text{mut.}}$. Suppose the other N_d individuals have mean offspring number $M_{\text{w.t.}}$ and let the selection coefficient s be defined by $1 + s = M_{\text{mut.}}/M_{\text{w.t.}}$. Let $n_{\text{mut.}}$ denote the (unsampled) number of offspring of the mutant and $n_{\text{w.t.}}$ the (unsampled) offspring of all the wild-type individuals. Let $N_{\text{mut.}}$ be the sampled number of mutant offspring. Then, sampling without replacement,

$$P[\,N_{\text{mut.}} = k\,|\,n_{\text{w.t.}} = n,\ n_{\text{mut.}} = n'\,] \quad =$$

$$\frac{\left(\begin{array}{c} n \\ k \end{array} \right) \left(\begin{array}{c} n' \\ N_d - k \end{array} \right)}{\left(\begin{array}{c} n + n' \\ N_d \end{array} \right)} \tag{21.8}$$

Using $n' \approx M_{\text{w.t.}} N_d$, Stirling's formula and the exponential limits $(1 \pm a/N)^{-N} \to \exp(\mp a)$, we find for large N_d

$$P[\, N_{\text{mut.}} = k \,|\, n_{\text{w.t.}} = n,\ n_{\text{mut.}} = n' \,] \approx \frac{n!}{(n-k)!\, k!} \left(\frac{M_{\text{w.t.}} - 1}{M_{\text{w.t.}}} \right)^n, \tag{21.9}$$

which yields

$$P[\, N_{\text{mut.}} = k \,] \approx \frac{(\, M_{\text{w.t.}} - 1 \,)^{-k}}{k!} \sum_{n=k}^{\infty} p_n \frac{n!}{(n-k)!} \left(\frac{M_{\text{w.t.}} - 1}{M_{\text{w.t.}}} \right)^n. \tag{21.10}$$

From (21.10) there follows directly that the sampled mean number of mutant offspring is $1 + s$ and

$$\text{Sampled VMR} = \text{vmr} \left(\frac{1+s}{M_{\text{w.t.}}} \right) + \left(1 - \frac{1+s}{M_{\text{w.t.}}} \right); \tag{21.11}$$

where "vmr" is the variance-to-mean ratio of the mutant's offspring distribution.

In another popular scheme, the mutant and wild-type have identical offspring distributions, but the mutant's offspring have a sampling advantage. Let the new generation be drawn without replacement from the offspring, with the chance of drawing a mutant being

$$p_s \equiv [\, n_{\text{mut.}} / (n_{\text{mut.}} + n_{\text{w.t.}}) \,] \, (1 + s). \tag{21.12}$$

Hence

$$P[\, N_{\text{mut.}} = k \,|\, n_{\text{mut.}} = n \,] = p_s^k \, (\, 1 - p_s \,)^{N_d - k} \left(\begin{array}{c} N_d \\ k \end{array} \right) \approx \frac{\xi_n^k}{k!} \exp(\, -\xi_n \,), \tag{21.13}$$

by Poisson's limit theorem, where $\xi_n = n\,(1+s)/M$. Therefore, for the sampled mutant offspring

$$P[\,N_{\text{mut.}} = k\,] \;\approx\; \sum_n \frac{[\,n\,(1+s)/M\,]^k}{k!}\,\exp\,(\,-n\,(1+s)/M\,)\,. \qquad (21.14)$$

From (21.14) there follows that the sampled mean is $1+s$ and

$$\text{Sampled VMR} \;=\; \left(\frac{1+s}{M}\right)\,\text{vmr} + 1. \qquad (21.15)$$

21.1 Exercises

1. Derive (21.2).

2. Write a program to simulate a classical, stochastic, population genetics-type dynamics directly from the definition. (I.e., assuming a fixed population and replacement sampling; to keep the run-time reasonable, take $N_d = 10^6$.) Compare the prediction of formula (21.2) with a typical stochastic trajectory.

3. Continuing from Exercise 2, adopt one of the models of sampling with large VMR from the chapter, implement it in your program, and simulate time-to-escape. Compare with the theoretical formula, (21.6).

21.2 Notes

Rouzine *et al.* [267] derived the formula (21.2), as well as a diffusion approximation for the stochastic regime of single-locus, classical genetics; this kind of approximation dates to the 1930s. That N_d/VMR is approximately the inverse probability of two PITs having the same parent was proved in [314], but similar results also date to the 1930s and can be found in any genetics text.

Chapter 22

Extinction Probabilities with Extra-Poisson Variation

In the simplest example of EPV adopted in our HIV infection model, there is one subtlety about extinction probabilities that needs to be addressed. It is worthwhile estimating the extinction probability of a continuous-time branching process with general offspring distribution.

Consider the simplest process, defined by: population variable X, taking integer values, jumps to $X + n$, $n = 0, 1, \ldots$, with rate $\rho_n X$, and to $X - 1$ with rate δX. Let $p_n = \rho_n/\delta$ and assume that $\sum_n p_n = 1$, which implies that each parent makes on average $M \equiv \sum_n p_n n$ offspring before dying. By introducing the probability-generating function of the process (see any branching-process text) or informal reasoning about the first event, the extinction probability, q, starting with one individual can be shown to be the root of

$$\psi(q) \equiv \sum_n p_n q^{n+1} - 2q + 1 = 0. \tag{22.1}$$

The quadratic approximation, obtained by expanding ψ to second order around $q = 1$, yields the approximation

$$\begin{aligned} q_{\text{quad.}} &= 1 - \left(\frac{2\left[\sum_n p_n n - 1\right]}{\sum_n n^2 + \sum_n n} \right) \\ &= 1 - \left(\frac{2\left[1 - 1/M\right]}{\text{VMR} + M + 1} \right), \end{aligned} \tag{22.2}$$

where VMR is the variance-to-mean ratio of the offspring distribution. Since the next term in the Taylor series is negative, $q_{\text{quad.}}$ only yields an upper

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bound on q, equivalent to a lower bound on $p_{\text{grow}} = 1 - q$:

$$p_{\text{grow}} \;\geq\; \frac{2\,(\,1 - 1/M\,)}{\text{VMR} + M + 1}. \tag{22.3}$$

To deduce interesting consequences of extra-Poisson variation, we need a lower bound on q, so consider the cubic approximation to ψ. Since $\psi(1) = 0$, the result is a quadratic with discriminate

$$D \;=\; [\,\psi''(1)\,]^2/4 \;-\; 2\,\psi'(1)\,\psi'''(1)/3. \tag{22.4}$$

As we will see shortly, the condition $D > 0$ rules out uninteresting cases with large VMR but which makes no contribution to the important probabilities. Assuming $D > 0$, the quadratic has two roots in $[0, 1]$ but the larger one gives the better estimate, which is now a lower bound on q since the next term in Taylor's formula is positive. Stated as a bound on survival probability, we obtain:

$$\begin{aligned} p_{\text{grow}} \quad &\leq \quad \frac{4\,(\,1 - 1/M\,)}{\text{VMR} + M + 1 + 2\,\sqrt{D}} \\ &\leq \quad \frac{4\,(\,1 - 1/M\,)}{\text{VMR} + M + 1}. \end{aligned} \tag{22.5}$$

i.e., the upper bound differs only by a factor of two from the lower bound using $q_{\text{quad.}}$.

To grasp the meaning of the condition on the third moment, consider the following cases with three non-zero offspring probabilities. First, let $p_0 + p_1 + p_K = 1$, with K large (e.g., $K = 10^4$) but $M = 1.5$. Then $\psi'' \approx p_K\,K^2$, $\psi''' \approx p_K\,K^3$, so

$$D \;\approx\; p_K\,K^3\,[\,p_K\,K/4 \;-\; 2\,(M-1)/3\,]. \tag{22.6}$$

Thus the condition reads: $M < 1 + (8/3)\,p_K\,K$, which says that the tail probability contributes substantially to the mean, which it must to make $M = 1.5$. That the bound (22.5) yields a very small p_{grow} follows intuitively from the fact that the process is dominated by individuals with zero or one offspring; so their lineage tends to go extinct unless rescued by a rare event. By contrast, the case: $p_1 + p_2 + p_K = 1$, again with K large and $M = 1.5$, can have large VMR but the branching-process need not have small survival probability.

22.1 Exercise

1. Derive formula (22.1) by reasoning about the first event. (Hint: what is $P\,[\,\text{Extinct}\,|\,n\,]$ in terms of $P\,[\,\text{Extinct}\,|\,1\,] = q$?)

Chapter 23

Computing the Boundaries

In this chapter we perform some computations relevant to the boundary between stability and escape for certain scenarios discussed in Chapters 7 and 8.

For the critical case $E = 3$, and assuming a simple dependence on number of mutations, let $\mathrm{LOF}(k : n)$ denote the loss-of-fitness of a strain with k mutations relative to one with n, and likewise $R_0(n)$ be the basic reproductive number of a strain with n mutations. In particular let

$$\begin{aligned} \mathrm{LOF}(1:0) &= 1 - \left\{ \frac{R_0(1)-1}{R_0(0)-1} \right\} \equiv r; \\ \mathrm{LOF}(2:1) &= 1 - \left\{ \frac{R_0(2)-1}{R_0(1)-1} \right\} \equiv s; \end{aligned} \tag{23.1}$$

which implies

$$\begin{aligned} \mathrm{LOF}(2:0) &= 1 - \left\{ \frac{R_0(2)-1}{R_0(0)-1} \right\} \\ &= 1 - (1-r)\,(1-s). \end{aligned} \tag{23.2}$$

The last result was used to compute the boundary with equivalent epitopes.

It is also of interest to ask whether epistasis is involved here. The condition "no epistasis" corresponds to $r = s$. Positive or negative epistasis corresponds to taking the greater-than sign, respectively less-than sign, in

$$\begin{aligned} 1 - \mathrm{LOF}(2:0) &\gtrless [\,1 - \mathrm{LOF}(1:0)\,]^2, \\ (1-r)\,(1-s) &\gtrless (1-r)^2; \end{aligned} \tag{23.3}$$

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hence positive epistasis is equivalent to $r > s$. Thus the escape boundary in this case corresponds to negative epistasis.

Concerning immunodominance, again consider the critical case of three responses and one relevant mutation locus per epitope. The interior of the no-replacement domain is given by the three inequalities:

$$\mathrm{REA}_1 \;<\; \mathrm{LOF}_1; \qquad \mathrm{REA}_2 \;<\; \mathrm{LOF}_2; \qquad \mathrm{REA}_3 \;<\; \mathrm{LOF}_3. \tag{23.4}$$

The necessary and sufficient condition for the existence of this set of favorable immune parameters is

$$\mathrm{SOL}: \qquad \mathrm{LOF}_1 \;+\; \mathrm{LOF}_2 \;+\; \mathrm{LOF}_3 \;>\; \sum_{m=1}^{3} \mathrm{REA}_m. \tag{23.5}$$

(The necessity is obvious; the sufficiency proof is left to the reader.) If each mutation totally abrogates recognition of one epitope, the REA_m's sum to one and the condition becomes

$$\mathrm{SOL}: \qquad \mathrm{LOF}_1 \;+\; \mathrm{LOF}_2 \;+\; \mathrm{LOF}_3 \;>\; 1. \tag{23.6}$$

To explore in a simple case the domain defined by these conditions, when it exists, consider the scenario: first response dominant, equal responses in the subdominant pair, total abrogation, and the relevant mutations in the subdominant epitopes have equal LOFs. Define $\mathrm{DR} = (\kappa_1 C_1)/(\kappa_2 C_2)$; the conditions (23.4) reduce to

$$\frac{1}{\mathrm{LOF}_2} \;-\; 2 \;<\; \mathrm{DR} \;<\; \frac{2\,\mathrm{LOF}_1}{1-\mathrm{LOF}_1}. \tag{23.7}$$

Using the sum SOL, we can rewrite the lower bound as:

$$2\left\{\frac{1}{\mathrm{SOL}-\mathrm{LOF}_1} \;-\; 1\right\} \;<\; \mathrm{DR}. \tag{23.8}$$

The lower bound is only interesting if it is somewhere larger than one, which occurs provided $\mathrm{LOF}_2 < \frac{1}{3}$, implying $\mathrm{SOL} < 1\,\frac{2}{3}$. The conclusions and Figure in Section 8.1 follow from these inequalities.

23.1 Exercises

1. Make a more comprehensive study of the relationship between immunodominance and the no-replacement domain defined by the Escape Formula. It might be interesting to consider many (e.g., 20) epitopes.

2. Adopt probability distributions for the LOF parameters, as in Section 7.2 (better: derive these distributions from published experiments), and study the expected "volume" of the no-escape domain as a function of the number of epitopes and immunodominance.

Chapter 24

Co-existence or Replacement?

In this section we consider whether it is possible for variants with escape mutations to co-exist with the wild-type. We define "possible co-existence" to mean that a mathematical steady-state with two or more large viral populations is neither ruled out by the structure of the model nor requires a "knife-edge" coincidence of parameter values. For simplicity in this discussion, we will assume the infection model without eclipse period or multiple types. Consider the case of three epitopes recognized ($E = 3$) and mutants that delete epitope one or epitope two. The definition of steady-state yields the familiar conditions (i.e., as in the derivation of the Escape Formula):

$$\begin{aligned} R_0(\text{w.t.}) &= 1 + (\,\kappa_1 C_1 + \kappa_2 C_2 + \kappa_3 C_3\,)/\delta; \\ R_0(\text{mut.;1}) &= 1 + (\,\kappa_2 C_2 + \kappa_3 C_3\,)/\delta; \\ R_0(\text{mut.;2}) &= 1 + (\,\kappa_1 C_1 + \kappa_3 C_3\,)/\delta. \end{aligned} \tag{24.1}$$

In these equations, assume the parameters, κ_1, κ_2, κ_3, δ, R_0(w.t.), R_0(mut.;1) and R_0(mut.;2) are known; then with a little algebra C_1, C_2 and C_3 can be found. All three are positive if and only if (Exercise 1):

$$\begin{aligned} R_0(\text{w.t.}) &> R_0(\text{mut.;1}); \\ R_0(\text{w.t.}) &> R_0(\text{mut.;2}); \\ R_0(\text{mut.;1}) + R_0(\text{mut.;2}) &> 1 + R_0(\text{w.t.}). \end{aligned} \tag{24.2}$$

The first two inequalities simply express the concept of LOF in 1-mutants, but the third is restrictive.

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Next let us enquire whether positive values exist for the steady-state viral populations. Let $Y_{\text{w.t.}}$, $Y_{\text{mut.};1}$, and $Y_{\text{mut.};2}$ denote the steady-state wild-type and mutant viral populations (PITs), that have deleted epitope 1 or 2, respectively. Again for simplicity, let the three clones differ only in their activation parameters, α_1, α_2, and α_3. With more simplifications in the CTL model, it is possible to reduce the steady-state ODE equations to the form (Exercise 2):

$$\begin{aligned} C_1 &= f\left(\alpha_1 \left\{ Y_{\text{w.t.}} + Y_{\text{mut.};2} \right\}\right); \\ C_2 &= f\left(\alpha_2 \left\{ Y_{\text{w.t.}} + Y_{\text{mut.};1} \right\}\right); \\ C_3 &= f\left(\alpha_3 \left\{ Y_{\text{w.t.}} + Y_{\text{mut.};1} + Y_{\text{mut.};2} \right\}\right). \end{aligned} \tag{24.3}$$

Moreover, the function $f(\cdot)$ enjoys the properties

$$f(0) = 0; \qquad f'(x) > 0; \qquad f(x^*) = \infty. \tag{24.4}$$

(The last means that f has a vertical asymptote at some positive value x^*.) Let us assume that positive values of C_1, C_2 and C_3 result from solving system (24.1). From the properties of $f(\cdot)$ it follows that the inverse function, $f^{-1}(\cdot)$, exists on the whole positive axis and we can define

$$A_i = f^{-1}(C_i)/\alpha_i; \qquad i = 1, 2, 3. \tag{24.5}$$

Positive solutions of system (24.3) exist if and only if (Exercise 3):

$$\begin{aligned} A_3 &> A_1; \\ A_3 &> A_2; \\ A_1 + A_2 &> A_3. \end{aligned} \tag{24.6}$$

Because (24.2) and (24.6) are systems of inequalities rather than equalities, which moreover are not contradictory, we conclude (although have not proved) that co-existence of wild-type and 1-mutants is possible in the model.

However, now add the 2-mutant, that has deleted epitopes 1 and 2. The first system has an additional equation:

$$R_0(\text{mut.};1,2) = 1 + (\kappa_3 C_3)/\delta; \tag{24.7}$$

and, in general, it is impossible to satisfy four equations with three unknowns (C_1, C_2 and C_3). The same impediment appears if we permit the other 1-mutant (that deletes epitope 3). Hence, co-existence of all these variants

with the wild-type is impossible, which explains the events shown in the simulations, Figures 4.4 and 8.6. Non-existence of a steady-state could imply oscillation rather than extinction, so again, we have not formally proven that the appearance of the 2-mutant, or all three 1-mutants, in fact dooms the wild-type to oblivion.

24.1 Exercises

1. Verify that the inequalities of system (24.2) are indeed the solvability conditions for system (24.1).

2. Consider the simplified cellular immune-system model of Chapter 19, Exercise 13. Show that the system obtained by setting the right-sides to zero in (19.17) can be collapsed to a single equation of form:

$$C \;=\; f\,(\alpha\, Y)\,. \tag{24.8}$$

   Verify that the properties in (24.4) hold for this function.

3. Verify that the inequalities of system (24.6) are indeed the solvability conditions for system (24.3).

Chapter 25

The Nef-Deletion Threshold Formulas

We derive the results stated in Chapter 9. The Nef-maintenance condition follows from the Escape Formula as usual (escape is impossible if $\text{REA}_m < \text{LOF}_m$) and can be derived from re-arranging the definition of REA to read

$$\begin{aligned} \text{REA}_m &= 1 - \left(\frac{\sum_{e=2}^{E} f\,\kappa_e\,C_e/\delta}{\sum_{e=1}^{E} \kappa_e\,C_e/\delta} \right) \\ &= 1 - f \times \text{FOE}. \end{aligned} \tag{25.1}$$

We next derive the replacement threshold for trading the defensive cloak for enhanced replication. Following the familiar route of Chapter 20, we have,

$$\begin{aligned} R_{\text{eff.}}(\text{w.t.}) &= \frac{R_0(\text{w.t.})}{1+\phi}; \\ \phi &= \sum_{e=1}^{E} \kappa_e\,C_e/\delta. \end{aligned} \tag{25.2}$$

Also for the mutant,

$$R_{\text{eff.}}(\text{mut.;m}) = \frac{R_0(\text{mut.;m})}{1+f\,\phi}. \tag{25.3}$$

Assuming a quasi-steady-state for the existing virus,

$$R_0(\text{w.t.}) - 1 = \phi; \tag{25.4}$$

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and replacement is possible if and only if

$$R_0(\text{mut.;m}) \;-\; 1 \;>\; f\,\phi. \tag{25.5}$$

Hence, dividing,

$$\frac{R_0(\text{mut.;m}) - 1}{R_0(\text{w.t.}) - 1} \;>\; f. \tag{25.6}$$

As stated in Chapter 9, all R_0's contain a factor of U; including this factor, we can write

$$\begin{aligned} R_0(\text{w.t.}) &= \psi_{\text{w.t.}}\,U; \\ R_0(\text{mut.;m}) &= \psi_{\text{mut.}}\,U. \end{aligned} \tag{25.7}$$

where the ψ-factors combine quantities such as infectivity and production. Inequality (25.6) becomes

$$F(U) \;=\; \frac{\psi_{\text{mut.}}\,U - 1}{\psi_{\text{w.t.}}\,U - 1} \;>\; f. \tag{25.8}$$

The threshold follows from an analysis of the function F. A calculus formula (see Exercise 1) is useful here. Note that from the definition of LOF in Chapter 20 and the quantities defined here,

$$\text{LOF} \;=\; 1 - \frac{\psi_{\text{mut.}}\,U - 1}{\psi_{\text{w.t.}}\,U - 1}; \tag{25.9}$$

so from the rule

$$d\text{LOF}/dU \;=\; -\,\frac{(\,\psi_{\text{w.t.}} - \psi_{\text{mut.}}\,)\,U}{(\,U\,\psi_{\text{w.t.}} - 1\,)^2}. \tag{25.10}$$

Thus, as we noted in Chapter 8, Section 8.9, if a mutation does not enhance intrinsic fitness then LOF is decreasing with increasing U—meaning LOF increases as U falls in late disease.

For the proof of the threshold formula, simply note these facts about F: enhancement means $\psi_{\text{mut.}} > \psi_{\text{w.t.}}$; hence $F'(U) < 0$; the graph of F has a horizontal asymptote at $\psi_{\text{mut.}}/\psi_{\text{w.t.}}$; the graph of F has a vertical asymptote at $1/\psi_{\text{w.t.}}$; and the inequality (9.2) assumed for the existence of the threshold. The exact threshold of (9.3) follows by inverting $F(U) = f$ and noting that the ratio $\psi_{\text{mut.}}/\psi_{\text{w.t.}} = R_0(\text{mut.;m})/R_0(\text{w.t.})$, independent of U.

25.1 Exercises

1. Prove the following calculus lemma:

   Lemma 1 *If*

   $$H(x) = \frac{ax+b}{cx+d}, \tag{25.11}$$

   then

   $$H'(x) = \frac{ad-bc}{(cx+d)^2}. \tag{25.12}$$

   The rule can be remembered as stating: "If you differentiate a ratio of linear expressions (a special case of a "rational fraction"), the answer is the determinant over the denominator, squared".

2. Prove the claims of the last paragraph of the chapter. (Make a sketch of the graph of F.)

Chapter 26

Modeling Retroviral Sex

The infected-cell-compartment variables are denoted $X_{(\mathbf{n};m;a)}$, where $\mathbf{n} = (n_0, n_1, \ldots, n_S)$, $n_i =$ is the number of wild-type proviruses in stage i; $m = 0, 1, 2$ indicates no mutant proviruses, one with the first mutation, or one with the second mutation, respectively; and a is the stage ("age") of the mutant provirus (if present). We will sometimes write $X_{\mathbf{g}}$ for $X_{(\mathbf{n};m;a)}$, abbreviating the provirus composition by $\mathbf{g} = (\mathbf{n}, m, a)$. Since the double-mutant is not represented by a cell-compartment, the indices on $X_{(\mathbf{n};m;a)}$ run over the range: $0 \leq \sum n_i + 1[m > 0] \leq \text{PVMAX} + 1$. By repeated use of indicator-function restriction, $1[\ldots]$, the rate-equations for the compartmental variables can be compactly expressed as:

$$dX_{(\mathbf{n};m;a)}/dt \;\; =$$

$$1[\, n_0 = 1; \sum_{2}^{S} n_k = 0; m = 0\,]\, U\, I_{w.t.} \tag{26.1}$$

$$+\, 1[\, n_0 = 0; \sum_{2}^{S} n_k = 0; m > 0\,]\, U\, I_m \tag{26.2}$$

$$+\, 1[\, n_0^{'} = n_0 - 1; n_0 > 0; n_i^{'} = n_i; i = 1 \ldots S; m' = m; a' = a\,]$$
$$\times\; 1[\, (n', m', a') \text{ is infectible}\,]\, X_{(\mathbf{n}';m';a')}\, I_{w.t.} \tag{26.3}$$

$$+\, \rho \sum_{i>0}^{S} 1[\, n_I > 0\,]\; (\, n_{i-1} + 1\,)\; X_{(n_0,\ldots n_{i-1}+1, n_i - 1, \ldots, n_S; m; a)} \tag{26.4}$$

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$$+\,\rho\,1[\,m>0; a\geq 1\,]\,X_{(n;m;a-1)} \tag{26.5}$$

$$-\,\rho\sum_{i<S} 1[n_i>0\,]\,n_i\,X_{(\mathbf{n};m;a)} \tag{26.6}$$

$$-\,\rho\,1[\,m>0\,]X_{(\mathbf{n};m;a)} \tag{26.7}$$

$$-\,\delta\,1[\,\mathrm{PIT}\,]\,X_{(\mathbf{n};m;a)} \tag{26.8}$$

$$-\,1[\,\mathrm{p.v.s}<\mathrm{PVMAX}\,]\,I_{w.t.}\,X_{(\mathbf{n};m;a)}. \tag{26.9}$$

$$-\,1[\,\mathrm{p.v.s}<\mathrm{PVMAX};\,m=0\,]\;(\,I_1+I_2\,)\;X_{(\mathbf{n};m;a)}. \tag{26.10}$$

Here U stands for uninfected target cells; ρ is the rate of progression through stages; $\delta=\delta_{\mathrm{IT}}+\kappa$ CTLs is the killing rate; $I_{w.t.}$ is the force-of-infection with wild-type proviruses; and I_m, $m=1,2$ is the force-of-infection by mutant proviruses. The latter are defined from the general expression for force-of-infection by a provirus of genome "g" due to virions made by PITs of composition $\mathbf{g}=(\mathbf{n},m,a)$:

$$\begin{aligned} I(g) &= \sum_{\mathbf{g}} \iota(g\,|\,\mathbf{g})\,X_{\mathbf{g}};\\ \iota(g\,|\,\mathbf{g}) &= \phi(\mathbf{g})\sum_{g',g''} p(g',g''\,|\,\mathbf{g})\,q(g\,|\,g',g''). \end{aligned} \tag{26.11}$$

Here

$\phi(\mathbf{g})$ = expected phenotype of a virion made by a PIT of composition $\mathbf{g}$;

$p(g',g''\,|\,\mathbf{g})$ = probability of making a virion packaging RNAs g' and g'', from a PIT of composition $\mathbf{g}$;

$q(g\,|\,g',g'')$ = probability of making a provirus of genome g from two RNAs of type g' and g''.

The phenomena of "phenotypic mixing" appears in formulating $\phi(\mathbf{g})$; e.g.,

$$\phi(\mathbf{g}) = \left(\frac{\iota}{M}\right)\sum_i 1[\,g_i \text{ is "mature"}\,]\,\phi_i;$$

$$\begin{aligned} M &= \sum_i 1[\, g_i \text{ is ``mature''}\,]; \\ \iota &= \left(R_0\, \delta_{\mathrm{PIT}} \right) / U. \end{aligned} \tag{26.12}$$

Note how R_0, the basic reproductive number of the infection process absent "intervention" (by drugs or the immune system), enters into the infectivity factor (ι); δ_{PIT} is the inverse lifetime of a PIT (minus CTL killing). Here ϕ_i is the phenotype (infectivity) of a "homozygous" virion of genome g_i; any intrinsic LOF due to a mutation enters at this point. Having absorbed the overall infectivity into factor ι, we define conventionally $\phi_i = 1$ for the wild-type provirus, and $\phi_i = 1 - \mathrm{LOF}_m$ for the provirus carrying mutation m.

The probability $p(g', g'' \,|\, \mathbf{g})$ is just drawing with replacement. In the third probability the mutation and recombination rates enter in:

$$\begin{aligned} q(g \,|\, g_1, g_2) &= \frac{1}{2}\,(1-R)\,\{\, Q_{11}\, Q_{12} + Q_{21}\, Q_{22}\,\} \quad + \\ & \quad \frac{1}{2}\, R\,\{\, Q_{11}\, Q_{22} + Q_{21}\, Q_{12}\,\}; \\ & \quad Q_{i,j} = 1 - \mu + (\,2\,\mu - 1\,)\,\left| g^{(j)} - g_i^{(j)} \right|; \\ & \quad g^{(j)} = \text{j-th gene in genome } g. \end{aligned} \tag{26.13}$$

Here μ is the point-mutation rate and R is the recombination rate (between two specified sites in the viral genome corresponding to mutations 1 and 2).

The probability intensity of creating the double-mutant provirus in a PIT by either route in the next cycle can be written in terms of these quantities, as follows. Let $g_{\mathrm{d.m.}}$ denote the double-mutant provirus. By stepwise mutation:

$$\lambda_{\mathrm{step}}(t) = I(g_{\mathrm{d.m.}}) \left(U + \sum_{\mathbf{g}} 1[\,\text{infectible}\,]\, X_{\mathbf{g}} \right). \tag{26.14}$$

(Here t denotes time.) The intensity of forming the double-mutant, $I(g_{\mathrm{d.m.}})$, is given by a special case of (26.11)–(26.13), with $R = 0$. By recombination:

$$\begin{aligned} \lambda_{\mathrm{rec.}}(t) &= \lambda_{\mathrm{rec.};1}(t) + \lambda_{\mathrm{rec.};2}(t); \\ \lambda_{\mathrm{rec.};1}(t) &= I_1 \times \left\{ \sum_{\mathbf{g}} 1[\,\text{infectible};\quad 2 \in \mathbf{g}\,]\, X_{\mathbf{g}}\, P_{\mathrm{rec.}}[\,\mathbf{g} \cup 1\,] \right\}, \end{aligned} \tag{26.15}$$

and the other case is similar. Here $2 \in \mathbf{g}$ means $\mathbf{g}$ contains a second-mutant provirus and $\mathbf{g} \cup 1$ denotes the union of $\mathbf{g}$ with a first-mutant provirus. $P_{\text{rec.}}[\,\mathbf{g}\,]$ is the probability that a PIT infected with multiple proviruses gives rise, by recombination, to a PIT infected by the double-mutant provirus in the next round of replication. Equation (26.15) simply states the rate a PIT with a second-gene-mutated provirus is reinfected with a first-gene-mutant provirus and then the genes are combined in the next cycle.

$P_{\text{rec.}}$ depends on the recombination probability and (a) the number of offspring PITs (Poisson distributed); (b) the "ages" of the mutant and wild-type proviruses; (c) the current PIT lifetime (which reflects CTL killing and natural death). These factors are combined as follows:

$$P_{\text{rec.}}[\,\mathbf{g}\,] = \Big\{ 1 - \exp\Big(-T_{\text{het.}}\, P_{\text{het.}}\, R_{\text{eff.}}\, \delta \Big) \Big\} \Pr[\text{rec.}]\,; \quad (26.16)$$

$$T_{\text{het.}} = \delta^{-1} - \max(a_1, a_w)\, \rho^{-1}; \quad (26.17)$$

$$P_{\text{het.}} = 2/[\,2 + \text{no. of mature w.t. proviruses}\,]^2; \quad (26.18)$$

$$R_{\text{eff.}} = (\,\iota\, U\,)\,/\delta. \quad (26.19)$$

$T_{\text{het.}}$ accounts for the time that passes before the "younger" mutant provirus matures, with δ the current inverse lifetime of a PIT; a_1 the "age" of the oldest mutant provirus; and a_w the "age" of the oldest wild-type provirus. $P_{\text{het.}}$ is simply the probability that a virion drawn from a PIT containing both mutant proviruses packages both (i.e., is a "heterozygous" virion). $R_{\text{eff.}}$ is the current effective reproductive number of the PIT (δ may depend on time because it reflects the present rate of CTL killing). Pr[rec.] is the probability of creating the double-mutant genome by recombination from a "heterozygous" infecting virion, given by $q(g_{\text{d.m.}} \,|\, g_1, g_2)$, where g_i has mutation i, in formula (26.13) with $\mu = 0$. Formula (26.19) expresses the probability, in a Poisson process whose rate is the product in the exponent, of a dually-infected PIT producing at least one offspring, times the probability that the double-mutant genome is created in that offspring by recombination.

The probability distribution of the time-of-appearance of the double-mutant is given in terms of the relevant intensity by

$$P[\,T_{\text{d.m.}} > t\,] = \exp\Big(-\int_0^t \lambda(s)\, ds \Big);$$

$$\lambda(t) = \lambda_{\text{step}}(t), \quad \text{for stepwise alone;}$$

$$\lambda(t) = \lambda_{\text{rec.;1}}(t) + \lambda_{\text{rec.;2}}(t), \quad \text{for recombination alone;}$$

$$\lambda(t) = \lambda_{\text{step}}(t) + \lambda_{\text{rec.;1}}(t) + \lambda_{\text{rec.;2}}(t), \quad \text{when including both.}$$

Note that this probability, despite its origin in a Poisson waiting-time problem, can also be evaluated by ODE methods, since

$$dP_t/dt \;=\; -P_t\,\lambda(t). \tag{26.20}$$

The dynamics of HIV-specific CD8 T-cells were also implemented as ODEs, according to the model of Chapter 3. The combined system, (26.10), the cellular immune system, and (26.20), was solved by standard techniques: Runge-Kutta, 4-th order, for the compartmental systems and, for the probability equation (26.20) (which may have a large negative eigenvalue), an implicit solver.

26.1 Notes

Concerning the mathematical tractability of the waiting-time problem beyond the approximation made here, Christiansen *et al.*, [59], in 1998 defined three levels of approximation for a classical Fisher-Wright population. The first level describes populations through the single-mutant stage by deterministic, compartmental laws, then derives the time-to-appearance distribution of the double-mutant; we followed this program in this chapter. In the second level, the single-mutant populations are regarded as independent branching-processes, and in the third the composite population is treated as fully stochastic. Besides the different mode of reproduction of retroviruses, interest here resides in a different fitness scenario (single-mutant disadvantage and double-mutant selected, which we call "escape"; for Fisher-Wright populations see [84, 156]), as opposed to genetic drift where "single-mutant types are neutral or weakly favored" ([59], p. 201). Concerning Christiansen *et al.*'s second level of approximation, given that the 1-mutants remain many orders-of-magnitude below the wild-type, even the presence of the immune response will not result in such large interactions (due e.g., to stimulating the same clones of CTLs), as to abrogate the branching-process approximation—although, for the primary-infection problem, the rate-constants for each process will be dependent on time. However, to develop this approximation scheme in our continuous-time, recombination model, mathematical methods will first have to be developed to evaluate functionals of type

$$\exp\left(-\int_0^t ds\,\sum a_{i,j}\,X_j^{(1)}(s)\,X_k^{(2)}(s)\right). \tag{26.21}$$

where $X^{(1)}(t)$ and $X^{(2)}(t)$ are independent branching-processes with time-dependent transition rates.

26.2 Exercise

1. In the last paragraph of the chapter we encountered an ODE of form

$$\begin{aligned} dx/dt &= F(x) \\ &= -\lambda\, x, \end{aligned} \tag{26.22}$$

which is certainly very simple if λ is a positive constant; the solution is $x = x_0 \exp(-\lambda\, t)$ and heads straight for the origin. Curiously, this equation is a conundrum for Euler or RK if λ is large. The Euler simulation method yields for $n+1$ time-units of h:

$$\begin{aligned} x_{n+1} &= x_n + h\, F(x_n); \\ x_{n+1} &= x_n - \lambda\, h\, x_n; \\ x_n &= x_0\, (1 - \lambda\, h)^n, \end{aligned} \tag{26.23}$$

which explodes to infinity if $h > 2/\lambda$ and oscillates around the origin if $2/\lambda > h > 1/\lambda$. An *implicit solver* generates the next x_n using the last and itself:

$$x_{n+1} = x_n + h\, F(x_{n+1}). \tag{26.24}$$

Show that this method generates a sequence which tends to zero for any h. Program an implicit solver for a general 1-compartment, time-inhomogeneous ODE and simulate the solution of

$$dx/dt = -10^3\, (1+t)\, x\, (1-x) \tag{26.25}$$

with $x_0 = 0.1$ and $x_0 = 1.1$ Compare with Euler or RK3 on this problem.

26.3 Notes

For a general implicit solver for ODEs, see [247], Chapter 15.6 (on so-called "stiff" equations, a term peculiar to numerical analysis for a hard problem).

Glossary

a.a.	Amino-acid; a building-block of a protein
Antibody	A molecule produced by B-cells; can bind and neutralize a virion
Antigen	Any molecule recognized by the immune system
APC	Antigen-presenting cell; includes macrophages, DCs and B cells
Assay	A laboratory test, for the presence of a molecule, cell, or virus
B cell	An immune-system cell; secretes antibodies and can act as an APC
CCR5	A membrane molecule called a co-receptor; found on some T-cells, used by variants of HIV to enter cells
CD4	A membrane molecule; found on some T-cells, used by HIV to enter cells
CD8	A membrane molecule; found on some T-cells

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Cell-cycle	The series of stages a proliferating cell passes through before dividing
Co-receptor	A molecule found on the membrane of CD4 T-cells that is required (in addition to CD4) for HIV entry
CTL	Cytotoxic T-lymphocyte; CD8 "killer" T-cell
CXCR4	A membrane molecule called a co-receptor; found on most T-cells, used by variants of HIV to enter cells
DC	Dendritic cell; an immune-system cell that takes up and presents antigen to T-cells (see also "APC")
Deterministic	Not random (stochastic); what Einstein meant by "God does not throw dice."
Dynamical (law)	A rule that defines the future evolution of the system
Env	Envelope; HIV proteins constituting part of its exterior shell; recognized by antibodies
EPV	Extra-Poisson variation (in replication); heterogeneity beyond a dart-game, a possible mechanism for diminished N_e
ESA	Effective selective advantage (of making a mutation); REA–LOF
Exponential (law)	A probability distribution for a waiting-time; required by the Markov property
Gag	An HIV structural protein; possibly harder to mutate and so often included in vaccines

Genome	The molecules that store an organism's genetic information; RNA or DNA (RNA in HIV; DNA in people)
Genotype	An organism's genetic information; as contrasted with phenotype
gp120	Glycoprotein 120; part of HIV's envelope (Env)
HAART	Highly-active antiretroviral therapy; aka ART, triple-combination therapy
Helper cell	A CD4 T-cell; helps B cells and CD8 T-cells by secreting stimulatory factors
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen; a molecule that presents antigen to T-cells
Homeostasis	Tendency to return to the original state when perturbed
IC	Immune-control; the theory that the chronic steady-state is established by CTLs
Killer cell	aka CTL; a CD8 T-cell that can kill a PIT
LOF	Loss-of-fitness; fractional loss in HIV replication due to a mutation
Lymphocyte	A cell frequently found in lymph nodes; a T- or B-cell
Markov (property)	A mathematical property of a dynamical law; future and past are independent, given the present

Mutation	Change in a genome; replacement of one or more nucleotides, may or may not affect phenotype
N_d	Demographic population size; the true number (e.g., of PITs)
N_e	Effective population size; perhaps lower than N_d; may reflect EPV
Nef	An HIV regulatory protein; mounts a partial defense against immune recognition
NHP	Non-human primate model; mainly SIV or SHIV injected into a macaque
Nucleotide	One of the bases (molecules) that makes up a genome
Peptide	A small molecule, made of amino acids strung together; usually derived from a protein
Phenotype	An organism's behavioral repertoire; as opposed to genotype
PIT	Productively-infected target cell; mainly activated, infected CD4 T-cells
PP	Programmed proliferation (of an activated T-cell); division continues for some cycles without re-exposure to antigen
R5	An HIV variant; uses the CCR5 receptor to enter T-cells
REA	Relative escape advantage; fraction of immune pressure relieved by a mutation

Reagent	A chemical used to detect another; a critical resource in most laboratory tests (see also "assay")
Recombinant	A virus formed from parts of the genome of two distinct viruses; result of retroviral sex
Set point	The VL after primary viremia but before AIDS; see also "steady-state"
SIV	Simian immunodeficiency virus
SHIV	A chimeric virus; an SIV backbone with HIV genes (often Env) substituted
Steady-state	A term from mathematics for stability, but not necessarily permanence or equilibrium; the situation after primary viremia but before AIDS
Stochastic	Random in the sense of dice-throwing; what Einstein objected to (as opposed to "deterministic")
T-cell	aka thymocyte; thymus-derived immune-system cell
TCD	Target-cell depletion; Phillip's 1996 theory of the chronic steady-state
Thymocyte	A cell frequently found in the thymus; see T-cell
Titer	Density of antibodies in serum; usually expressed as dilutions necessary to eliminate detectible activity
Viremia	Ongoing or increased viral replication
Virion	A viral particle

VL	Viral load (aka viremia level); usually virions per ml in blood
X4	An HIV variant; uses the CXCR4 receptor to enter T-cells

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