Detection of HIV-2 and HIV-1/2 Dual Infections

HIV-2 is a retrovirus with a structure and replication program similar to HIV-1 but is distinct from HIV-1 in origin, epidemiology, spread, disease progression, genetic organization, and nucleic acid sequence[37,38,267,268] (see Chapter 169). HIV-2 infections originally reported in West Africa are now present in Europe, especially Portugal, and in South Asia, including India.[269] The CDC reports that African countries with at least 1% HIV-2 prevalence include Angola, Cape Verde, Côte d'Ivoire, Gambia, Guinea-Bissau, Mali, Mauritania, Mozambique, Nigeria, and Sierra Leone. In addition, Benin, Burkina-Faso, Ghana, Guinea, Liberia, Niger, São Tomé, Senegal, and Togo are reported to have significant numbers of HIV-2 infections.[270] New initiatives have been implemented to track HIV-2 epidemiology.[271] Infection with HIV-2 does not prevent infection with HIV-1,[272] sequential HIV-2 to HIV-1 infections have been documented,[273] and dual HIV-1 plus HIV-2 infections are reported in West Africa and in India.[269,274,275] The prevalence of HIV-2 remains low and may be decreasing in some areas of West Africa.[276] In the United States HIV-2 remains infrequent but not rare.[277] The CDC reported 242 cases in 1987–2010[278]; Torian and colleagues[279] documented 62 HIV-2 infections in New York City between 2000 and 2008,[279] and Hollenbeck and coworkers[280] identified 12 patients in Providence, Rhode Island. The current prevalence in the United States is not known precisely. Individuals from countries with substantial populations of HIV-2 in West Africa and elsewhere emigrate to the United States, and the understanding of geographic distribution is useful in patient evaluation; as summarized by Gandhi and coworkers,[281] the HIV-2 epidemic has undergone complex dynamics and may be declining in West Africa, especially in younger individuals.

The ability to detect HIV-2 infection, discriminate HIV-2 from HIV-1, and identify HIV-1 plus HIV-2 dual infections has always been important from an epidemiologic and blood safety standpoint, but specific diagnosis has become essential in the era of effective ART. Specifically, HIV-2 reverse transcriptase is not sensitive to nonnucleoside reverse-transcriptase inhibitor (NNRTI) antiretrovirals, such as nevirapine, efavirenz, etravirine, or delavirdine. This is due to a Y188L polymorphism naturally occurring in HIV-2. As a result, patients with HIV-2 infection (or HIV-1/2 coinfection) should not be treated with NNRTI-based regimens, and misdiagnosis of HIV-1/2 coinfection as HIV-1 monoinfection may rapidly lead to complex drug resistance.

Initially, 60% to 90% of sera from HIV-2 infected individuals reacted with HIV-1 ELISA kits[282–284]; in the United States specific screening assays for HIV-2 were not initially developed because of the relatively few HIV-2 infections. Instead, combination screening assays[285] were developed to improve the sensitivity of HIV-1 assays, often incorporating synthetic peptides, including those from immunodominant domains of HIV glycoproteins.[286–292] Use of cross-absorption tests,[293] competitive ELISA,[294,295] and PCR assays were reported to distinguish infections.[296]

The development of an HIV-1/2 ELISA platform (e.g., GEENIUS [Bio-Rad]) that distinguishes whether reactivity is derived from HIV-1 or HIV-2 represents a particularly useful advance. The combination of rapid screening assay and point-of-care supplemental confirmatory assay offered, for the first time, an FDA-approved method for diagnosing HIV-2 infection in the United States.

Methodology for HIV-2 RNA levels has not been standardized to the same degree as HIV-1, and no FDA-approved tests are available for diagnosis; HIV-2 RNA assays are available for monitoring established disease.[268] IFAs have been used to distinguish HIV-1 and HIV-2 infection,[232] but currently there are no FDA-approved HIV-2 IFA test kits. HIV-2 cultivation from infected individuals has been reported,[297] but the frequency of infectious virus recovery was lower than that detected with HIV-1.[297]

As newly diagnosed cases of HIV are frequently referred for early initiation of ART, identifying the presence of HIV-2 either as a single infection or as HIV-1/2 dual infection is an absolute imperative. Gottleib and coworkers[268] have highlighted that achieving 90-90-90 targets for HIV-2 has lagged behind HIV-1, with a number of potential explanations. Torian and her colleagues[279] have documented a prolonged (>1 year) delay in documenting the presence of HIV-2 after a first diagnosis with HIV infection. In light of consequences for the emergence of drug resistance if NNRTI-containing regimens are initially used, careful assessment for HIV-infection must be considered in all individuals with HIV infection originating from, or ties to, West Africa. The CDC recommends HIV-2 testing for sex partners or needle-sharing partners of HIV-2–infected persons or individuals from countries with significant HIV-2 populations, individuals who have received transfusions or nonsterile needle exposures in countries with significant HIV-2 populations, and children of women known to be HIV-2 infected. Thoughtful consideration for HIV-2 must also be considered in individuals from West Africa who have been previously identified with HIV-1 and in whom HIV-2 has not been specifically evaluated; Torian and coworkers[279] noted that greater than 60% of the HIV-2 diagnoses had an initial diagnosis of HIV-1 only; the absence of testing does not mean the absence of infection.

Little is known regarding the HIV-2 window period; recently Cazals and colleagues[298] described HIV-2 seroconversion in detail in a single patient with seroconversion dynamics similar to that reported for HIV-1. An individual with symptoms consistent with HIV seroconversion had ELISA seroreactivity and p24 antigen reactivity within 21 days of symptom onset with an HIV Ag-Ab combination[298]; HIV-2 WB was indeterminate, but additional WB 50 days after symptom onset was positive. Performance characteristics of HIV testing assays in acute or recent HIV-2 infection have not been otherwise analyzed; third-generation HIV1/2 ELISA assays were less sensitive in detecting early HIV-2 infection.[299] There are few available seroconversion samples for HIV-2; use of experimentally infected primates with simian immunodeficiency virus (SIV; a close relative of HIV-2) has been suggested, but this method relies on the efficiency of detecting cross-species antibody, and the kinetics of an immune response may not be identical to HIV-2 infection in humans.

Detecting Non-B HIV-1 Types and Subtypes Other Than Subtype B

HIV-1 is composed of four principal groups: a large group, M (main), representing the majority of HIV-1 pandemic viruses; O (outlier); N (not M and not O[300,301]), and the recently isolated group P. The latter strain was identified in 2009 in a Cameroonian woman, and it is closely related to gorilla SIV (SIVgor), suggesting a different cross-species transmission compared with SIV in chimpanzees (SIVcpz).[302]

Group M viruses have been classified by phylogenetic analysis into a series of nine subtypes (A–J, except for E), five sub-subtypes, a growing number of circulating recombinant forms (circulating recombinant forms [CRFs], 58 so far), and an immeasurable number of unique recombinant forms (URFs). Non-B viruses account for almost 90% of HIV-1 infections globally and, due to travels and migratory waves, showed an increasing prevalence in previously subtype B–restricted areas (i.e., Europe and North America). In the past such variants were relatively difficult to detect with same sensitivity as subtype B, although more recent versions of ELISA assays have been optimized to include detection of non-B subtypes.[303,304] Group O viruses are more distantly related to the group M, have been identified at relatively low frequency in localized areas of central Africa,[305] and may have emerged after zoonotic events from chimpanzee or gorilla reservoirs.[306,307] Other groups are quite rare; group N ($N = 13$) and P ($N = 2$) viruses detected in West Africa are likely zoonotic events from nonhuman primates. Of note, all types and groups are detected by HIV ELISA assays, even groups N and P.[308]

The performance characteristics for many HIV-1 kits have been optimized using HIV-1 subtype B. Incorporation of HIV peptides with broad cross-reactivity into third-generation ELISAs has enabled detection of non-B subtypes, including more distantly related type O.[309–312] A peptide-based ELISA assay has been reported using V3 peptides to accurately discriminate among HIV and SIV viruses,[313]including group O, which detected group M and was used to identify the existence of group N isolates.[300] HIV-1 M subtypes, group O, and group N viruses are detected by ELISA, WB, and fourth-generation assays techniques. Qualitative TMA/HPA testing has also performed well in detecting non-B subtypes; all A, C, D, E, F, G, N, and O subtypes were detected, although as described later ("HIV Testing for Blood Donor Screening"), some viruses have not been detected in blood product surveillance. As

discussed before, PCR-based assays for viral load measurements also have difficulty detecting and reliably quantifying HIV-1 RNA when testing diverse genetic variants of HIV-1, especially group O and N. Different assays may yield discordant viral load results. No extensive data are available regarding detection of CRFs.[54]

Detecting HIV-1 Dual Infections

Two strains of HIV can infrequently infect the same person before host immunity develops, known as coinfection; otherwise, if the second strain is transmitted after seroconversion, this condition is named superinfection. One of the strongest markers of superinfection is the high frequency, at least 20% of the HIV pandemic, of recombinant viral strains worldwide. Clinical implications of HIV dual infection are acquisition of drug-resistant strains during effective therapy, thus leading to virologic failure and onward transmission of drug resistance if recombination occurs, and the impact on disease progression. Cornelissen and coworkers[314] showed that dual infection is associated with faster CD4 decline in patients with recent HIV infection. Detecting superinfection is difficult and hinges on detecting genetic differences between viral strains within the same individual: Frequent sampling; specific diagnostic assays; and sensitive sequencing methods, such as single-genome sequencing or next-generation sequencing, are fundamental. A new multiregion hybridization PCR assay in three regions of the HIV-1 genome (*gag p17, vpu,* and *nef*) was developed to detect the most common subtypes and CRFs in West Central Africa,[315] providing sufficient sensitivity and specificity so that it could be used as a screening tool in areas where multiple subtypes cocirculate with high prevalence, including areas of Europe.[316] Dual infections are difficult to detect and therefore difficult to quantify; studies show wide incidence variations, from no detected cases to rates close to the rate of incident HIV in the background population. This is due to wide variation of laboratory methods, sample timing and frequency, HIV-1 subtype, ongoing risk behavior, and ART use.

HIV Testing During Pregnancy or at Delivery

Diagnosis of HIV in pregnant women is a public health priority; the advent of effective therapy to reduce the risk of transmission has made such arguments more compelling. Diagnosis in pregnancy may be complicated by increased false-positive rates of ELISA screening assays with increased parity. In addition, women may present at delivery without previous evaluation, and rapid tests may be the only available modality to evaluate HIV infection expeditiously. In a study of 858 women diagnosed in labor, Constantine and coworkers[317,318] have suggested that a single-screening ELISA at delivery is sensitive, although the use of two rapid ELISA assays have performance characteristics as for the standard ELISA-WB strategy. Current guidelines for standard prenatal care include information regarding HIV testing; routine opt-out testing, except in jurisdictions where testing requires written informed consent; and testing early in pregnancy and in the third trimester in individuals at high risk for HIV infection. Some experts recommend repeat testing for all pregnant women in the third trimester. For women who present at delivery with unknown serostatus, 24-hour availability of rapid testing along with initiation of ART based on a single positive rapid result is recommended.[319]

HIV in Perinatal Diagnosis

Serodiagnosis of HIV infection in the perinatal period is complicated by the prolonged persistence of maternal antibody for approximately 18 months after delivery. Methods for diagnosing infection include the serial Aptima HIV-1 qualitative assay (plasma) and DNA PCR testing of peripheral blood mononuclear cells (whole blood or dried blood spots[320,321]). The sensitivity and specificity of DNA and RNA testing methods have been reviewed[322]; detection of non-B subtypes by qualitative RNA testing (Aptima) has had excellent reported results, and DNA PCR may be suboptimal with false-negative results documented.[323] The recent FDA approval of qualitative RNA testing (Aptima) for use in diagnosis (including pediatric diagnosis) may make this test the preferred modality. Alternative techniques to measure the presence of early HIV infection include direct HIV cultivation using techniques standardized by the AIDS Clinical Trials Group to evaluate HIV infection; a recent summary reports use of sequential sampling and analysis using DNA/RNA or p24 testing. Two positive samples obtained after ≥1 month and after ≥4 months indicates the presence of HIV infection. Negative HIV infection is based on two DNA or RNA or p24 determinations at ≥1 month and ≥4 months of age, or two negative serologic tests obtained separately at ≥6 months of age; repeating serologic evaluation at 18 months in individuals with prior negative results is common.[322] HIV-1 NAT early after birth must be interpreted with care because infection may take place at parturition, and HIV-1 RNA levels in plasma may be relatively low at delivery even in true infection. Early introduction of ART after delivery may also confound diagnosis; HIV RNA and DNA levels may be suppressed as a result of therapy, and complete antibody seroconversion may not take place in the setting of early ART. In such circumstances perhaps the only method to identify the presence of infection is close monitoring and serial testing after completion of a defined course of ART.

Maternal-fetal transmission of HIV-2 is much less common than HIV-1 (mother-to-child transmission rate of 4% vs. 15%–45%, respectively) but may occur especially if women are infected during pregnancy. Diagnosis of HIV-2 infection is similar to HIV-1, although Faye and coworkers[324] have reported that assays may be less sensitive than those for HIV-1. A full discussion of diagnosis of HIV infection in newborn can be found in the report by Read.[322]

HIV Testing for Blood Donor Screening

Laboratory methods for HIV detection were first implemented in populations as screening tools for blood products; more than 14,000 recipients of blood products as a risk factor for HIV infection have developed AIDS. Cases have decreased markedly; in the United States 40 adults and adolescents have been infected or developed AIDS after receiving blood screened as negative for HIV antibody,[325] but none since 2008.[326] Elsewhere, rare infections have occurred since the advent of NAT testing[327–330]; in some cases, transmissions are due to genetic differences of transmitted virus not detected by NAT, highlighting the need for vigilance in understanding genetic variability of HIV within countries. The residual risk for HIV infection in the United States is currently <1 : 1,000,000 units transfused.[331]

Blood surveillance procedures are designed to provide optimum safety of blood products; the screening phase begins with an interview and questionnaire, and donors may be screened out based on history; donors not screened out sign consent for donation and for HIV-1/2 testing with HIV-1/2 ELISA and NAT. Any sample reactive on either assay is discarded, and the sample is repeated in both screening assays; if the repeat screen is negative, a second screening assay is performed. If the second screening assay is negative, the sample is considered negative and the donor is not deferred. If the second screening assay is positive, the sample is considered repeatedly reactive and sent for confirmatory WB; positive WBs are repeated with a separate sample; and the results of ELISA and WB are reported to the patient with appropriate posttest counseling and referral. An individual with donor status of positive screening and with a negative confirmatory test remains under consideration. WHO recommendations for screening for blood safety involves a single screening assay for HIV infection; if reactive in a single test, the donation is discarded and the test repeated. The risk from blood donation in developed countries is relatively low[332]; recent analysis demonstrated the successful expansion of NAT screening over the 1998–2008 period, both in terms of numbers of countries screening and in terms of advances in technology and automation.[333] The majority of NAT screening is now conducted with multiplexed HIV/hepatitis C virus (HCV)/hepatitis B virus assays, based on either transcription-mediated amplification (TIGRIS [Gen-Probe]/ULTRIO [Grifols, Los Angeles, CA] systems) or multiplex polymerase chain reaction (MPX), performed on highly automated instrument platforms that ensure reliable results with excellent sensitivity and specificity.[334] Serologic screening must, however, be maintained, even with the most sensitive NAT testing performed on individual donations; rare low-viremic HIV-infected subjects (i.e., elite controllers) may indeed be seropositive but NAT

negative, given their unique control over viral replication. Overall, in the United States the estimated risk of HIV from transfusion is <1 per 10^6 units transfused.

Worldwide, blood safety in general varies considerably; in an estimated 39 countries, WHO reported that blood donations are not routinely tested for HIV, and 47% of donated units in low-income countries are tested in the absence of quality assurance (http://www.who.int/bloodsafety/global_database/GDBS_Summary_Report_2011.pdf). Dedicated efforts to improve safety have been implemented; in 2003 the President's Emergency Plan for AIDS Relief (PEPFAR) began supporting programs in 14 targeted countries in sub-Saharan Africa and the Carribean,[156] with improvements in blood donation infrastructure, increases in numbers of units donated, and decreases in HIV-reactive units. Challenges persist: a UNAIDS survey recently reported that >99% of all donations are appropriately screened for HIV in high-income and upper-middle-income countries; 76.2% to 83.2% of donations are appropriately screened for HIV in lower-middle-income and low-income countries.[335]

NAT has demonstrated a substantial impact worldwide. More than 270 million units have been screened by HIV NAT since its inception, and 244 (0.9/1 million) were NAT positive/serology negative. Although residual risk calculations may be overestimates,[336,337] the residual risk for HIV infection in the United States remains <1:1,000,000 units transfused.[331] Rare infections have occurred since the advent of NAT testing[327–330]; not all contaminated units result in infection.[338] In some cases transmissions are due to genetic differences of transmitted virus not detected by NAT, highlighting the need for vigilance in understanding genetic variability of HIV within countries.[337] Worldwide the demonstrated success of NAT has not yet been realized in all countries. The WHO Global Database on Blood Safety survey[339] reported that in 2013 176 of 180 reporting countries screened blood donations for HIV; 70 countries used antibody testing only, 106 reported use of antigen + antibody testing, and a total of 44 countries used NAT.

HIV Testing in Tissue Procurement for Transplantation

HIV transmission from organs transplanted from infected individuals occurred before the availability of sensitive and specific testing. In adults and adolescents there have been at least 50 cases of HIV transmission through transplantation or artificial insemination, sometimes involving multiple recipients. Strikingly, a 38-year-old male donor who tested negative for HIV and HCV antibodies at the time of donation transmitted HIV and HCV to four recipients.[340] A transmission event happened in 2009 from a living donor that resulted negative for HIV antibodies 79 days before organ recovery; a retrospective investigation revealed a reactive NAT on a stored sample obtained 11 days before transplant.[341] Both in the United States and internationally, donor screening before organ recovery has been based on ELISA testing since its introduction in 1985; after the implementation of NAT assays, greater than 50% of organ procurement organizations recently adopted this additional test to overcome the risk associated with the antibody window period. Such further screening raised the issue of losing more life-saving organs due to false-positive results: HIV transmission is an extremely rare event in the United States, and there is no nationwide policy for HIV screening for living donors. The CDC recommendations address (1) repeated donor testing with a combination of an HIV serologic test and HIV NAT as close to the time of organ donation as feasible logistically, but no longer than 7 days preceding organ donation; (2) all living donors should be advised of their obligation to avoid behaviors that would place them at risk for acquiring HIV infection before transplant surgery; and (3) for living donors with a history of high-risk behaviors (e.g., high-risk sexual activity or injection drug use) identified during evaluation, individualized counseling and a detailed discussion of specific strategies to avoid high-risk behaviors should be provided.

HIV Testing in Vaccine, Prevention, Postexposure, and Gene Therapy Studies

One recent issue concern in laboratory evaluation of HIV-1 infection is evaluation of patients enrolled in vaccine trials using one or more HIV antigens for immunization. If the vaccines themselves are effective immunogens, it is possible that patients will artifacually "seroconvert" for HIV in the absence of true infection. For example, individuals immunized with a single gene product (e.g., gp160) may develop specific gp160 reactivity.[342] If such individuals already have a nonspecific reactivity for p24, then the combination of reactivity to gp160 and to p24 will result in positive HIV screening and supplemental assays. Current and planned candidate vaccines include multiple HIV components, and effective immune responses may affect positive routine testing. Vaccine-induced seropositivity has important social implications, and vaccine recruitment trials clearly and plainly address possible outcomes.[343] Alternatively, patients in vaccine trials may become truly infected, and authentic infection requires identification. Careful history and physical examination are essential; application of NAT in such circumstances may yield useful results in establishing the presence of infection,[344] and individuals receiving HIV DNA vaccines have not yielded positive HIV NATs. CD4 counts and percentage of CD4 cells in peripheral blood are also useful because serial samples are likely available for comparison in vaccine studies.

HIV Testing in Gene Therapy Studies Using Lentiviral Vectors

The recent advent of lentiviral vectors for gene therapy raises possibilities that portions of the vector may be expressed and potentially result in false-positive HIV testing. The backbone for a number of gene therapy vectors is a lentivirus (typically HIV), which can infect nondividing cells, thereby expanding their range of cell types in which therapeutic genes can be introduced. The genomes of these vectors are highly deleted, but RNA produced by the vectors can contain HIV and can therefore be detected by HIV RNA assays. These vectors do not encode HIV *gag* or *env* genes, and serologic assays for HIV antigens or p24 assays have been nonreactive. As a consequence, such individuals may have HIV testing results similar to recent HIV infection (HIV RNA positive/antibody negative). An early analysis of large series of individuals who have received autologous cells transduced with lentiviral vectors did not document reactive serologies or detectable retroviral nucleic acid in plasma.[345] More recently, however, patients undergoing gene therapy have had detectable HIV RNA in plasma using conventional quantitative HIV RNA assays.[346–348] In all of these cases detailed analysis revealed that authentic HIV infection was not present. It is imperative that analyses of such cases proceed in a rigorous, methodical, and thoughtful fashion. Thorough understanding of the vectors and viral RNA assay are essential. Analysis of sequential samples is necessary; sequencing the nucleic acid present in plasma, if present in sufficient quantities, will provide useful information, and patient-derived sequences identical to vector-derived sequences will identify the source of the positive HIV RNA result as the vector.

In certain gene therapy settings, defective retroviral vectors have been used to complement patient gene deficiencies; cells from which these defective viruses are propagated include other "helper" viruses and may contribute to development of malignancy in certain situations.[349] Neither the nucleic acids nor gene products from the vectors or the helper cell lines are remotely related to HIV and are not sources of cross-reaction.

HIV Infection but Viral RNA Less Than 50 Copies/mL: HIV Elite Controllers

A small fraction of individuals infected with HIV do not progress to CD4 lymphopenia and do not have HIV viral RNA levels >50 copies/mL plasma in commercial assays; more sensitive single-copy assays do detect the presence of HIV in these so-called "elite controller" patients, and ongoing low-level HIV replication is detectable using sufficiently sensitive assays.[219,350,351] Despite the low level of plasma viremia and substantial CD4 cell numbers, all such patients are ELISA reactive and fully WB positive. As a result, patients who are newly diagnosed with HIV and have reactive HIV-1/2 ELISA tests and are WB positive but have HIV RNA levels <50 copies/mL are likely to be elite controllers; the presence of a fully positive WB makes early infection much less likely. All such patients should be repeatedly evaluated to document

the presence of HIV infection and absence of viremia; such patients are actively studied in tertiary care centers,[352–355] and referral for additional evaluation is often useful.

HIV Testing in the Setting of Acute Retroviral Syndrome or Other Acute Illness

Primary HIV infection may present as an acute viral syndrome with fever, lymphadenopathy, and rash (see Chapter 122). Diagnosis near the time of infection has obvious social, medical, and public health benefits, but not all screening strategies are equally effective in detecting primary HIV infection. TMA/HTA assays (Aptima[356]) are currently the most sensitive qualitative FDA-approved assays for acute HIV-1 infection. FDA-approved rapid/simple assays may not be as sensitive as standard third-generation ELISA assays. New fourth-generation ELISAs, as described earlier, have excellent performance characteristics and can detect antigens approximately 5 to 7 days after the appearance of nucleic acid. Of importance, the data published to date indicate the assays available in the United States are capable of detecting acute HIV infection in greater than 80% of individuals who are NAT positive but nonreactive or indeterminate in antibody-only assays. The window period for HIV infection may vary from patient to patient, and thus the value of a negative screening test must be weighed carefully but not exclusively. As outlined earlier, other acute infections may yield false reactivity in HIV screening assays. Similarly, primary HIV infection may present with relatively nonspecific symptoms and may give false-positive results for acute EBV infection. Thus review of such cases should be directed and comprehensive.

HIV Testing in Occupational Exposure

HIV-1 infection may be transmitted as an occupational exposure (see Chapter 298) in the hospital setting as a result of needlestick injury, transmission during surgery, and so forth; in outpatient dental circumstances; or other instances where contact favoring transmission may occur. It is essential that the serostatus of the donor patient be established as expeditiously as possible. Although testing in these circumstances is time sensitive, it still requires the same elements of counseling, consent, and confidentiality as standard HIV-1 testing. In the United States, in the opinion of the American Medical Association Council of Ethical and Judicial Affairs, involuntary HIV testing is acceptable in the event a health care professional underwent exposure but the patient refuses to be tested.[357] By contrast, WHO has unequivocally rejected mandatory HIV testing,[75] except in screening of blood or blood-manufactured materials or in screening of donors before procedures involving transfer of bodily fluids, for instance, organ donation, artificial insemination, and transplantation.

In occupational exposures it is important to test the exposed worker at the time the event took place to establish baseline serostatus. Counseling and HIV testing should be performed on the exposed worker immediately, to rule out the possibility of preexisting but undiagnosed infection. Rapid ELISA testing may be used, especially if the donor is not thought to be acutely HIV-1 infected. In the event of a nonreactive rapid ELISA, it would be prudent to obtain routine ELISA and TMA/HPA tests to address the unlikely possibility that the rapid assay was insensitive to an early HIV-1 infection. Judicious use of RNA assays may also be used. It is essential to carefully scrutinize testing of donors in this circumstance because hospitalized or acutely ill patients may have a variety of conditions that may yield a falsely reactive HIV-1/2 ELISA. In the event the serostatus cannot be established, initiating ART is an appropriate option (see Chapter 298).

HIV Testing in Idiopathic CD4 Lymphopenia

Idiopathic CD4 lymphopenia (ICL) is an immunodeficiency characterized by repeated CD4 cell numbers <300 cells/μL (<20%), without a coexisting condition thought to be a cause of immunodeficiency, and the patient is HIV negative.[358] ICL is obviously completely distinct from HIV infection, but patients with persistent CD4 lymphopenia and HIV nonreactive ELISA may be referred for additional evaluation. Careful history and repeated HIV testing with judicious use of appropriate NAT will clarify these cases quickly so that they may be referred for appropriate and targeted care.

Factitious HIV Infection and AIDS Phobia

Cases of factitious HIV infection have been reported in the context of psychiatric dysfunction.[359–362] The advent of multiple testing modalities, including home collection systems, may complicate patient presentation in factitious HIV. Careful history, application of routine algorithms, repeat standard assays, use of appropriate counseling or psychiatric consult services, and common sense may resolve most of these delicate cases.

Similarly, a number of individuals have reported substantial fear of HIV infection because of experiencing flulike symptoms. These cases are typically isolated occurrences but may occur in groups.[363] In general these cases are typically resolved by routine evaluation and sequential testing; in the setting of coexisting obsessive-compulsive, anxiety, or paranoia disorders, such patients obviously require primary therapy for underlying illness as well, and infectious disease consultants play a key role in providing information, counseling, and dispelling myths while performing appropriate testing.

HIV Testing to Estimate Duration of Infection and Incidence

The HIV epidemic has been tracked by prevalence using rates of clinical disease (AIDS) and more recently by HIV seroprevalence. Estimates of incidence rates of HIV infection have been relatively difficult to obtain but are an important characteristic of epidemics, especially in area where infection rates may be changing rapidly. Application of serologic and nucleic acid assays has been useful in estimating duration of infection,[364] with a general goal of identifying individuals within 24 to 48 weeks of infection.

Serologic assays have been developed taking advantage of two general characteristics of early infection: (1) decreased avidity of early IgG and IgM antibodies relative to avidity of antibodies in established infection and (2) delayed emergence of antibodies to p31 until after several months of infection. Assays based on either observation have been developed. Initial studies used "sensitive/less sensitive" (S/LS) ELISA assays and quantitative immunocapture assays for estimates of HIV-1 incidence rates.[124,365,366] An S/LS (or "detuned") ELISA approach uses a dual ELISA assay strategy that consists of a standard sensitive third-generation ELISA to detect all HIV infections and a detuned ELISA that does not detect early infections because of the decreased antibody quantity and avidity early in infection. Samples that are HIV reactive using the standard assay and nonreactive on the detuned assay are classified as recent infections. S/LS assays are generally applicable in epidemiologic studies of HIV-1 to characterize high-risk populations, incidence trends, and peak age incidence of infection.[367–369] Subtype performance has not been extensively studied, and the assay is somewhat labor intensive.

A quantitative immunocapture assay (Aware BED; Calypte Biomedical, Portland, OR) represents a second-generation incidence assay designed to address some limitations of S/LS approach.[370] Quantitative immunocapture assay measures the relative levels of HIV gp41-specific IgG as a fraction of the total IgG. The gp41 peptide is a synthetic peptide consisting of an 18–amino-acid immunodominant region of gp41 from subtypes B, E, and D, each ligated to a spacer, aminobutyric acid, and then conjugated in parallel as a branched-chain peptide.[370] Field testing with >600 samples revealed a linear relationship between standardized detection of the BED peptide and duration of infection. The BED assay has been used in the United States[124,371] and elsewhere[372–375] to detect recent infections and estimate incidence rates.

In addition, an avidity index assay can be performed to discriminate between recent seroconversions and established infections; this assay is based on the comparison of test signal (measured as sample/cutoff ratio) between two replicates of fourth-generation assays in which the second replicate is added with guanidine hydrochloride, a denaturation agent that elutes low-avidity and -affinity antibodies after Ag-Ab bonds have formed. This application has been effective in discriminating recent from chronic HIV infections in field testing of subtype B and non-B HIV.[376]

Anti-p31 antibodies are detected in newer discriminating supplemental assays; the absence of anti-p31, indicating relatively early HIV

infection (Fiebig stage V, HIV infection likely <100 days). Not all assays detect antibody to p31 with equal sensitivity; Tuaillon and coworkers[377] reported the GEENIUS assay to be less sensitive in detecting anti-p31 compared with New Lav Blot I WB (Bio-Rad) or INNO-LIA HIV I/II Score Dot Blot (Fujirebio, Malvern, PA) assays; as such GEENIUS may overestimate early infection compared with other assays. Overall, as described by Ananworanich and coworkers,[378] the development of fourth-generation and NAT assays has permitted a reevaluation of standard staging of early HIV infection and a new (fourth-generation) staging proposal that correlates with T-, B-, and natural killer–cell concentrations and cell-associated HIV DNA levels. As Robb and coworkers[379] have reported, early HIV infection is heterogeneous, with wide variation in HIV RNA levels. New approaches developed to discriminate HIV reactivity[380,381] may lead to improved diagnostics and greater understanding of this critical period.

Nucleic acid assays have also been used to estimate duration of HIV infection. Kouyos and coworkers[382] from the SWISS HIV Cohort evaluated whether HIV viral evolution and sequence diversity could correlate with time from infection. During acute/early HIV infection, viral quasispecies are highly homogeneous, whereas they become more and more diverse within the following years. Sequence data obtained from routine genotyping using Sanger sequencing for clinical purposes (i.e., evaluation of drug resistance in protease and reverse transcriptase) show that the number of ambiguous nucleotides increased with the age of infection, and a fraction of ambiguous nucleotides greater than 0.5% provides strong evidence against a recent infection event, 1 year before sampling.[382] Andersson and coworkers[383] and Grossman and coworkers[384] demonstrated that this approach is useful in non-B subtypes. Additional approaches have been developed using other genome regions such as *gag*,[385] new next-generation sequencing assays instead of Sanger sequencing,[386–388] and additional measures of genetic variation (e.g., generalized or Shannon entropy[385,387]). High-resolution amplicon melting temperature,[389] clustering algorithms,[390] Bayesian-derived time to recent common ancestor,[391] and Hamming distance[392] have also been used. Assays are useful with plasma but at present not with dried blood spots.[393,394]

Recency assays, including both serologic and nucleic acid–based testing, have been limited by elevated "false recency" rates, including misclassifying advanced HIV infection as recent. A combined biomarker approach has demonstrated success superior to single assays alone.[395] Laeyendecker and colleagues[394] demonstrated the combination of LAg (limiting antigen) Avidity and Bio-Rad Avidity assays, combined with elevated viral RNA levels, were superior to single-assay approaches. These approaches, resulting in recent infection testing algorithms (RITAs), which integrate early infection serologic assays with viral RNA levels, provide critical information regarding trends in the course of the HIV epidemic. These approaches have clear usefulness in epidemiologic assays for HIV infection; they also serve useful benefit in patient management, especially in partner notification and contact tracing. Surveys of HIV specialists in England and Northern Ireland, where RITAs have been available for years, revealed that physicians were well acquainted with assays and were confident in communicating essential information, including contact tracing without undue patient anxiety.[396]

Home Collection and Self-Testing for HIV

The development of simple/rapid tests present new opportunities and the possibility of HIV diagnosis outside traditional testing facilities. Two kinds of HIV testing are available outside the health care facilities: home collection devices and home testing. Home collection devices are available in the United States and are FDA approved (Home Access; Home Access Health, Hoffman Estates, IL). Patients purchasing a home collection kit must contact the distributor using a toll-free telephone number, register a unique identifier contained in the kit, provide demographic data, and receive an educational pretest counseling session. Packaged material contains consent documents, and consent is implied when the individual registers the test by telephone. Patients prepare the dried blood spot sample, which is shipped for laboratory testing. After a short (3–7 days) processing time, patients contact the toll-free number for results, which are available through an interactive voice response system or directly from a counselor. Testing includes both screening with HIV-1 ELISA (window period of 3 months) and confirmatory testing (immunofluorescence) for reactive screening results.[236] All indeterminate and positive results are delivered by trained counselors. Home collection is considered a safe, confidential, and FDA-approved service and has been available in the United States since 1996.

In July 2012 FDA approved the OraQuick InHome HIV Test (OraSure Technologies, Bethlehem, PA) (see Table 120.1), intended as an over-the-counter test for consumer use as an aid in the diagnosis of HIV-1 and HIV-2. Such a test is not to be used by individuals younger than 17 years and is not intended to be used with specimens other than oral fluid. The kit provides a test result in 20 minutes, and results are for screening only; additional confirmatory testing is essential. Of critical concern, the window period for in-home testing (3 months) is relatively long, and nonreactive results obtained shortly after potential transmission events may provide a false sense of security in the setting of a potentially infectious period.

Self-testing expands the population that tests for HIV[397] and can increase frequency of testing; it is unclear whether lack of additional counseling may miss opportunities to address risk behavior.[398] Home testing can be quite reliable, and extensive review of performance characteristics[399] in general revealed only minor difficulties in self-testing related to sample collection and overall directions. Implementation is not uniformly successful in all circumstances, however; in a population of commercial sex workers in Uganda, Oldenberg and colleagues[400] measured a 15% to 23% incorrect interpretation of test images of strong positive, strong negative, and inconclusive home test results, and as high as a 61% incorrect interpretation of weak positive results. Study participants were peer-trained with the home testing kits and had limited health education and concomitant drug use, which represented possible correlates for increased frequency of incorrect interpretation, although such limitations do approximate real-world conditions. In addition, self-testing has been used successfully, can alleviate stigma, and can reach a patient population that does not typically engage the health care system or HIV testing.[397] Self-testing may be attractive for use as a "point-of-sex" procedure.[401–403] As the window period for home testing remains considerable, widespread replacement of clinic-based testing with self-testing could actually result in increased spread in high-prevalence circumstances[404]; health care and public health–efforts workers caring for at-risk individuals who use self-testing should incorporate detailed and ongoing education discussions and posttest counseling. In a randomized study comparing standard care or provision of self-test kits with or without additional active follow-up, Wray and coworkers[405] found individuals receiving self-test kits and follow-up telephone contacts with counselors had increased rates of repeat testing, risk reduction counseling, and PrEP referrals.

Self-testing has gained demonstrated acceptance in a number of studies worldwide[406–409] and is recommended as an additional approach for HIV testing by WHO.[410] In 2015 a large initiative, HIV Self-Testing Africa (STAR), was implemented in Africa; by 2018 STAR distributed over 2 million self-testing kits in Eswatini, Lesotho, Malawi, South Africa, Zambia, and Zimbabwe, and new clinical trials (https://clinicaltrials.gov/ct2/show/NCT02718274) are investigating the role of additional engagement to initiate ART. STAR will also evaluate acceptance and economic benefits of self-testing.[411,412] Current challenges include issues of implementation scale-up,[413,414] case reporting, and partner notification requirements.[410] New studies are in process to investigate HIV self-testing in a number of at-risk populations (https://clinicaltrials.gov/ct2/results?cond=HIV+self+testing&term=&cntry=&state=&city=&dist=). These new studies will evaluate the utility of self-testing in various settings, ranging from deployment of self-testing kits in villages in Africa and China, to evaluating health approaches to expand home testing in individuals not typically engaged in health care in the United States. These and similar studies are likely to yield important new insights regarding self-testing and linkage to care that may widely expand the numbers of individuals undergoing testing. As a consequence, health care professionals engaging individuals newly identified with HIV infection should be well acquainted with the performance characteristics and limitations of these assays.

Other Human Retroviral Infections and New Retroviral Zoonoses

HIV-1 and HIV-2 epidemics arose from two independent zoonotic infections from distinct primate species, with zoonotic events unlikely. There are more than 40 SIV variants in nonhuman primates, with only a few having established infection with human-to-human transmission. Several key viral variants may have contributed to enhancing transmission.[415] The precise circumstances of these cross-species events remain uncertain, and practices and behaviors continue so that new zoonotic retroviral infection remains possible. Evaluation of patients with immunodeficiency will undoubtedly involve testing for known retroviruses, and the possibility of novel infections should be considered. Primate hunter/butcherers exposed to animals in which infection with SIV is endemic may show evidence of exposure using sensitive laboratory-based assays to detect cell-mediated immunity to SIV[416] but are consistently negative in HIV-1/2 ELISAs. There are, however, numerous distinct retroviruses in primate species, and the sensitivity of currently approved assays for the detection of more distantly related viruses is uncertain but is likely to be low. Yang and coworkers[417] have reported the design of new peptide ELISA assays using Env gp120 V3 sequences from several HIV-1 M, N, and O isolates and from diverse SIV isolates. Such assays, which have demonstrated robust performance in field detection of HIV and SIV, may have additional utility in screening potentially contaminated bushmeat and thereby prevent future zoonotic events.[418]

New zoonotic infections have been detected in persons from Central Africa with extensive contact with primate blood and body fluids. Using sequential strategy of HTLV serology, followed by amplification using primers with sequences shared by primate T-cell leukemia viruses, two primate retroviruses were identified in humans.[419] Primate T-cell viruses are genetically distinct from HIV-1 and HIV-2 and should not yield reactive HIV-1/2 ELISA or NAT results.

Spumaretroviruses are members of the Spumaretrovirinae, a subfamily of Retroviridae and are distinct from the Orthoretrovirinae subfamily to which HIV belongs. Spumaviruses are present in a number of mammal families (e.g., bovine, equine, feline), including numerous nonhuman primate species and Old and New World monkeys, chimpanzees, gorillas, baboons, and oranguatans.[420] Most macaques are seroreactive by 3 years of age. Spumaviruses of cows, cats, and horses do not infect humans, but simian spumaviruses are readily transmissible to humans. Typically, primate veterinary handlers, bushmeat hunters, and others with occupational or other direct primate contact[421,422] may be infected. Although foamy virus infection in vitro results in dramatic cytopathic effect, and simian foamy virus (SFV) may augment SIV pathogenicity,[421] foamy viruses have not been associated with disease in humans.[421] In the Democratic Republic of Congo, Switzer and coworkers[423] measured a seroprevalence rate of 0.5% among individuals in rural villages with routine and close contact with nonhuman primates, and higher rates (2.2%–11.1% PCR positive) have been reported by Engel and coworkers[424] in Bangladesh and by Muniz and coworkers[425] in Brazil, where 18% of primate handlers were seropositive for SFV, but additional study is essential to document human-to-human transmission. SFV is not closely related to HIV or SIV and should not be responsible for reactive HIV-1 serology or NAT.

Cross-species transmission of retroviruses from other species has been considered as a possible consequence of xenotransplantation, as candidate species for tissue donation all harbor endogenous retroviruses, especially porcine endogenous retrovirus (PERV) in pig species.[426,427] Endogenous retroviruses present in animal genomes may not be expressed in the host species but may be inducible upon culture in vitro and transmitted to human cells.[428] Receptors for PERV are expressed in a variety of tissues,[429] and PERV is resistant to most current antiretrovirals.[430] Thus one concern is that endogenous retrovirus production may not be detected at time of harvest but may be induced after transplantation. Concerns for potential retroviral expression with development of zoonotic disease from other porcine and other tissue has prompted debate and recommendations on testing of xenotransplanted tissue for the presence of retroviral sequences and the ability to induce retroviruses in vitro from candidate tissues.[431,432] No evidence, however, of PERV transmission to humans has been demonstrated,[433] and PERV genomes undergo inactivation due to extensive APOBEC (a cellular cytidine deaminase)–mediated hypermutation.[434] Similarly, studies in nonhuman primates have not detected PERV in experimental xenotransplants,[435] although transmission to severe combined immunodeficiency disease (SCID) or nucleotide oligomerization domain (NOD)/SCID mice has been reported in pancreatic islet transplants.[435,436] PERV antigens are serologically distinct from HIV.[437]

REGULATORY ISSUES

Regulation of HIV Testing Modalities

HIV infection and AIDS are conditions with active constituencies among the public and relatively high-profile media attention. Although the scientific debate over the cause of AIDS has justifiably been put to rest,[438] and HIV testing has been largely accepted, material in the lay press continues to circulate that has erroneously criticized or misinterpreted HIV testing sensitivity and accuracy (e.g., www.aliveandwell.org, www.duesberg.com, virusmyth.net, http://ed-sherbeyn.com). Physicians, scientists, and the public require accurate information regarding the materials and procedures used for HIV testing (UNAIDS.org/special). Numerous individual kits are available for HIV testing; in the United States the CDC surveys laboratories performing HIV testing through routine performance evaluations (MPEP), and FDA reviews quality control and licensure of these products. A list of FDA-approved tests is maintained at www.fda.gov/cber/products/testkits.htm. Worldwide, WHO has supported distribution of kits to resource-poor countries since 1998 and maintains a list of kits meeting WHO performance specifications (www.WHO.int); WHO recommends that any kit manufactured for distribution should be approved by regulatory agencies in the home country of manufacture.

Regulation of HIV Reporting

HIV prevalence and incidence data represent critical strategic information and the basis for public health response to the epidemic. Reliable and responsible reporting of HIV infection is essential to achieve accurate surveillance and maintain public trust. Integrating reporting into public health programs planning eradication of HIV infection and clinical programs that link individuals to care will maximize the use of these data. The availability of home testing and anonymous testing modalities expands the populations that may access HIV testing, especially among key populations, but will provide, at best, haphazard reporting. An exhaustive analysis[439] reached the critical conclusion that although passive epidemiologic approaches have been useful, active data collection from key populations is essential for comprehensive understanding of the HIV epidemic, to develop useful datasets for use in monitoring programs addressing HIV-infected individuals and to evaluate their effectiveness.[439] Achieving "90-90-90" objectives requires use of an expanded HIV diagnostic database. Development and use of unique identifiers for patients, especially in key populations, will be useful in linking individuals across clinical and epidemiologic platforms and may address limitations of current data collection strategies.

CONCLUSION

HIV diagnosis is performed for a variety of purposes: epidemiologic surveillance, individual testing, and protecting supply of blood products and tissues for transplantation. Establishing the diagnosis of HIV infection in individuals is a process that optimally requires a coordinated effort among patients, health professionals, and the laboratories. Of critical importance, the use of HIV testing modalities in PrEP and PEP circumstances places a substantial burden on the performance characteristics of testing modalities to detect the presence of HIV in low-prevalence populations and in early HIV infection. Not all testing modalities are equally useful for these demanding circumstances. The contribution of infectious disease specialist in diagnosis will be invaluable and should proceed from medical history and physical examination to laboratory diagnostic testing.

ACKNOWLEDGMENTS

We thank John Coffin, Henry Masur, H. Clifford Lane, John Mellors, Richard T. Davey, Michael A. Polis, Olivia Fankuchen, Joseph Kovacs, Malcolm A. Martin, Klaus Strebel, Francois Clavel, Indira Hewlett, Susan Leitman, Julia A. Metcalf, and Tina Levin for insightful discussions, and are grateful to NIH Infectious Diseases fellows for useful conversations.

Key References

The complete reference list is available online at Expert Consult.

67. Liu A, Coleman K, Bojan K, et al. Developing a mobile app (LYNX) to support linkage to HIV/sexually transmitted infection testing and pre-exposure prophylaxis for young men who have sex with men: protocol for a randomized controlled trial. *JMIR Res Protoc*. 2019;8:e10659.
81. Ackermann N, Marosevic D, Hormansdorfer S, et al. Screening for infectious diseases among newly arrived asylum seekers, Bavaria, Germany, 2015. *Euro Surveill*. 2018;23.
187. Lavoie S, Caswell D, Gill MJ, et al. Heterophilic interference in specimens yielding false-reactive results on the Abbott 4th generation ARCHITECT HIV Ag/Ab Combo assay. *J Clin Virol*. 2018;104:23–28.
223. Baylis SA, Wallace P, McCulloch E, et al. Standardization of nucleic acid tests: the approach of the World Health Organization. *J Clin Microbiol*. 2019;57.
259. Riddell J, Amico KR, Mayer KH. HIV Preexposure prophylaxis: a review. *JAMA*. 2018;319:1261–1268.
262. Cohen SE, Sachdev D, Lee SA, et al. Acquisition of tenofovir-susceptible, emtricitabine-resistant HIV despite high adherence to daily pre-exposure prophylaxis: a case report. *Lancet HIV*. 2018.
268. Gottlieb GS, Raugi DN, Smith RA. 90-90-90 for HIV-2? Ending the HIV-2 epidemic by enhancing care and clinical management of patients infected with HIV-2. *Lancet HIV*. 2018;5:e390–e399.
298. Cazals N, Le Hingrat Q, Abraham B, et al. HIV-2 primary infection in a French 69-year-old bisexual man. *Open Forum Infect Dis*. 2018;5:ofy223.
331. Busch MP, Bloch EM, Kleinman SH. Prevention of transfusion transmitted infections. *Blood*. 2019;133:1854–1864.
338. Cappy P, Barlet V, Lucas Q, et al. Transfusion of HIV-infected blood products despite highly sensitive nucleic acid testing. *Transfusion*. 2019.
343. Couderc M. Being "false positive": an "inconvenience"? debates and questions regarding the notion of vaccine-induced seropositivity (VISP) in the recruitment of healthy volunteers for a preventive anti-HIV vaccine trial. *J Acquir Immune Defic Syndr*. 2018;79(suppl 1): S20–S29.
346. Laetsch TW, Maude SL, Milone MC, et al. False-positive results with select HIV-1 NAT methods following lentivirus-based tisagenlecleucel therapy. *Blood*. 2018;131:2596–2598.
388. Dennis AM, Zhou S, Sellers CJ, et al. Using Primer-ID deep sequencing to detect recent human immunodeficiency virus type 1 infection. *J Infect Dis*. 2018;218:1777–1782.
393. Eisenberg AL, Patel EU, Packman ZR, et al. Short communication: dried blood spots stored at room temperature should not be used for HIV incidence testing. *AIDS Res Hum Retroviruses*. 2018;34:1013–1016.
394. Laeyendecker O, Konikoff J, Morrison DE, et al. Identification and validation of a multi-assay algorithm for cross-sectional HIV incidence estimation in populations with subtype C infection. *J Int AIDS Soc*. 2018;21.
395. Grebe E, Welte A, Johnson LF, et al. Population-level HIV incidence estimates using a combination of synthetic cohort and recency biomarker approaches in KwaZulu-Natal, South Africa. *PLoS ONE*. 2018;13:e0203638.
397. Patel AV, Abrams SM, Gaydos CA, et al. Increasing HIV testing engagement through provision of home HIV self-testing kits for patients who decline testing in the emergency department: a pilot randomisation study. *Sex Transm Infect*. 2018;pii: sextrans-2018-053592.
398. Katz DA, Golden MR, Hughes JP, et al. HIV self-testing increases HIV testing frequency in high-risk men who have sex with men: a randomized controlled trial. *J Acquir Immune Defic Syndr*. 2018;78:505–512.
399. Figueroa C, Johnson C, Ford N, et al. Reliability of HIV rapid diagnostic tests for self-testing compared with testing by health-care workers: a systematic review and meta-analysis. *Lancet HIV*. 2018;5:e277–e290.
400. Ortblad KF, Musoke DK, Ngabirano T, et al. Female sex workers often incorrectly interpret HIV self-test results in Uganda. *J Acquir Immune Defic Syndr*. 2018;79: e42–e45.
401. Wei C, Yan L, Lippman SA, et al. Prevalence and correlates of point-of-sex human immunodeficiency virus self-testing among human immunodeficiency virus-negative men who have sex with men in China. *Sex Transm Dis*. 2018;45:818–822.
405. Wray TB, Chan PA, Simpanen E, et al. A pilot, randomized controlled trial of HIV self-testing and real-time post-test counseling/referral on screening and preventative care among men who have sex with men. *AIDS Patient Care STDS*. 2018;32:360–367.
406. Tun W, Vu L, Dirisu O, et al. Uptake of HIV self-testing and linkage to treatment among men who have sex with men (MSM) in Nigeria: a pilot programme using key opinion leaders to reach MSM. *J Int AIDS Soc*. 2018;21(suppl 5):e25124.
407. Choko AT, Nanfuka M, Birungi J, et al. A pilot trial of the peer-based distribution of HIV self-test kits among fishermen in Bulisa, Uganda. *PLoS ONE*. 2018;13:e0208191.
408. Harichund C, Karim QA, Kunene P, et al. HIV self-testing as part of a differentiated HIV testing approach: exploring urban and rural adult experiences from KwaZulu-Natal, South Africa using a cross-over study design. *BMC Public Health*. 2019;19:53.
409. Sabapathy K, Mulubwa C, Mathema H, et al. Is home-based HIV testing universally acceptable? Findings from a case-control study nested within the HPTN 071 (PopART) trial. *Trop Med Int Health*. 2018;23:678–690.
411. Neuman M, Indravudh P, Chilongosi R, et al. The effectiveness and cost-effectiveness of community-based lay distribution of HIV self-tests in increasing uptake of HIV testing among adults in rural Malawi and rural and peri-urban Zambia: protocol for STAR (self-testing for Africa) cluster randomized evaluations. *BMC Public Health*. 2018;18:1234.
412. George G, Chetty T, Strauss M, et al. Costing analysis of an SMS-based intervention to promote HIV self-testing amongst truckers and sex workers in Kenya. *PLoS ONE*. 2018;13:e0197305.
413. Qin Y, Han L, Babbitt A, et al. Experiences using and organizing HIV self-testing. *AIDS*. 2018;32:371–381.
415. Sauter D, Kirchhoff F. Key viral adaptations preceding the AIDS pandemic. *Cell Host Microbe*. 2019;25:27–38.
420. Khan AS, Bodem J, Buseyne F, et al. Spumaretroviruses: updated taxonomy and nomenclature. *Virology*. 2018;516:158–164.
432. Fishman JA. Infectious disease risks in xenotransplantation. *Am J Transplant*. 2018;18:1857–1864.
434. Jin SY, Choi HY, Kim HS, et al. Human-APOBEC3G-dependent restriction of porcine endogenous retrovirus replication is mediated by cytidine deamination and inhibition of DNA strand transfer during reverse transcription. *Arch Virol*. 2018;163:1907–1914.
439. Weir SS, Baral SD, Edwards JK, et al. Opportunities for enhanced strategic use of surveys, medical records, and program data for HIV surveillance of key populations: scoping review. *JMIR Public Health Surveill*. 2018;4:e28.

References

1. World Health Organization. HIV/AIDS Data and Statistics 2017. https://www.who.int/hiv/data/en/. Accessed June 20, 2019.
2. Staveteig S, Croft TN, Kampa KT, et al. Reaching the 'first 90': gaps in coverage of HIV testing among people living with HIV in 16 African countries. *PLoS ONE*. 2017;12:e0186316.
3. Shah M, Risher K, Berry SA, et al. The epidemiologic and economic impact of improving HIV testing, linkage, and retention in care in the United States. *Clin Infect Dis*. 2016;62:220–229.
4. Human Resources and Services Administration. National HIV/AIDS Strategy for the United States. Updated to 2020. HIV/AIDS Bureau, 2015. https://hab.hrsa.gov/about-ryan-white-hivaids-program/national-hivaids-strategy-updated-2020.
5. Gostin LO, Webber DW. HIV infection and AIDS in the public health and health care systems: the role of law and litigation. *JAMA*. 1998;279:1108–1113.
6. Malpractice suit falls within statute of limitations. *AIDS Policy Law*. 1997;12:5.
7. Galel SA, Lifson JD, Engleman EG. Prevention of AIDS transmission through screening of the blood supply. *Annu Rev Immunol*. 1995;13:201–227.
8. Sarngadharan MG, Popovic M, Bruch L, et al. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science*. 1984;224:506–508.
9. Safai B, Sarngadharan MG, Groopman JE, et al. Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome. *Lancet*. 1984;1:1438–1440.
10. Popovic M, Sarngadharan MG, Read E, et al. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*. 1984;224:497–500.
11. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science*. 1984;224:500–503.
12. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*. 1983;220:868–871.
13. Folks T, Benn S, Rabson A, et al. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. *Proc Natl Acad Sci USA*. 1985;82:4539–4543.
14. Gazzard BG, Shanson DC, Farthing C, et al. Clinical findings and serological evidence of HTLV-III infection in homosexual contacts of patients with AIDS and persistent generalised lymphadenopathy in London. *Lancet*. 1984;2:480–483.
15. Brun-Vezinet F, Rouzioux C, Barre-Sinoussi F, et al. Detection of IgG antibodies to lymphadenopathy-associated virus in patients with AIDS or lymphadenopathy syndrome. *Lancet*. 1984;1:1253–1256.
16. Weiss SH, Goedert JJ, Sarngadharan MG, et al. Screening test for HTLV-III (AIDS agent) antibodies. Specificity, sensitivity, and applications. *JAMA*. 1985;253:221–225.
17. Centers for Disease Control and Prevention. Current trends update: Public Health Service workshop on human T-lymphotropic virus Type III antibody testing – United States. *MMWR Morb Mortal Wkly Rep*. 1985;34:477–478.
18. Marwick C. Blood banks give HTLV-III test positive appraisal at five months. *JAMA*. 1985;254:1681–1683.
19. Ward JW, Holmberg SD, Allen JR, et al. Transmission of human immunodeficiency virus (HIV) by blood transfusions screened as negative for HIV antibody. *N Engl J Med*. 1988;318:473–478.
20. Steckelberg JM, Cockerill FR 3rd. Serologic testing for human immunodeficiency virus antibodies. *Mayo Clin Proc*. 1988;63:373–380.
21. De Majo E, Ravina A, Ricciarelli L, et al. Screening and confirmatory tests for antibody to HTLV-III/LAV retrovirus in individuals at risk for AIDS. *Ric Clin Lab*. 1986;16:489–494.
22. Gallo D, Diggs JL, Shell GR, et al. Comparison of detection of antibody to the acquired immune deficiency syndrome virus by enzyme immunoassay, immunofluorescence, and Western blot methods. *J Clin Microbiol*. 1986;23:1049–1051.
23. Kunz C, Heinz FX. Detection of HTLV-III antibodies using ELISA and the Western blot. *Wien Klin Wochenschr*. 1985;97:829–832.
24. Fisher AG, Collalti E, Ratner L, et al. A molecular clone of HTLV-III with biological activity. *Nature*. 1985;316:262–265.
25. Levy JA, Cheng-Mayer C, Dina D, et al. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. *Science*. 1986;232:998–1001.
26. Adachi A, Gendelman HE, Koenig S, et al. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol*. 1986;59:284–291.
27. Hardy CT, Damrow TA, Villareal DB, et al. Evaluation of viral-lysate enzyme-linked immunosorbent assay kits for detecting human immunodeficiency virus (type 1) infections using human sera standardized by quantitative Western blotting. *J Virol Methods*. 1992;37:259–273.
28. Ng VL, Chiang CS, Debouck C, et al. Reliable confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) with an enzyme-linked immunoassay using recombinant antigens derived from the HIV-1 *gag, pol,* and *env* genes. *J Clin Microbiol*. 1989;27:977–982.
29. Gauthier DK, Turner JG. Anti-HIV antibody testing: procedures and precautions. *Am J Infect Control*. 1989;17:213–225.
30. Hellings JA, Theunissen H, Keur W, et al. New developments in ELISA verification of anti-HIV screening of blood donors. *J Virol Methods*. 1987;17:11–17.
31. Weber B, Hess G, Koberstein R, et al. Evaluation of the automated 'Enzymen-Test Anti HIV-1 + 2' and 'Enzymen-Test Anti HIV-1/2 selective' for the combined detection and differentiation of anti-HIV-1 and anti-HIV-2 antibodies. *J Virol Methods*. 1993;44:251–260.
32. Lee YS. A comparison of reverse transcriptase and antigen capture assays for the detection of HIV. *J Virol Methods*. 1988;20:89–93.
33. Goudsmit J, de Wolf F, Paul DA, et al. Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet*. 1986;2:177–180.
34. Allain JP, Laurian Y, Paul DA, et al. Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. Potential clinical importance. *N Engl J Med*. 1987;317:1114–1121.
35. Simmonds P, Lainson FA, Cuthbert R, et al. HIV antigen and antibody detection: variable responses to infection in the Edinburgh haemophiliac cohort. *Br Med J (Clin Res Ed)*. 1988;296:593–598.
36. von Sydow M, Gaines H, Sonnerborg A, et al. Antigen detection in primary HIV infection. *Br Med J (Clin Res Ed)*. 1988;296:238–240.
37. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. *Science*. 1986;233:343–346.
38. Clavel F, Guyader M, Guetard D, et al. Molecular cloning and polymorphism of the human immune deficiency virus type 2. *Nature*. 1986;324:691–695.
39. AIDS due to HIV-2 infection–New Jersey. *Can Dis Wkly Rep*. 1988;14:50–51.
40. Marquart KH, Muller HA, Brede HD. HIV-2 in West Germany. *AIDS*. 1988;2:141.
41. Newmark P. HIV-2 detected in UK. *Nature*. 1988;332:295.
42. Georgoulias V, Fountouli D, Karvela-Agelakis A, et al. HIV-1 and HIV-2 double infection in Greece. *Ann Intern Med*. 1988;108:155.
43. Neumann PW, Lepine D, Woodside M, et al. HIV-2 infection detected in Canada. *Can Dis Wkly Rep*. 1988;14:125–126.
44. Courouce AM. A prospective study of HIV-2 prevalence in France. *AIDS*. 1988;2:261–265.
45. Starkey CA, Yen-Lieberman B, Proffitt MR. Evaluation of the recombigen HIV-1 latex agglutination test. *J Clin Microbiol*. 1990;28:819–822.
46. Gallo D, George JR, Fitchen JH, et al. Evaluation of a system using oral mucosal transudate for HIV-1 antibody screening and confirmatory testing. OraSure HIV Clinical Trials Group. *JAMA*. 1997;277:254–258.
47. Archibald DW, Zon LI, Groopman JE, et al. Salivary antibodies as a means of detecting human T cell lymphotropic virus type III/lymphadenopathy-associated virus infection. *J Clin Microbiol*. 1986;24:873–875.
48. Archibald DW, Zon L, Groopman JE, et al. Antibodies to human T-lymphotropic virus type III (HTLV-III) in saliva of acquired immunodeficiency syndrome (AIDS) patients and in persons at risk for AIDS. *Blood*. 1986;67:831–834.
49. Parry JV, Perry KR, Mortimer PP. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. *Lancet*. 1987;2:72–75.
50. Tamashiro H, Constantine NT. Serological diagnosis of HIV infection using oral fluid samples. *Bull World Health Organ*. 1994;72:135–143.
51. van Binsbergen J, Keur W, Siebelink A, et al. Strongly enhanced sensitivity of a direct anti-HIV-1/-2 assay in seroconversion by incorporation of HIV p24 Ag detection: a new generation vironostika HIV Uni-Form II. *J Virol Methods*. 1998;76:59–71.
52. Weber B, Fall EH, Berger A, et al. Reduction of diagnostic window by new fourth-generation human immunodeficiency virus screening assays. *J Clin Microbiol*. 1998;36:2235–2239.
53. Vargo J, Smith K, Knott C, et al. Clinical specificity and sensitivity of a blood screening assay for detection of HIV-1 and HCV RNA. *Transfusion*. 2002;42:876–885.
54. Aptima HIV-1 RNA qualitative package insert, 2006. Gen-Probe, San Diego, CA.
55. Brust S, Duttmann H, Feldner J, et al. Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV-1/2/O screening test. *J Virol Methods*. 2000;90:153–165.
56. Saville RD, Constantine NT, Cleghorn FR, et al. Fourth-generation enzyme-linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody. *J Clin Microbiol*. 2001;39:2518–2524.
57. Weber B, Gurtler L, Thorstensson R, et al. Multicenter evaluation of a new automated fourth-generation human immunodeficiency virus screening assay with a sensitive antigen detection module and high specificity. *J Clin Microbiol*. 2002;40:1938–1946.
58. Owen M, Patel P, Wesolowski L, et al. Evaluation of the Abbott ARCHITECT Ag/Ab Combo Assay, an antigen/antibody combination test: implications for US HIV testing programs. Presented at the 16th Conference on Retroviruses and Opportunistic Infections. Montreal, Canada; 2009.
59. Griffin CW 3rd. Model performance evaluation program (HIV testing): program overview. *Infect Control Hosp Epidemiol*. 1988;9:364.
60. Valdiserri RO, Taylor RN, Hearn TL, et al. Centers for Disease Control perspective on quality assurance for human immunodeficiency virus type 1 antibody testing. Model Performance Evaluation Program. *Arch Pathol Lab Med*. 1990;114:263–267.
61. Schalla WO, Hearn TL, Taylor RN, et al. CDC's Model Performance Evaluation Program: assessment of the quality of laboratory performance for HIV-1 antibody testing. *Public Health Rep*. 1990;105:167–171.
62. Snell JJ, Supran EM, Esparza J, et al. World Health Organization quality assessment programme on HIV testing. *AIDS*. 1990;4:803–806.
63. Taylor RN, Przybyszewski VA. Summary of the Centers for Disease Control human immunodeficiency virus (HIV) performance evaluation surveys for 1985 and 1986. *Am J Clin Pathol*. 1988;89:1–13.
64. Benenson AS, Peddecord M, Hofherr LK, et al. Reporting the results of human immunodeficiency virus testing. *JAMA*. 1989;262:3435–3438.
65. Hancock JS, Taylor RN, Johnson CA, et al. Quality of laboratory performance in testing for human immunodeficiency virus type 1 antibody. Identification of variables associated with laboratory performance. *Arch Pathol Lab Med*. 1993;117:1148–1155.
66. Astles JR, Lipman HB, Schalla WO, et al. Impact of quality control on accuracy in enzyme immunoassay testing for human immunodeficiency virus type 1 antibodies. *Arch Pathol Lab Med*. 1998;122:700–707.
67. Liu A, Coleman K, Bojan K, et al. Developing a mobile app (LYNX) to support linkage to HIV/sexually transmitted infection testing and pre-exposure prophylaxis for young men who have sex with men: protocol for a randomized controlled trial. *JMIR RES Protoc*. 2019;8:e10659.
68. Centers for Disease Control and Prevention. Human T-lymphotropic virus type III/lymphadenopathy-associated virus antibody testing at alternate sites. *MMWR Morb Mortal Wkly Rep*. 1986;35:284–287.
69. Shilts R. *And the Band Played on: Politics, People and the AIDS Epidemic*. New York: Penquin Books; 1988.
70. Centers for Disease Control and Prevention. Persons tested for HIV–United States, 2006. *MMWR Morb Mortal Wkly Rep*. 2008;57:845–849.
71. Branson BM, Handsfield HH, Lampe MA, et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Recomm Rep*. 2006;55(RR–14):1–17, quiz CE11-CE14.
72. Delpierre C, Cuzin L, Lert F. Routine testing to reduce late HIV diagnosis in France. *BMJ*. 2007;334:1354–1356.
73. Cherutich P, Bunnell R, Mermin J. HIV testing: current practice and future directions. *Curr HIV/AIDS Rep*. 2013;10:134–141.
74. WHO/UNAIDS. WHO/UNAIDS Statement on HIV Testing Services: New Opportunities and Ongoing Challenges. 2017. http://www.unaids.org/en/resources/presscentre/featurestories/2017/august/20170829_HIV-testing-services.

75. UNAIDS policy on HIV testing and counseling. In: *UNAIDS Policy Statement (UNAIDS 972)*. Geneva/New York: United Nations; 1997.
76. Jacobson v. Commonwealth of Massachusetts. 197 U.S. 11, Edited by U.S. Court; 1905.
77. Braithwaite RL, Arriola KR. Male prisoners and HIV prevention: a call for action ignored. *Am J Public Health*. 2003;93:759–763.
78. Hammett TM. HIV/AIDS and other infectious diseases among correctional inmates: transmission, burden, and an appropriate response. *Am J Public Health*. 2006;96:974–978.
79. Gagnon M, Jacob JD, Cormier L. Total control: a critical analysis of mandatory HIV testing in U.S. prisons. *J Forensic Nurs*. 2013;9:154–161.
80. Green A. President of Zambia declares HIV testing mandatory. *Lancet*. 2017;390:925.
81. Ackermann N, Marosevic D, Hormansdorfer S, et al. Screening for infectious diseases among newly arrived asylum seekers, Bavaria, Germany, 2015. *Euro Surveill*. 2018;23.
82. Centers for Disease Control and Prevention. Update: trends in AIDS incidence – United States, 1996. *MMWR Morb Mortal Wkly Rep*. 1997;46:861–867.
83. New York governor signs bill to track HIV cases by name. *AIDS Policy Law*. 1998;13:1–2.
84. World Health Organization (WHO). *Global Programme on AIDS: Unlinked Anonymous Screening for the Public Health Surveillance of HIV Infections*. Geneva, Switzerland: WHO; 1989. https://apps.who.int/iris/bitstream/handle/10665/64004/GPA_SFI_89.3.pdf?sequence=1&isAllowed=y. Accessed June 20, 2019.
85. Centers for Disease Control and Prevention. Rapid HIV testing in outreach and other community settings—United States, 2004-2006. *MMWR Morb Mortal Wkly Rep*. 2007;56:1233–1237.
86. Muessig KE, Nekkanti M, Bauermeister J, et al. A systematic review of recent smartphone, Internet and Web 2.0 interventions to address the HIV continuum of care. *Curr HIV/AIDS Rep*. 2015;12:173–190.
87. Davey RT Jr, Lane HC. Laboratory methods in the diagnosis and prognostic staging of infection with human immunodeficiency virus type 1. *Rev Infect Dis*. 1990;12:912–930.
88. Keele BF, Giorgi EE, Salazar-Gonzalez JF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci USA*. 2008;105:7552–7557.
89. Kearney M, Maldarelli F, Shao W, et al. Human immunodeficiency virus type 1 population genetics and adaptation in newly infected individuals. *J Virol*. 2009;83:2715–2727.
90. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med*. 2001;193:169–180.
91. Wilson JD, Ogg GS, Allen RL, et al. Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection. *AIDS*. 2000;14:225–233.
92. Appay V, Papagno L, Spina CA, et al. Dynamics of T cell responses in HIV infection. *J Immunol*. 2002;168:3660–3666.
93. McRae B, Lange JA, Ascher MS, et al. Immune response to HIV p24 core protein during the early phases of human immunodeficiency virus infection. *AIDS Res Hum Retroviruses*. 1991;7:637–643.
94. Cooper DA, Imrie AA, Penny R. Antibody response to human immunodeficiency virus after primary infection. *J Infect Dis*. 1987;155:1113–1118.
95. Healey DS, Maskill WJ, Gust ID. Detection of anti-HIV immunoglobulin M by particle agglutination following acute HIV infection. *AIDS*. 1989;3:301–304.
96. Gallarda JL, Henrard DR, Liu D, et al. Early detection of antibody to human immunodeficiency virus type 1 by using an antigen conjugate immunoassay correlates with the presence of immunoglobulin M antibody. *J Clin Microbiol*. 1992;30:2379–2384.
97. Muller F, Muller KH. Detection of anti-HIV-1 immunoglobulin M antibodies in patients with serologically proved HIV-1 infection. *Infection*. 1988;16:115–118.
98. Gaines H, von Sydow M, Parry JV, et al. Detection of immunoglobulin M antibody in primary human immunodeficiency virus infection. *AIDS*. 1988;2:11–15.
99. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet*. 1989;2:637–640.
100. Loveday C, Tedder RS. Enzyme-linked immunosorbent assays for the measurement of human immunodeficiency virus, type 1 reverse transcriptase antigen and antibodies. *J Virol Methods*. 1993;41:181–192.
101. Constantine NT, van der Groen G, Belsey EM, et al. Sensitivity of HIV-antibody assays determined by seroconversion panels. *AIDS*. 1994;8:1715–1720.
102. U.S. Department of Health and Human Services, AIDS Information; FDA Talking Paper. FDA Approves First Nucleic Acid Test (NAT) System to Screen Whole Blood Donors for Infections With Human Immunodeficiency Virus (HIV and Hepatitis C Virus (HCV). https://aidsinfo.nih.gov/news/593/fda-approves-first-nucleic-acid-test–nat–system-to-screen-whole-blood-donors-for-infections-with-human-immunodeficiency-virus–hiv–and-hepatitis-c-virus–hcv-.
103. Montagnier L, Brenner C, Chamaret S, et al. Human immunodeficiency virus infection and AIDS in a person with negative serology. *J Infect Dis*. 1997;175:955–959.
104. Centers for Disease Control and Prevention. Persistent lack of detectable HIV-1 antibody in a person with HIV infection–Utah, 1995. *MMWR Morb Mortal Wkly Rep*. 1996;45:181–185.
105. Sullivan PS, Schable C, Koch W, et al. Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results. Seronegative AIDS Clinical Study Group. *AIDS*. 1999;13:89–96.
106. Barthel HR, Wallace DJ. False-positive human immunodeficiency virus testing in patients with lupus erythematosus. *Semin Arthritis Rheum*. 1993;23:1–7.
107. Soriano V, Dronda F, Gonzalez-Lopez A, et al. HIV-1 causing AIDS and death in a seronegative individual. *Vox Sang*. 1994;67:410–411.
108. Candotti D, Adu-Sarkodie Y, Davies F, et al. AIDS in an HIV-seronegative Ghanaian woman with intersubtype A/G recombinant HIV-1 infection. *J Med Virol*. 2000;62:1–8.
109. Oka S, Ida S, Shioda T, et al. Genetic analysis of HIV-1 during rapid progression to AIDS in an apparently healthy man. *AIDS Res Hum Retroviruses*. 1994;10:271–277.
110. Wegner S, Ohl C, DeNobile J, et al. Case report of a woman with seronegative HIV-1 infection. *AIDS Res Hum Retroviruses*. 1995;11:S173.
111. Ellenberger DL, Sullivan PS, Dorn J, et al. Viral and immunologic examination of human immunodeficiency virus type 1-infected, persistently seronegative persons. *J Infect Dis*. 1999;180:1033–1042.
112. Kopko P, Calhoun L, Petz L. Distinguishing immunosilent AIDS from the acute retroviral syndrome in a frequent blood donor. *Transfusion*. 1999;39:383–386.
113. Michael NL, Brown AE, Voigt RF, et al. Rapid disease progression without seroconversion following primary human immunodeficiency virus type 1 infection–evidence for highly susceptible human hosts. *J Infect Dis*. 1997;175:1352–1359.
114. Apetrei C, Tamalet C, Edlinger C, et al. Delayed HIV-1 seroconversion after antiretroviral therapy. *AIDS*. 1998;12:1935–1936.
115. Chockalingam M, Clarke L, McCormack WM. HIV infection with negative serological tests: development of seropositivity in association with highly active antiretroviral therapy. *AIDS Patient Care STDS*. 2000;14:305–308.
116. De Rossi A, Giaquinto C, Del Mistro A, et al. Onset of HIV-1 antibody production after highly active antiretroviral therapy in a seronegative HIV-1-infected child. *AIDS*. 2000;14:1284–1286.
117. Morris L, Binley JM, Clas BA, et al. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J Exp Med*. 1998;188:233–245.
118. O'Connell RJ, Merritt TM, Malia JA, et al. Performance of the OraQuick rapid antibody test for diagnosis of human immunodeficiency virus type 1 infection in patients with various levels of exposure to highly active antiretroviral therapy. *J Clin Microbiol*. 2003;41:2153–2155.
119. Kenealy W, Reed D, Cybulski R, et al. Analysis of human serum antibodies to human immunodeficiency virus (HIV) using recombinant ENV and GAG antigens. *AIDS Res Hum Retroviruses*. 1987;3:95–105.
120. Dawson GJ, Heller JS, Wood CA, et al. Reliable detection of individuals seropositive for the human immunodeficiency virus (HIV) by competitive immunoassays using *Escherichia coli*-expressed HIV structural proteins. *J Infect Dis*. 1988;157:149–155.
121. Ragni MV, O'Brien TA, Reed D, et al. Prognostic importance of antibodies to human immunodeficiency virus by recombinant immunoassay and Western blot techniques in HIV antibody-positive hemophiliacs. *AIDS Res Hum Retroviruses*. 1988;4:223–231.
122. Higgins JR, Pedersen NC, Carlson JR. Detection and differentiation by sandwich enzyme-linked immunosorbent assay of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus- and acquired immunodeficiency syndrome-associated retroviruslike clinical isolates. *J Clin Microbiol*. 1986;24:424–430.
123. Beelaert G, Vercauteren G, Fransen K, et al. Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV. *J Virol Methods*. 2002;105:197–206.
124. Hall HI, Song R, Rhodes P, et al. Estimation of HIV incidence in the United States. *JAMA*. 2008;300:520–529.
125. Francis HL, Kabeya M, Kafuama N, et al. Comparison of sensitivities and specificities of latex agglutination and an enzyme-linked immunosorbent assay for detection of antibodies to the human immunodeficiency virus in African sera. *J Clin Microbiol*. 1988;26:2462–2464.
126. Windsor IM, Gomes dos Santos ML, de la Hunt LI, et al. An evaluation of the capillus HIV-1/HIV-2 latex agglutination test using serum and whole blood. *Int J STD AIDS*. 1997;8:192–195.
127. Lepine DG, Neumann PW, Frenette SL, et al. Evaluation of a human immunodeficiency virus test algorithm utilizing a recombinant protein enzyme immunoassay. *J Clin Microbiol*. 1990;28:1169–1171.
128. Elavia AJ, Thomas A, Nandi J, et al. Performance evaluation of a particle agglutination test for antibody to human immunodeficiency virus 1: comparison with enzyme immunoassay. *Vox Sang*. 1995;69:23–26.
129. Poljak M, Zener N, Seme K, et al. Particle agglutination test "Serodia HIV-1/2" as a novel anti-HIV-1/2 screening test: comparative study on 3311 serum samples. *Folia Biol (Praha)*. 1997;43:171–173.
130. Parry JV, Mortimer PP. An immunoglobulin G antibody capture particle-adherence test (GACPAT) for antibody to HIV-1 and HTLV-I that allows economical large-scale screening. *AIDS*. 1989;3:173–176.
131. Houck JA, Sedmak DD, Grose MP, et al. Sensitivity and interobserver variability of the Recombigen-HIV-1 LA test. *Am J Clin Pathol*. 1990;93:538–540.
132. Louisirirotchanakul S, Kanoksinsombat C, Thongput A, et al. Anti-HIV-1 antibody testing using modified gelatin particle agglutination: a large field study. *J Med Assoc Thai*. 2001;84:1708–1713.
133. Ramalingam S, Kannangai R, Raj AA, et al. Rapid particle agglutination test for human immunodeficiency virus: hospital-based evaluation. *J Clin Microbiol*. 2002;40:1553–1554.
134. Lien TX, Tien NT, Chanpong GF, et al. Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg*. 2000;62:301–309.
135. Jourbert J, Dewar JB, Weinberg J, et al. A cost-effective particle agglutination assay to detect viral antibodies in dried blood spots—a simple solution to HIV and HCV screening. *Cent Afr J Med*. 2003;49:127–130.
136. Makuwa M, Souquiere S, Niangui MT, et al. Reliability of rapid diagnostic tests for HIV variant infection. *J Virol Methods*. 2002;103:183–190.
137. Essential Medicines and Health Products Information Portal/World Health Organization. *Operational Characteristics of Commercially Available Assays to Determine Antibodies to HIV-1 and/or HIV-2 in Human Sera*. Geneva: United Nations; 1999:1–63.
138. Brauer M, De Villiers JC, Mayaphi SH. Evaluation of the Determine fourth generation HIV rapid assay. *J Virol Methods*. 2013;189:180–183.
139. Lyamuya EFAS, Urassa WK, Sufi J, et al. Evaluation of simple rapid HIV assays and development of national rapid HIV test algorithms in Dar es Salaam, Tanzania. *BMC Infect Dis*. 2009;9:19.
140. Klarkowski DBWJ, Lokuge KM, Shanks L, et al. The evaluation of a rapid in situ HIV confirmation test in a programme with a high failure rate of the WHO HIV two-test diagnostic algorithm. *PLoS ONE*. 2009;4:e4351.
141. Kassler WJ, Dillon BA, Haley C, et al. On-site, rapid HIV testing with same-day results and counseling. *AIDS*. 1997;11:1045–1051.
142. Keenan PA, Keenan JM. Rapid HIV testing in urban outreach: a strategy for improving posttest counseling rates. *AIDS Educ Prev*. 2001;13:541–550.
143. Kelen GD, Shahan JB, Quinn TC. Emergency department-based HIV screening and counseling: experience with rapid and standard serologic testing. *Ann Emerg Med*. 1999;33:147–155.
144. Centers for Disease Control and Prevention. Update: HIV counseling and testing using rapid tests—United States, 1995. *MMWR Morb Mortal Wkly Rep*. 1998;47:211–215.
145. Malonza IM, Richardson BA, Kreiss JK, et al. The effect of rapid HIV-1 testing on uptake of perinatal HIV-1 interventions: a randomized clinical trial. *AIDS*. 2003;17:113–118.
146. Owen SM, Yang C, Spira T, et al. Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. *J Clin Microbiol*. 2008;46:1588–1595.
147. Barr CE, Miller LK, Lopez MR, et al. Recovery of infectious HIV-1 from whole saliva. *J Am Dent Assoc*. 1992;123:36–37, 39–48; discussion 38.

148. Chohan BH, Lavreys L, Mandaliya KN, et al. Validation of a modified commercial enzyme-linked immunoassay for detection of human immunodeficiency virus type 1 immunoglobulin G antibodies in saliva. *Clin Diagn Lab Immunol*. 2001;8:346–348.
149. Wilson E, Tanzosh T, Maldarelli F. HIV diagnosis and testing: what every healthcare professional can do (and why they should). *Oral Dis*. 2013;19:431–439.
150. Connell JA, Parry JV, Mortimer PP, et al. Novel assay for the detection of immunoglobulin G antihuman immunodeficiency virus in untreated saliva and urine. *J Med Virol*. 1993;41:159–164.
151. Raux M, Finkielsztejn L, Salmon-Ceron D, et al. IgG subclass distribution in serum and various mucosal fluids of HIV type 1-infected subjects. *AIDS Res Hum Retroviruses*. 2000;16:583–594.
152. Lu XS, Delfraissy JF, Grangeot-Keros L, et al. Rapid and constant detection of HIV antibody response in saliva of HIV-infected patients; selective distribution of anti-HIV activity in the IgG isotype. *Res Virol*. 1994;145:369–377.
153. Cartry O, Moja P, Quesnel A, et al. Quantification of IgA and IgG and specificities of antibodies to viral proteins in parotid saliva at different stages of HIV-1 infection. *Clin Exp Immunol*. 1997;109:47–53.
154. Martinez PM, Torres AR, Ortiz de Lejarazu R, et al. Human immunodeficiency virus antibody testing by enzyme-linked fluorescent and Western blot assays using serum, gingival-crevicular transudate, and urine samples. *J Clin Microbiol*. 1999;37:1100–1106.
155. Granade TC, Phillips SK, Parekh B, et al. Detection of antibodies to human immunodeficiency virus type 1 in oral fluids: a large-scale evaluation of immunoassay performance. *Clin Diagn Lab Immunol*. 1998;5:171–175.
156. Centers for Disease Control and Prevention. Progress toward strengthening blood transfusion services—14 countries, 2003-2007. *MMWR Morb Mortal Wkly Rep*. 2008;57:1273–1277.
157. Facente S, Dowling T, Vittinghoff E, et al. False-positive rate of rapid oral fluid HIV tests increases as kits near expiration date. Present at the 16th Conference on Retroviruses and Opportunistic Infections. Montreal, Canada; 2009.
158. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. *Oral Microbiol Immunol*. 1996;11:289–293.
159. Maticic M, Poljak M, Kramar B, et al. Proviral HIV-1 DNA in gingival crevicular fluid of HIV-1-infected patients in various stages of HIV disease. *J Dent Res*. 2000;79:1496–1501.
160. Zuckerman RA, Whittington WL, Celum CL, et al. Factors associated with oropharyngeal human immunodeficiency virus shedding. *J Infect Dis*. 2003;188:142–145.
161. Weiblen BJ, Lee FK, Cooper ER, et al. Early diagnosis of HIV infection in infants by detection of IgA HIV antibodies. *Lancet*. 1990;335:988–990.
162. Morgado de Moura Machedo JE, Kayita J, Bakaki P, et al. IgA antibodies to human immunodeficiency virus in serum, saliva and urine for early diagnosis of immunodeficiency virus infection in Ugandan infants. *Pediatr Infect Dis J*. 2003;22:193–195.
163. Jackson S, Prince S, Kulhavy R, et al. False positivity of enzyme-linked immunosorbent assay for measurement of secretory IgA antibodies directed at HIV type 1 antigens. *AIDS Res Hum Retroviruses*. 2000;16:595–602.
164. George JR, Schochetman G. *AIDS Testing: A Comprehensive Guide to Medical Social Legal and Managemnt Issues*. New York: Springer-Verlag; 1994.
165. Constantine NT, Zhang X, Li L, et al. Application of a rapid assay for detection of antibodies to human immunodeficiency virus in urine. *Am J Clin Pathol*. 1994;101:157–161.
166. Urnovitz HB, Sturge JC, Gottfried TD, et al. Urine antibody tests: new insights into the dynamics of HIV-1 infection. *Clin Chem*. 1999;45:1602–1613.
167. Kacena KA, Dohnal K, Benesova V, et al. Chlamydia, gonorrhea, and HIV-1 prevalence among five populations of women in the Czech and Slovak Republics. *Sex Transm Dis*. 2001;28:356–362.
168. Gaines H, von Sydow M, Sonnerborg A, et al. Antibody response in primary human immunodeficiency virus infection. *Lancet*. 1987;1:1249–1253.
169. Henrard DR, Wu S, Phillips J, et al. Detection of p24 antigen with and without immune complex dissociation for longitudinal monitoring of human immunodeficiency virus type 1 infection. *J Clin Microbiol*. 1995;33:72–75.
170. Schupbach J. Measurement of HIV-1 p24 antigen by signal-amplification-boosted ELISA of heat-denatured plasma is a simple and inexpensive alternative to tests for viral RNA. *AIDS Rev*. 2002;4:83–92.
171. Sutthent R, Gaudart N, Chokpaibulkit K, et al. p24 Antigen detection assay modified with a booster step for diagnosis and monitoring of human immunodeficiency virus type 1 infection. *J Clin Microbiol*. 2003;41:1016–1022.
172. Tehe A, Maurice C, Hanson DL, et al. Quantification of HIV-1 p24 by a highly improved ELISA: an alternative to HIV-1 RNA based treatment monitoring in patients from Abidjan, Côte d'Ivoire. *J Clin Virol*. 2006;37:199–205.
173. Schupbach J, Tomasik Z, Knuchel M, et al. Optimized virus disruption improves detection of HIV-1 p24 in particles and uncovers a p24 reactivity in patients with undetectable HIV-1 RNA under long-term HAART. *J Med Virol*. 2006;78:1003–1010.
174. Vetter BN, Orlowski V, Fransen K, et al. Generation of a recombinant Gag virus-like-particle panel for the evaluation of p24 antigen detection by diagnostic HIV tests. *PLoS ONE*. 2014;9:e111552.
175. Seed CR, Margaritis AR, Bolton WV, et al. Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. *Transfusion*. 2003;43:226–234.
176. Morell A, Barandun S, Locher G. HTLV-III seroconversion in a homosexual patient with common variable immunodeficiency. *N Engl J Med*. 1986;315:456–457.
177. Bowen DL, Lane HC, Fauci AS. Immunopathogenesis of the acquired immunodeficiency syndrome. *Ann Intern Med*. 1985;103:704–709.
178. Preiser W, Brink NS, Hayman A, et al. False-negative HIV antibody test results. *J Med Virol*. 2000;60:43–47.
179. McAlpine L, Parry JV, Shanson D, et al. False negative results in enzyme linked immunosorbent assays using synthetic HIV antigens. *J Clin Pathol*. 1995;48:490–493.
180. Parry JV, Mortimer PP, Friderich P, et al. Faulty washers and soiled micropipettors may generate false positive serological results. *Clin Diagn Virol*. 1997;7:173–181.
181. Evans BG, Parry JV, Mortimer PP. HIV antibody assay that gave false negative results: multicentre collaborative study. *BMJ*. 1997;315:772–774.
182. Bouillon M, Aubin E, Roberge C, et al. Reduced frequency of blood donors with false-positive HIV-1 and -2 antibody EIA reactivity after elution of low-affinity nonspecific natural antibodies. *Transfusion*. 2002;42:1046–1052.
183. Wai CT, Tambyah PA. False-positive HIV-1 ELISA in patients with hepatitis B. *Am J Med*. 2002;112:737.
184. Pearlman ES, Ballas SK. False-positive human immunodeficiency virus screening test related to rabies vaccination. *Arch Pathol Lab Med*. 1994;118:805–806.
185. Plotkin SA, Loupi E, Blondeau C. False-positive human immunodeficiency virus screening test related to rabies vaccination. *Arch Pathol Lab Med*. 1995;119:679.
186. Henderson S, Leibnitz G, Turnbull M, et al. False-positive human immunodeficiency virus seroconversion is not common following rabies vaccination. *Clin Diagn Lab Immunol*. 2002;9:942–943.
187. Lavoie S, Caswell D, Gill MJ, et al. Heterophilic interference in specimens yielding false-reactive results on the Abbott 4th generation ARCHITECT HIV Ag/Ab Combo assay. *J Clin Virol*. 2018;104:23–28.
188. Andrade VL, Avelleira JC, Marques A, et al. Leprosy as cause of false-positive results in serological assays for the detection of antibodies to HIV-1. *Int J Lepr Other Mycobact Dis*. 1991;59:125–126.
189. ShivRaj L, Patil SA, Girdhar A, et al. Antibodies to HIV-1 in sera from patients with mycobacterial infections. *Int J Lepr Other Mycobact Dis*. 1988;56:546–551.
190. Kashala O, Marlink R, Ilunga M, et al. Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts: correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. *J Infect Dis*. 1994;169:296–304.
191. Sterne JA, Turner AC, Fine PE, et al. Testing for antibody to human immunodeficiency virus type 1 in a population in which mycobacterial diseases are endemic. *J Infect Dis*. 1995;172:543–546.
192. Walensky RP, Rosenberg ES, Ferraro MJ, et al. Investigation of primary human immunodeficiency virus infection in patients who test positive for heterophile antibody. *Clin Infect Dis*. 2001;33:570–572.
193. Zehender G, De Maddalena C, Gianotto M, et al. High prevalence of false-negative anti-HTLV type I/II enzyme-linked immunosorbent assay results in HIV type 1-positive patients. *AIDS Res Hum Retroviruses*. 1997;13:1141–1146.
194. Ferrer-Canabate J, Agullo-Ortuno T, Garcia-Mancebo ML, et al. Propylene glycol interference in the Vidas HIV DUO assay. *Clin Biochem*. 2002;35:333–334.
195. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time RT-PCR assay with single-copy sensitivity for HIV-1 RNA in plasma. *J Clin Microbiol*. 2003;41:4531–4536.
196. Candotti D, Richetin A, Cant B, et al. Evaluation of a transcription-mediated amplification-based HCV and HIV-1 RNA duplex assay for screening individual blood donations: a comparison with a minipool testing system. *Transfusion*. 2003;43:215–225.
197. Lelie PN, van Drimmelen HA, Cuypers HT, et al. Sensitivity of HCV RNA and HIV RNA blood screening assays. *Transfusion*. 2002;42:527–536.
198. Stramer SL. US NAT yield: where are we after 2 years? *Transfus Med*. 2002;12:243–253.
199. Tomono T, Murokawa H, Minegishi K, et al. Status of NAT screening for HCV, HIV and HBV: experience in Japan. *Dev Biol (Basel)*. 2002;108:29–39.
200. Roth WK, Weber M, Buhr S, et al. Yield of HCV and HIV-1 NAT after screening of 3.6 million blood donations in central Europe. *Transfusion*. 2002;42:862–868.
201. Fang CT, Field SP, Busch MP, et al. Human immunodeficiency virus-1 and hepatitis C virus RNA among South African blood donors: estimation of residual transfusion risk and yield of nucleic acid testing. *Vox Sang*. 2003;85:9–19.
202. Jongerius JM, Sjerps M, Cuijpers HT, et al. Validation of the NucliSens Extractor combined with the AmpliScreen HIV version 1.5 and HCV version 2.0 test for application in NAT minipool screening. *Transfusion*. 2002;42:792–797.
203. Aprili G, Gandini G, Piccoli P, et al. Detection of an early HIV-1 infection by HIV RNA testing in an Italian blood donor during the preseroconversion window period. *Transfusion*. 2003;43:848–852.
204. Dreier J, Gotting C, Wolff C, et al. Recent experience with human immunodeficiency virus transmission by cellular blood products in Germany: antibody screening is not sufficient to prevent transmission. *Vox Sang*. 2002;82:80–83.
205. Kopko PM, Fernando LP, Bonney EN, et al. HIV transmissions from a window-period platelet donation. *Am J Clin Pathol*. 2001;116:562–566.
206. Toro C, Rodes B, Colino F, et al. Transmission of HIV type 1 through blood transfusion from an antibody-negative/p24 antigen-negative donor. *AIDS Res Hum Retroviruses*. 2003;19:447–448.
207. Suarez AA, Sokol-Anderson ML, Creer M, et al. Case report. Diagnosis of early HIV-1 infection. *AIDS Patient Care STDS*. 2001;15:237–241.
208. Ling AE, Robbins KE, Brown TM, et al. Failure of routine HIV-1 tests in a case involving transmission with preseroconversion blood components during the infectious window period. *JAMA*. 2000;284:210–214.
209. Wang B, Schreiber GB, Glynn SA, et al. Prevalence of transfusion-transmissible viral infections in first-time US blood donors by donation site. *Transfusion*. 2003;43:705–712.
210. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion*. 2002;42:975–979.
211. Seed CR, Cheng A, Ismay SL, et al. Assessing the accuracy of three viral risk models in predicting the outcome of implementing HIV and HCV NAT donor screening in Australia and the implications for future HBV NAT. *Transfusion*. 2002;42:1365–1372.
212. Weusten JJ, van Drimmelen HA, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. *Transfusion*. 2002;42:537–548.
213. Jackson BR, Busch MP, Stramer SL, et al. The cost-effectiveness of NAT for HIV, HCV, and HBV in whole-blood donations. *Transfusion*. 2003;43:721–729.
214. Holmes H, Davis C, Heath A, et al. An international collaborative study to establish the 1st international standard for HIV-1 RNA for use in nucleic acid-based techniques. *J Virol Methods*. 2001;92:141–150.
215. Rich JD, Merriman NA, Mylonakis E, et al. Misdiagnosis of HIV infection by HIV-1 plasma viral load testing: a case series. *Ann Intern Med*. 1999;130:37–39.
216. Hecht FM, Busch MP, Rawal B, et al. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS*. 2002;16:1119–1129.
217. Brambilla D, Jennings C, Aldrovandi G, et al. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. *J Clin Microbiol*. 2003;41:1888–1893.
218. Migueles SA, Sabbaghian MS, Shupert WL, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci USA*. 2000;97:2709–2714.
219. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. *Immunity*. 2008;29:1009–1021.
220. Stramer SL, Yu G, Herron R, et al. Two human immunodeficiency virus type 2 cases in US blood donors

including serologic, molecular, and genomic characterization of an epidemiologically unusual case. *Transfusion*. 2016;56(6 Pt 2):1560–1568.

221. Holmes H, Davis C, Heath A. Development of the 1st International Reference Panel for HIV-1 RNA genotypes for use in nucleic acid-based techniques. *J Virol Methods*. 2008;154:86–91.
222. Foglieni B, Candotti D, Guarnori I, et al. A cluster of human immunodeficiency virus Type 1 recombinant form escaping detection by commercial genomic amplification assays. *Transfusion*. 2011;51:719–730.
223. Baylis SA, Wallace P, McCulloch E, et al. Standardization of nucleic acid tests: the approach of the World Health Organization. *J Clin Microbiol*. 2019;57:pii: e01056-18.
224. Valkirs GE, Barton R. ImmunoConcentration—a new format for solid-phase immunoassays. *Clin Chem*. 1985;31:1427–1431.
225. Wesolowski LG, Delaney KP, Hart C, et al. Performance of an alternative laboratory-based algorithm for diagnosis of HIV infection utilizing a third generation immunoassay, a rapid HIV-1/HIV-2 differentiation test and a DNA or RNA-based nucleic acid amplification test in persons with established HIV-1 infection and blood donors. *J Clin Virol*. 2011;52(suppl 1):S45–S49.
226. Nasrullah M, Ethridge SF, Delaney KP, et al. Comparison of alternative interpretive criteria for the HIV-1 Western blot and results of the Multispot HIV-1/HIV-2 Rapid Test for classifying HIV-1 and HIV-2 infections. *J Clin Virol*. 2011;52(suppl 1):S23–S27.
227. Delaney KP, Heffelfinger JD, Wesolowski LG, et al. Performance of an alternative laboratory-based algorithm for HIV diagnosis in a high-risk population. *J Clin Virol*. 2011;52(suppl 1):S5–S10.
228. Masciotra S, McDougal JS, Feldman J, et al. Evaluation of an alternative HIV diagnostic algorithm using specimens from seroconversion panels and persons with established HIV infections. *J Clin Virol*. 2011;52(suppl 1):S17–S22.
229. Sandstrom EG, Schooley RT, Ho DD, et al. Detection of human anti-HTLV-III antibodies by indirect immunofluorescence using fixed cells. *Transfusion*. 1985;25:308–312.
230. Hedenskog M, Dewhurst S, Ludvigsen C, et al. Testing for antibodies to AIDS-associated retrovirus (HTLV-III/LAV) by indirect fixed cell immunofluorescence: specificity, sensitivity, and applications. *J Med Virol*. 1986; 19:325–334.
231. van der Groen G, Vercauteren G, Piot P. Immunofluorescence tests for HIV antibody and their value as confirmatory tests. *J Virol Methods*. 1987;17: 35–43.
232. Kvinesdal BB, Nielsen CM, Poulsen AG, et al. Immunofluorescence assay for detection of antibodies to human immunodeficiency virus type 2. *J Clin Microbiol*. 1989;27:2502–2504.
233. Sullivan MT, Mucke H, Kadey SD, et al. Evaluation of an indirect immunofluorescence assay for confirmation of human immunodeficiency virus type 1 antibody in U.S. blood donor sera. *J Clin Microbiol*. 1992;30:2509–2510.
234. Boshell J, Alvarez C, Marrugo S, et al. Indirect immunofluorescence as a supplementary test for confirming HIV-1 infection: the experience of the National Institute of Health, 1993-2000. *Biomedica*. 2002;22:30–38.
235. Mahony J, Rosenthal K, Chernesky M, et al. Agreement study between two laboratories of immunofluorescence as a confirmatory test for human immunodeficiency virus type 1 antibody screening. *J Clin Microbiol*. 1989;27:1234–1237.
236. Home Access Health. Home Access HIV-1 Test System; 1996.
237. Huisman JG, Winkel IN, Lelie PN, et al. Detection of early anti-p24 HIV responses in EIA- and immunoblot-negative individuals. Implications for confirmatory testing. *Vox Sang*. 1987;53:31–36.
238. Pinter A, Honnen WJ. A sensitive radioimmunoprecipitation assay for human immunodeficiency virus (HIV). *J Immunol Methods*. 1988;112:235–241.
239. Resnick L, Shapshak P. Serologic characterization of human immunodeficiency virus infection by Western blot and radioimmunoprecipitation assays. *Arch Pathol Lab Med*. 1987;111:1040–1044.
240. Saah AJ, Farzadegan H, Fox R, et al. Detection of early antibodies in human immunodeficiency virus infection by enzyme-linked immunosorbent assay, Western blot, and radioimmunoprecipitation. *J Clin Microbiol*. 1987;25:1605–1610.
241. Roberts CR, Longfield JN, Platte RC, et al. Transfusion-associated human immunodeficiency virus type 1 from screened antibody-negative blood donors. *Arch Pathol Lab Med*. 1994;118:1188–1192.
242. Simon F, Cot MC, Lesager C, et al. Differentiation between HIV-1 and HIV-2 infection by radioimmunoprecipitation and synthetic peptides in double reactive sera. *AIDS*. 1989;3:401–402.
243. Grimes DA, Schulz KF. Uses and abuses of screening tests. *Lancet*. 2002;359:881–884.

243a. Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. 2003;17:1871–1879.

244. Frank AP, Wandell MG, Headings MD, et al. Anonymous HIV testing using home collection and telemedicine counseling. A multicenter evaluation. *Arch Intern Med*. 1997;157:309–314.
245. Tsu RC, Burm ML, Gilhooly JA, et al. Telephone vs. face-to-face notification of HIV results in high-risk youth. *J Adolesc Health*. 2002;30:154–160.
246. Aidoo S, Ampofo WK, Brandful JA, et al. Suitability of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus in Ghana, West Africa. *J Clin Microbiol*. 2001;39:2572–2575.
247. Downing RG, Otten RA, Marum E, et al. Optimizing the delivery of HIV counseling and testing services: the Uganda experience using rapid HIV antibody test algorithms. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;18:384–388.
248. Urassa W, Nozohoor S, Jaffer S, et al. Evaluation of an alternative confirmatory strategy for the diagnosis of HIV infection in Dar Es Salaam, Tanzania, based on simple rapid assays. *J Virol Methods*. 2002;100:115–120.
249. Urassa W, Godoy K, Killewo J, et al. The accuracy of an alternative confirmatory strategy for detection of antibodies to HIV-1: experience from a regional laboratory in Kagera, Tanzania. *J Clin Virol*. 1999;14:25–29.
250. Nkengasong JN, Maurice C, Koblavi S, et al. Evaluation of HIV serial and parallel serologic testing algorithms in Abidjan, Côte d'Ivoire. *AIDS*. 1999;13:109–117.
251. Rehle TM, Mattke P, Liomba GN, et al. Evaluation of a quantitative double ELISA strategy for confirmation and differentiation of HIV infection. *J Virol Methods*. 1997;66:203–209.
252. Mylonakis E, Paliou M, Lally M, et al. Laboratory testing for infection with the human immunodeficiency virus: established and novel approaches. *Am J Med*. 2000;109:568–576.
253. Constantine NT, Ketema F. Rapid confirmation of HIV infection. *Int J Infect Dis*. 2002;6:170–177.
254. Stetler HC, Granade TC, Nunez CA, et al. Field evaluation of rapid HIV serologic tests for screening and confirming HIV-1 infection in Honduras. *AIDS*. 1997;11:369–375.
255. Johnson CC, Fonner V, Sands A, et al. To err is human, to correct is public health: a systematic review examining poor quality testing and misdiagnosis of HIV status. *J Int AIDS Soc*. 2017;20(suppl 6):21755.
256. Joint United Nations Programme on HIV/AIDS (UNAIDS)-WHO Revised recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec*. 1997;72:81–88.
257. Sato PA, Maskill WJ, Tamashiro H, et al. Strategies for laboratory HIV testing: an examination of alternative approaches not requiring Western blot. *Bull World Health Organ*. 1994;72:129–134.
258. World Health Organization. Consolidated Guidelines on HIV Testing Services. 2015. https://www.who.int/hiv/pub/guidelines/hiv-testing-services/en/.
259. Riddell JT, Amico KR, Mayer KH. HIV preexposure prophylaxis: a review. *JAMA*. 2018;319:1261–1268.
260. Interim guidance: preexposure prophylaxis for the prevention of HIV infection in men who have sex with men. *MMWR Morb Mortal Wkly Rep*. 2011;60:65–68.
261. Sivay MV, Li M, Piwowar-Manning E, et al. Characterization of HIV seroconverters in a TDF/FTC PrEP study: HPTN 067/ADAPT. *J Acquir Immune Defic Syndr*. 2017;75:271–279.
262. Cohen SE, Sachdev D, Lee SA, et al. Acquisition of tenofovir-susceptible, emtricitabine-resistant HIV despite high adherence to daily pre-exposure prophylaxis: a case report. *Lancet HIV*. 2018.
263. Curlin ME, Gvetadze R, Leelawiwat W, et al. Analysis of false-negative human immunodeficiency virus rapid tests performed on oral fluid in 3 international clinical research studies. *Clin Infect Dis*. 2017;64:1663–1669.
264. Delaugerre C, Antoni G, Mahjoub N, et al. Assessment of HIV screening tests for use in preexposure prophylaxis programs. *J Infect Dis*. 2017;216:382–386.
265. Livant E, Heaps A, Kelly C, et al. The fourth generation Alere(TM) HIV Combo rapid test improves detection of acute infection in MTN-003 (VOICE) samples. *J Clin Virol*. 2017;94:15–21.
266. Suntharasamai P, Martin M, Choopanya K, et al. Assessment of oral fluid HIV test performance in an HIV pre-exposure prophylaxis trial in Bangkok, Thailand. *PLoS ONE*. 2015;10:e0145859.
267. Lemey P, Pybus OG, Wang B, et al. Tracing the origin and history of the HIV-2 epidemic. *Proc Natl Acad Sci USA*. 2003;100:6588–6592.
268. Gottlieb GS, Raugi DN, Smith RA. 90-90-90 for HIV-2? Ending the HIV-2 epidemic by enhancing care and clinical management of patients infected with HIV-2. *Lancet HIV*. 2018;5:e390–e399.
269. Rubsamen-Waigmann H, Maniar J, Gerte S, et al. High proportion of HIV-2 and HIV-1/2 double-reactive sera in two Indian states, Maharashtra and Goa: first appearance of an HIV-2 epidemic along with an HIV-1 epidemic outside of Africa. *Zentralbl Bakteriol*. 1994;280:398–402.
270. Visseaux B, Damond F, Matheron S, et al. HIV-2 molecular epidemiology. *Infect Genet Evol*. 2016;46:233–240.
271. Abeler-Dörner L, Grabowski MK, Rambaut A, et al; PANGEA consortium. PANGEA-HIV 2: Phylogenetics and Networks for Generalised Epidemics in Africa. *Curr Opin HIV AIDS*. 2019;14:173–180.
272. Norrgren H, Andersson S, Biague AJ, et al. Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, West Africa: no protection of HIV-2 against HIV-1 infection. *AIDS*. 1999;13:701–707.
273. van der Loeff MF, Aaby P, Aryioshi K, et al. HIV-2 does not protect against HIV-1 infection in a rural community in Guinea-Bissau. *AIDS*. 2001;15:2303–2310.
274. Alabi AS, Jaffar S, Ariyoshi K, et al. Plasma viral load, CD4 cell percentage, HLA and survival of HIV-1, HIV-2, and dually infected Gambian patients. *AIDS*. 2003;17: 1513–1520.
275. Schim van der Loeff MF, Jaffar S, Aveika AA, et al. Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS*. 2002;16:1775–1783.
276. da Silva ZJ, Oliveira I, Andersen A, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *AIDS*. 2008;22:1195–1202.
277. Torian LVSR, Branson B, Owen M, et al. HIV-2 infection surveillance - United States, 1987-2009. *MMWR Morb Mortal Wkly Rep*. 2011;60:985–988.
278. Centers for Disease Control and Prevention. HIV-2 infection surveillance—United States, 1987-2009. *MMWR Morb Mortal Wkly Rep*. 2011;60:985–988.
279. Torian LV, Eavey JJ, Punsalang AP, et al. HIV type 2 in New York City, 2000-2008. *Clin Infect Dis*. 2010;51: 1334–1342.
280. Hollenbeck BL, Beckwith CG. HIV-2 infection in Providence, Rhode Island from 2002 to 2011. *HIV Med*. 2013;14:115–119.
281. Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. *Clin Infect Dis*. 2011;52:780–787.
282. Bottiger B, Karlsson A, Andreasson PA, et al. Envelope cross-reactivity between human immunodeficiency virus types 1 and 2 detected by different serological methods: correlation between cross-neutralization and reactivity against the main neutralizing site. *J Virol*. 1990;64:3492–3499.
283. George JR, Rayfield MA, Phillips S, et al. Efficacies of US Food and Drug Administration-licensed HIV-1-screening enzyme immunoassays for detecting antibodies to HIV-2. *AIDS*. 1990;4:321–326.
284. Sazama K, Kuramoto IK, Holland PV, et al. Detection of antibodies to human immunodeficiency virus type 2 (HIV-2) in blood donor sera using United States assay methods for anti-HIV type 1. *Transfusion*. 1992;32: 398–401.
285. Constantine NT, Callahan JD, Watts DM. Time for HIV-1/HIV-2 combination tests? *J Virol Methods*. 1989;26:219–221.
286. Smith RS, Parks E. Synthetic peptide assays to detect human immunodeficiency virus types 1 and 2 in seropositive individuals. *Arch Pathol Lab Med*. 1990;114:254–258.
287. Broliden PA, Ruden U, Ouattara AS, et al. Specific synthetic peptides for detection of and discrimination between HIV-1 and HIV-2 infection. *J Acquir Immune Defic Syndr*. 1991;4:952–958.
288. Sprengers ED, Hellings JA, Theunissen HJ, et al. A microELISA system for the detection of both anti-HIV-1 and anti-HIV-2 antibodies. *J Immunol Methods*. 1991;139:77–82.
289. Sondergard-Andersen J, Lauritzen E, Lind K, et al. Covalently linked peptides for enzyme-linked immunosorbent assay. *J Immunol Methods*. 1990;131: 99–104.
290. Manocha M, Chitralekha KT, Thakar M, et al. Comparing modified and plain peptide linked enzyme immunosorbent assay (ELISA) for detection of human immunodeficiency virus type-1 (HIV-1) and type-2 (HIV-2) antibodies. *Immunol Lett*. 2003;85:275–278.

291. Kannangai R, Ramalingam S, Prakash KJ, et al. A peptide enzyme linked immunosorbent assay (ELISA) for the detection of human immunodeficiency virus type-2 (HIV-2) antibodies: an evaluation on polymerase chain reaction (PCR) confirmed samples. *J Clin Virol*. 2001;22:41–46.
292. Gonzalez L, Boyle RW, Zhang M, et al. Synthetic-peptide-based enzyme-linked immunosorbent assay for screening human serum or plasma for antibodies to human immunodeficiency virus type 1 and type 2. *Clin Diagn Lab Immunol*. 1997;4:598–603.
293. Imai M, Hayashi T, Kondo M, et al. Differentiation of human immunodeficiency virus type 1 (HIV-1) infections with HIV-2-cross-reacting antibody from mixed infections with HIV-1 and HIV-2 by serological absorption test. *J Clin Microbiol*. 1995;33:1727–1729.
294. Berry NJ, Salker R, Contreras M, et al. A comparison of four enzyme immunoassays for the simultaneous detection of HIV-1- and HIV-2-specific antibody. *J Virol Methods*. 1991;34:91–100.
295. Berry N, Pepin J, Gaye I, et al. Competitive EIA for anti-HIV-2 detection in The Gambia: use as a screening assay and to identify possible dual infections. *J Med Virol*. 1993;39:101–108.
296. Leonard G, Chaput A, Courgnaud V, et al. Characterization of dual HIV-1 and HIV-2 serological profiles by polymerase chain reaction. *AIDS*. 1993;7:1185–1189.
297. Simon F, Matheron S, Tamalet C, et al. Cellular and plasma viral load in patients infected with HIV-2. *AIDS*. 1993;7:1411–1417.
298. Cazals N, Le Hingrat Q, Abraham B, et al. HIV-2 Primary infection in a French 69-year-old bisexual man. *Open Forum Infect Dis*. 2018;5:ofy223.
299. Christiansen CB, Jessen TE, Nielsen C, et al. False negative anti-HIV-1/HIV-2 ELISAs in acute HIV-2 infection. *Vox Sang*. 1996;70:144–147.
300. Simon F, Mauclere P, Roques P, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat Med*. 1998;4:1032–1037.
301. Ayouba A, Souquieres S, Njinku B, et al. HIV-1 group N among HIV-1-seropositive individuals in Cameroon. *AIDS*. 2000;14:2623–2625.
302. Plantier JC, Leoz M, Dickerson JE, et al. A new human immunodeficiency virus derived from gorillas. *Nat Med*. 2009;15:871–872.
303. Dorn J, Masciotra S, Yang C, et al. Analysis of genetic variability within the immunodominant epitopes of envelope gp41 from human immunodeficiency virus type 1 (HIV-1) group M and its impact on HIV-1 antibody detection. *J Clin Microbiol*. 2000;38:773–780.
304. Koch WH, Sullivan PS, Roberts C, et al. Evaluation of United States-licensed human immunodeficiency virus immunoassays for detection of group M viral variants. *J Clin Microbiol*. 2001;39:1017–1020.
305. Peeters M, Gueye A, Mboup S, et al. Geographical distribution of HIV-1 group O viruses in Africa. *AIDS*. 1997;11:493–498.
306. Takehisa J, Kraus MH, Ayouba A, et al. Origin and biology of simian immunodeficiency virus in wild-living western gorillas. *J Virol*. 2009;83:1635–1648.
307. Van Heuverswyn F, Li Y, Neel C, et al. Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature*. 2006;444:164.
308. Branson BM. State of the art for diagnosis of HIV infection. *Clin Infect Dis*. 2007;45(suppl 4):S221–S225.
309. Ondoa P, Willems B, Fransen K, et al. Evaluation of different V3 peptides in an enzyme immunoassay for specific HIV type 1 group O antibody detection. *AIDS Res Hum Retroviruses*. 1998;14:963–972.
310. van Binsbergen J, Keur W, vd Graaf M, et al. Reactivity of a new HIV-1 group O third generation A-HIV-1/-2 assay with an unusual HIV-1 seroconversion panel and HIV-1 group O/group M subtyped samples. *J Virol Methods*. 1997;69:29–37.
311. Mauclere P, Damond F, Apetrei C, et al. Synthetic peptide ELISAs for detection of and discrimination between group M and group O HIV type 1 infection. *AIDS Res Hum Retroviruses*. 1997;13:987–993.
312. Jongerius JM, van der Poel CL, van Loon AM, et al. Human immunodeficiency virus (HIV) antibodies detected by new assays that are enhanced for HIV-1 subtype O. *Transfusion*. 1997;37:841–844.
313. Mauclere P, Loussert-Ajaka I, Damond F, et al. Serological and virological characterization of HIV-1 group O infection in Cameroon. *AIDS*. 1997;11:445–453.
314. Cornelissen M, Pasternak AO, Grijsen ML, et al. HIV-1 dual infection is associated with faster CD4+ T-cell decline in a cohort of men with primary HIV infection. *Clin Infect Dis*. 2012;54:539–547.
315. Freitas FB, Esteves A, Piedade J, et al. Novel multiregion hybridization assay for the identification of the most prevalent genetic forms of the human immunodeficiency virus type 1 circulating in Portugal. *AIDS Res Hum Retroviruses*. 2013;29:318–328.
316. Carvalho A, Costa P, Triunfante V, et al. Analysis of a local HIV-1 epidemic in Portugal highlights established transmission of non-B and non-G subtypes. *J Clin Microbiol*. 2015;53:1506–1514.
317. Van Doornum GJ, Buimer M, Gobbers E, et al. Evaluation of an expanded two-ELISA approach for confirmation of reactive serum samples in an HIV-screening programme for pregnant women. *J Med Virol*. 1998;54:285–290.
318. Nogueira SA, Lambert JS, Albuquerque AL, et al. Assessment of a rapid HIV test strategy during labor: a pilot study from Rio de Janeiro, Brazil. *J Hum Virol*. 2001;4:278–282.
319. American Academy of Pediatrics Committee on Pediatric AIDS. HIV testing and prophylaxis to prevent mother-to-child transmission in the United States. *Pediatrics*. 2008;122:1127–1134.
320. Cassol S, Butcher A, Kinard S, et al. Rapid screening for early detection of mother-to-child transmission of human immunodeficiency virus type 1. *J Clin Microbiol*. 1994;32:2641–2645.
321. Nielsen K, Bryson YJ. Diagnosis of HIV infection in children. *Pediatr Clin North Am*. 2000;47:39–63.
322. Read JS. Diagnosis of HIV-1 infection in children younger than 18 months in the United States. *Pediatrics*. 2007;120:e1547–e1562.
323. Cunningham P, Marriott D, Harris C, et al. False negative HIV-1 proviral DNA polymerase chain reaction in a patient with primary infection acquired in Thailand. *J Clin Virol*. 2003;26:163–169.
324. Faye A, Burgard M, Crosnier H, et al. Human immunodeficiency virus type 2 infection in children. *J Pediatr*. 1997;130:994–997.
325. Centers for Disease Control and Prevention/Department of Health and Human Services. *HIV/AIDS Surveillance Report. US HIV and AIDS Cases Reported Through June 1997*. Vol. 9. Atlanta: Centers for Disease Control and Prevention/Department of Health and Human Services; 1997.
326. HIV transmission through transfusion — Missouri and Colorado, 2008. *MMWR Morb Mortal Wkly Rep*. 2010;59:1335–1339.
327. Schmidt M, Korn K, Nubling CM, et al. First transmission of human immunodeficiency virus Type 1 by a cellular blood product after mandatory nucleic acid screening in Germany. *Transfusion*. 2009;49:1836–1844.
328. Chudy M, Weber-Schehl M, Pichl L, et al. Blood screening nucleic acid amplification tests for human immunodeficiency virus Type 1 may require two different amplification targets. *Transfusion*. 2012;52:431–439.
329. Alvarez M, Luis-Hidalgo M, Bracho MA, et al. Transmission of human immunodeficiency virus type-1 by fresh-frozen plasma treated with methylene blue and light. *Transfusion*. 2016;56:831–836.
330. Sobata R, Shinohara N, Matsumoto C, et al. First report of human immunodeficiency virus transmission via a blood donation that tested negative by 20-minipool nucleic acid amplification in Japan. *Transfusion*. 2014;54:2361–2362.
331. Busch MP, Bloch EM, Kleinman SH. Prevention of transfusion transmitted infections. *Blood*. 2019;133:1854–1864.
332. Hourfar MK, Jork C, Schottstedt V, et al. Experience of German Red Cross blood donor services with nucleic acid testing: results of screening more than 30 million blood donations for human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus. *Transfusion*. 2008;48:1558–1566.
333. Roth WK, Busch MP, Schuller A, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. *Vox Sang*. 2012;102:82–90.
334. Stramer SL, Krysztof DE, Brodsky JP, et al. Comparative analysis of triplex nucleic acid test assays in United States blood donors. *Transfusion*. 2013;53(10 Pt 2):2525–2537.
335. World Health Organization. Blood Safety and Availability; May 29, 2019. https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability. Accessed June 19, 2019.
336. Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. *Transfusion*. 2009;49:2454–2489.
337. Candotti D, Allain JP. Molecular virology in transfusion medicine laboratory. *Blood Transfus*. 2013;11:203–216.
338. Cappy P, Barlet V, Lucas Q, et al. Transfusion of HIV-infected blood products despite highly sensitive nucleic acid testing. *Transfusion*. 2019.
339. World Health Organization (WHO). *Global Status Report on Blood Safety and Availability, 2016*. Geneva: WHO; 2017.
340. Ahn J, Cohen SM. Transmission of human immunodeficiency virus and hepatitis C virus through liver transplantation. *Liver Transpl*. 2008;14:1603–1608.
341. HIV transmitted from a living organ donor—New York City, 2009. *Am J Transplant*. 2011;11:1334–1337.
342. Connor RI, Korber BT, Graham BS, et al. Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines. *J Virol*. 1998;72:1552–1576.
343. Couderc M. Being "false positive": an "inconvenience"? debates and questions regarding the notion of vaccine-induced seropositivity (VISP) in the recruitment of healthy volunteers for a preventive anti-HIV vaccine trial. *J Acquir Immune Defic Syndr*. 2018;79(suppl 1):S20–S29.
344. Weber J. Distinguishing between response to HIV vaccine and response to HIV. *Lancet*. 1997;350:230–231.
345. McGarrity GJ, Hoyah G, Winemiller A, et al. Patient monitoring and follow-up in lentiviral clinical trials. *J Gene Med*. 2013;15:78–82.
346. Laetsch TW, Maude SL, Milone MC, et al. False-positive results with select HIV-1 NAT methods following lentivirus-based tisagenlecleucel therapy. *Blood*. 2018;131:2596–2598.
347. Ariza-Heredia EJ, Granwehr BP, Viola GM, et al. False-positive HIV nucleic acid amplification testing during CAR T-cell therapy. *Diagn Microbiol Infect Dis*. 2017;88:305–307.
348. De Ravin SS, Gray JT, Throm RE, et al. False-positive HIV PCR test following ex vivo lentiviral gene transfer treatment of X-linked severe combined immunodeficiency vector. *Mol Ther*. 2014;22:244–245.
349. Check E. A tragic setback. *Nature*. 2002;420:116–118.
350. Dinoso JB, Kim SY, Siliciano RF, et al. A comparison of viral loads between HIV-1-infected elite suppressors and individuals who receive suppressive highly active antiretroviral therapy. *Clin Infect Dis*. 2008;47:102–104.
351. Mens H, Kearney M, Wiegand A, et al. HIV-1 continues to replicate and evolve in patients with natural control of HIV infection. *J Virol*. 2010;84:12971–12981.
352. Hatano H, Delwart EL, Norris PJ, et al. Evidence for persistent low-level viremia in individuals who control human immunodeficiency virus in the absence of antiretroviral therapy. *J Virol*. 2009;83:329–335.
353. Migueles SA, Connors M. Success and failure of the cellular immune response against HIV-1. *Nat Immunol*. 2015;16:563–570.
354. Sajadi M, Redfield RR. Long-term nonprogressive disease among individuals with untreated HIV infection. *JAMA*. 2010;304:1784–1785, author reply 1785–1786.
355. Walker BD, Yu XG. Unravelling the mechanisms of durable control of HIV-1. *Nat Rev Immunol*. 2013;13:487–498.
356. Aptima HIV-1 RNA Qualitative Assay. Marlborough, MA, Hologic, 2015.
357. American Medical Association Council of Ethical and Judicial Affairs. Code of Medical Ethics Opinion E-2.23, HIV Testing, 2003.
358. Zonios DI, Falloon J, Bennett JE, et al. Idiopathic CD4+ lymphocytopenia: natural history and prognostic factors. *Blood*. 2008;112:287–294.
359. Craven DE, Steger KA, La Chapelle R, et al. Factitious HIV infection: the importance of documenting infection. *Ann Intern Med*. 1994;121:763–766.
360. Sacks M, Burton W, Dermatis H, et al. HIV-related cases among 2,094 admissions to a psychiatric hospital. *Psychiatr Serv*. 1995;46:131–135.
361. Wu AW, Kennedy CJ, Paradise M. Factitious false-positive test for HIV. *JAMA*. 1988;259:1647.
362. Zuger A, O'Dowd MA. The baron has AIDS: a case of factitious human immunodeficiency virus infection and review. *Clin Infect Dis*. 1992;14:211–216.
363. Li YL, Li TS, Xie J, et al. An analysis of clinical characteristics of forty-six AIDS phobia patients. *Zhonghua Nei Ke Za Zhi*. 2011;50:650–653.
364. Moyo S, Wilkinson E, Novitsky V, et al. Identifying recent HIV infections: from serological assays to genomics. *Viruses*. 2015;7:5508–5524.
365. Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA*. 1998;280:42–48.
366. Rutherford GW, Schwarcz SK, McFarland W. Surveillance for incident HIV infection: new technology and new opportunities. *J Acquir Immune Defic Syndr*. 2000;25(suppl 2):S115–S119.
367. Gouws E, Williams BG, Sheppard HW, et al. High incidence of HIV-1 in South Africa using a standardized algorithm for recent HIV seroconversion. *J Acquir Immune Defic Syndr*. 2002;29:531–535.
368. Kral AH, Lorvick J, Gee L, et al. Trends in human immunodeficiency virus seroincidence among

street-recruited injection drug users in San Francisco, 1987-1998. *Am J Epidemiol*. 2003;157:915–922.

369. McFarland W, Busch MP, Kellogg TA, et al. Detection of early HIV infection and estimation of incidence using a sensitive/less-sensitive enzyme immunoassay testing strategy at anonymous counseling and testing sites in San Francisco. *J Acquir Immune Defic Syndr*. 1999;22:484–489.
370. Parekh BS, Kennedy MS, Dobbs T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Res Hum Retroviruses*. 2002;18:295–307.
371. Nesheim S, Parekh B, Sullivan K, et al. Temporal trends in HIV Type 1 incidence among inner-city childbearing women in Atlanta: use of the IgG-capture BED-enzyme immunoassay. *AIDS Res Hum Retroviruses*. 2005;21: 537–544.
372. Hu DJ, Vanichseni S, Mock PA, et al. HIV type 1 incidence estimates by detection of recent infection from a cross-sectional sampling of injection drug users in Bangkok: use of the IgG capture BED enzyme immunoassay. *AIDS Res Hum Retroviruses*. 2003;19: 727–730.
373. UNAIDS Reference Group on estimates, modelling and projections–statement on the use of the BED assay for the estimation of HIV-1 incidence for surveillance or epidemic monitoring. *Wkly Epidemiol Rec*. 2006;81:40.
374. Wolday D, Meles H, Hailu E, et al. Temporal trends in the incidence of HIV infection in antenatal clinic attendees in Addis Ababa, Ethiopia, 1995-2003. *J Intern Med*. 2007;261:132–137.
375. Karita E, Price M, Hunter E, et al. Investigating the utility of the HIV-1 BED capture enzyme immunoassay using cross-sectional and longitudinal seroconverter specimens from Africa. *AIDS*. 2007;21:403–408.
376. Suligoi B, Regine V, Raimondo M, et al. HIV avidity index performance using a modified fourth-generation immunoassay to detect recent HIV infections. *Clin Chem Lab Med*. 2017;55:2010–2019.
377. Tuaillon E, Sanosyan A, Pisoni A, et al. Staging of recent HIV-1 infection using Geenius rapid confirmatory assay compared to INNO-LIA, New Lav and Blot 2.2 assays. *J Clin Virol*. 2017;95:47–51.
378. Ananworanich J, Fletcher JL, Pinyakorn S, et al. A novel acute HIV infection staging system based on 4th generation immunoassay. *Retrovirology*. 2013;10:56.
379. Robb ML, Eller LA, Kibuuka H, et al. Prospective study of acute HIV-1 infection in adults in East Africa and Thailand. *N Engl J Med*. 2016;374:2120–2130.
380. Mendoza D, Johnson SA, Peterson BA, et al. Comprehensive analysis of unique cases with extraordinary control over HIV replication. *Blood*. 2012;119:4645–4655.
381. Burbelo PD, Bren KE, Ching KH, et al. LIPS arrays for simultaneous detection of antibodies against partial and whole proteomes of HCV, HIV and EBV. *Mol Biosyst*. 2011;7:1453–1462.
382. Kouyos RD, von Wyl V, Yerly S, et al. Ambiguous nucleotide calls from population-based sequencing of HIV-1 are a marker for viral diversity and the age of infection. *Clin Infect Dis*. 2011;52:532–539.
383. Andersson E, Shao W, Bontell I, et al. Evaluation of sequence ambiguities of the HIV-1 pol gene as a method to identify recent HIV-1 infection in transmitted drug resistance surveys. *Infect Genet Evol*. 2013;18:125–131.
384. Grossman Z, Avidor B, Mor Z, et al. A population-structured HIV epidemic in Israel: roles of risk and ethnicity. *PLoS ONE*. 2015;10:e0135061.
385. Wu JW, Patterson-Lomba O, Novitsky V, et al. A generalized entropy measure of within-host viral diversity for identifying recent HIV-1 Infections. *Medicine (Baltimore)*. 2015;94:e1865.
386. Puller V, Neher R, Albert J. Estimating time of HIV-1 infection from next-generation sequence diversity. *PLoS Comput Biol*. 2017;13:e1005775.
387. Kafando A, Fournier E, Serhir B, et al. HIV-1 envelope sequence-based diversity measures for identifying recent infections. *PLoS ONE*. 2017;12:e0189999.
388. Dennis AM, Zhou S, Sellers CJ, et al. Using Primer-ID Deep Sequencing to detect recent human immunodeficiency virus type 1 infection. *J Infect Dis*. 2018;218:1777–1782.
389. Cousins MM, Konikoff J, Sabin D, et al. A comparison of two measures of HIV diversity in multi-assay algorithms for HIV incidence estimation. *PLoS ONE*. 2014;9:e101043.
390. Xia XY, Ge M, Hsi JH, et al. High-accuracy identification of incident HIV-1 infections using a sequence clustering based diversity measure. *PLoS ONE*. 2014;9:e100081.
391. Moyo S, Wilkinson E, Vandormael A, et al. Pairwise diversity and tMRCA as potential markers for HIV infection recency. *Medicine (Baltimore)*. 2017;96:e6041.
392. Park SY, Love TMT, Reynell L, et al. The HIV genomic incidence assay meets false recency rate and mean duration of recency infection performance standards. *Sci Rep*. 2017;7:7480.
393. Eisenberg AL, Patel EU, Packman ZR, et al. Short communication: dried blood spots stored at room temperature should not be used for HIV incidence testing. *AIDS Res Hum Retroviruses*. 2018;34:1013–1016.
394. Laeyendecker O, Konikoff J, Morrison DE, et al. Identification and validation of a multi-assay algorithm for cross-sectional HIV incidence estimation in populations with subtype C infection. *J Int AIDS Soc*. 2018;21.
395. Grebe E, Welte A, Johnson LF, et al. Population-level HIV incidence estimates using a combination of synthetic cohort and recency biomarker approaches in KwaZulu-Natal, South Africa. *PLoS ONE*. 2018;13:e0203638.
396. Garrett N, Lattimore S, Gilbart V, et al. The Recent Infection Testing Algorithm (RITA) in clinical practice: a survey of HIV clinicians in England and Northern Ireland. *HIV Med*. 2012;13:444–447.
397. Patel AV, Abrams SM, Gaydos CA, et al. Increasing HIV testing engagement through provision of home HIV self-testing kits for patients who decline testing in the emergency department: a pilot randomisation study. *Sex Transm Infect*. 2018;pii: sextrans-2018-053592.
398. Katz DA, Golden MR, Hughes JP, et al. HIV self-testing increases HIV testing frequency in high-risk men who have sex with men: a randomized controlled trial. *J Acquir Immune Defic Syndr*. 2018;78:505–512.
399. Figueroa C, Johnson C, Ford N, et al. Reliability of HIV rapid diagnostic tests for self-testing compared with testing by health-care workers: a systematic review and meta-analysis. *Lancet HIV*. 2018;5:e277–e290.
400. Ortblad KF, Musoke DK, Ngabirano T, et al. Female sex workers often incorrectly interpret HIV self-test results in Uganda. *J Acquir Immune Defic Syndr*. 2018;79:e42–e45.
401. Wei C, Yan L, Lippman SA, et al. Prevalence and correlates of point-of-sex human immunodeficiency virus self-testing among human immunodeficiency virus-negative men who have sex with men in China. *Sex Transm Dis*. 2018;45:818–822.
402. Ventuneac A, Carballo-Dieguez A, Leu CS, et al. Use of a rapid HIV home test to screen sexual partners: an evaluation of its possible use and relative risk. *AIDS Behav*. 2009;13:731–737.
403. Grov C, Rendina HJ, Breslow AS, et al. Characteristics of men who have sex with men (MSM) who attend sex parties: results from a national online sample in the USA. *Sex Transm Infect*. 2014;90:26–32.
404. Katz DA, Cassels SL, Stekler JD. Replacing clinic-based tests with home-use tests may increase HIV prevalence among Seattle men who have sex with men: evidence from a mathematical model. *Sex Transm Dis*. 2014;41:2–9.
405. Wray TB, Chan PA, Simpanen E, et al. A pilot, randomized controlled trial of HIV self-testing and real-time post-test counseling/referral on screening and preventative care among men who have sex with men. *AIDS Patient Care STDS*. 2018;32:360–367.
406. Tun W, Vu L, Dirisu O, et al. Uptake of HIV self-testing and linkage to treatment among men who have sex with men (MSM) in Nigeria: a pilot programme using key opinion leaders to reach MSM. *J Int AIDS Soc*. 2018;21(suppl 5):e25124.
407. Choko AT, Nanfuka M, Birungi J, et al. A pilot trial of the peer-based distribution of HIV self-test kits among fishermen in Bulisa, Uganda. *PLoS ONE*. 2018;13: e0208191.
408. Harichund C, Karim QA, Kunene P, et al. HIV self-testing as part of a differentiated HIV testing approach: exploring urban and rural adult experiences from KwaZulu-Natal, South Africa using a cross-over study design. *BMC Public Health*. 2019;19:53.
409. Sabapathy K, Mulubwa C, Mathema H, et al. Is home-based HIV testing universally acceptable? Findings from a case-control study nested within the HPTN 071 (PopART) trial. *Trop Med Int Health*. 2018;23:678–690.
410. World Health Organization. Guidelines on HIV Self-testing and Partner Notification. Supplement to Consolidated Guidelines on HIV Testing Services. 2016. https://www.who.int/hiv/pub/vct/hiv-self-testing-guidelines/en/.
411. Neuman M, Indravudh P, Chilongosi R, et al. The effectiveness and cost-effectiveness of community-based lay distribution of HIV self-tests in increasing uptake of HIV testing among adults in rural Malawi and rural and peri-urban Zambia: protocol for STAR (self-testing for Africa) cluster randomized evaluations. *BMC Public Health*. 2018;18:1234.
412. George G, Chetty T, Strauss M, et al. Costing analysis of an SMS-based intervention to promote HIV self-testing amongst truckers and sex workers in Kenya. *PLoS ONE*. 2018;13:e0197305.
413. Qin Y, Han L, Babbitt A, et al. Experiences using and organizing HIV self-testing. *AIDS*. 2018;32:371–381.
414. Tucker JD, Wei C, Pendse R, et al. HIV self-testing among key populations: an implementation science approach to evaluating self-testing. *J Virus Erad*. 2015;1:38–42.
415. Sauter D, Kirchhoff F. Key viral adaptations preceding the AIDS pandemic. *Cell Host Microbe*. 2019;25:27–38.
416. Kalish ML, Ndongmo CB, Wolfe ND, et al. Evidence for continued exposure to and possible infection of humans with SIV. In: *HIV Dynamics and Evolution 10th International Workshop*. Lake Arrowhead, CA: 2003.
417. Yang C, Dash BC, Simon F, et al. Detection of diverse variants of human immunodeficiency virus-1 groups M, N, and O and simian immunodeficiency viruses from chimpanzees by using generic pol and env primer pairs. *J Infect Dis*. 2000;181:1791–1795.
418. Simon F, Souquiere S, Damond F, et al. Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses. *AIDS Res Hum Retroviruses*. 2001;17:937–952.
419. Wolfe ND, Heneine W, Carr JK, et al. Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. *Proc Natl Acad Sci USA*. 2005;102:7994–7999.
420. Khan AS, Bodem J, Buseyne F, et al. Spumaretroviruses: updated taxonomy and nomenclature. *Virology*. 2018;516:158–164.
421. Pinto-Santini DM, Stenbak CR, Linial ML. Foamy virus zoonotic infections. *Retrovirology*. 2017;14:55.
422. Switzer WM, Bhullar V, Shanmugam V, et al. Frequent simian foamy virus infection in persons occupationally exposed to nonhuman primates. *J Virol*. 2004;78: 2780–2789.
423. Switzer WM, Tang S, Ahuka-Mundeke S, et al. Novel simian foamy virus infections from multiple monkey species in women from the Democratic Republic of Congo. *Retrovirology*. 2012;9:100.
424. Engel GA, Small CT, Soliven K, et al. Zoonotic simian foamy virus in Bangladesh reflects diverse patterns of transmission and co-infection. *Emerg Microbes Infect*. 2013;2:e58.
425. Muniz CP, Cavalcante LTF, Jia H, et al. Zoonotic infection of Brazilian primate workers with New World simian foamy virus. *PLoS ONE*. 2017;12:e0184502.
426. Stoye JP, Le Tissier P, Takeuchi Y, et al. Endogenous retroviruses: a potential problem for xenotransplantation? *Ann N Y Acad Sci*. 1998;862:67–74.
427. Stoye JP, Coffin JM. The dangers of xenotransplantation. *Nat Med*. 1995;1:1100.
428. Blusch JH, Patience C, Martin U. Pig endogenous retroviruses and xenotransplantation. *Xenotransplantation*. 2002;9:242–251.
429. Ericsson TA, Takeuchi Y, Templin C, et al. Identification of receptors for pig endogenous retrovirus. *Proc Natl Acad Sci USA*. 2003;100:6759–6764.
430. Qari SH, Magre S, Garcia-Lerma JG, et al. Susceptibility of the porcine endogenous retrovirus to reverse transcriptase and protease inhibitors. *J Virol*. 2001;75:1048–1053.
431. US Food and Drug Administration. Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans: Guidance for Industry; December 2016. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/source-animal-product-preclinical-and-clinical-issues-concerning-use-xenotransplantation-products. Accessed June 20, 2019.
432. Fishman JA. Infectious disease risks in xenotransplantation. *Am J Transplant*. 2018;18:1857–1864.
433. Birmingham K. FDA subcommittee finds no evidence of PERV transmission. *Nat Med*. 1999;5:855.
434. Jin SY, Choi HY, Kim HS, et al. Human-APOBEC3G-dependent restriction of porcine endogenous retrovirus replication is mediated by cytidine deamination and inhibition of DNA strand transfer during reverse transcription. *Arch Virol*. 2018;163:1907–1914.
435. Switzer WM, Michler RE, Shanmugam V, et al. Lack of cross-species transmission of porcine endogenous retrovirus infection to nonhuman primate recipients of porcine cells, tissues, or organs. *Transplantation*. 2001;71: 959–965.
436. van der Laan LJ, Lockey C, Griffeth BC, et al. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature*. 2000;407:90–94.
437. Matthews AL, Brown J, Switzer W, et al. Development and validation of a Western immunoblot assay for detection of antibodies to porcine endogenous retrovirus. *Transplantation*. 1999;67:939–943.

438. HIV causes AIDS: To argue otherwise cost lives. *New York Times*. July 2, 2000.
439. Weir SS, Baral SD, Edwards JK, et al. Opportunities for enhanced strategic use of surveys, medical records, and program data for HIV surveillance of key populations: scoping review. *JMIR Public Health Surveill*. 2018;4:e28.
440. US Food and Drug Administration. APTIMA HIV-1 RNA Qualitative Assay; page last updated February 27, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm149922.htm. Accessed April 11, 2019.
441. US Food and Drug Administration. Elecsys HIV combi PT; page last updated March 21, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm564192.htm. Accessed April 11, 2019.
442. US Food and Drug Administration. VITROS Immunodiagnostic Products HIV Combo Reagent Pack & VITROS Immunodiagnostic Products HIV Combo Calibrator; page last updated December 14, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm588917.htm. Accessed April 11, 2019.
443. US Food and Drug Administration. BioPlex 2200 HIV Ag-Ab Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm455818.htm. Accessed April 11, 2019.
444. US Food and Drug Administration. ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm450386.htm. Accessed April 11, 2019.
445. US Food and Drug Administration. Abbott ARCHITECT HIV Ag/Ab Combo; page last updated February 28, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm216291.htm. Accessed April 11, 2019.
446. US Food and Drug Administration. GS HIV Combo Ag/Ab EIA; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm264723.htm. Accessed April 11, 2019.
447. US Food and Drug Administration. Alere Determine HIV-1/2 Ag/Ab Combo; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm364651.htm. Accessed April 11, 2019.
448. US Food and Drug Administration. Geenius HIV 1/2 Supplemental Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm420713.htm. Accessed April 11, 2019.
449. US Food and Drug Administration. Chembio SURE CHECK HIV 1/2 Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091240.htm. Accessed April 11, 2019.
450. US Food and Drug Administration. Chembio HIV 1/2 STAT-PAK Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091243.htm. Accessed April 11, 2019.
451. US Food and Drug Administration. OraQuick ADVANCE Rapid HIV/1/2 Antibody Test; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091491.htm. Accessed April 11, 2019.
452. US Food and Drug Administration. Chembio DPP HIV 1/2 Assay; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm333601.htm. Accessed April 11, 2019.
453. US Food and Drug Administration. INSTI HIV-1/HIV-2 Antibody Test; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm235024.htm. Accessed April 11, 2019.
454. US Food and Drug Administration. MedMira Reveal Rapid HIV-1 Antibody Test; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091478.htm. Accessed April 11, 2019.
455. US Food and Drug Administration. Uni-Gold Recombigen HIV-1/2; page last updated July 24, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091636.htm. Accessed April 11, 2019.
456. US Food and Drug Administration. Genetic Systems HIV-1/HIV-2 Plus O EIA; page last updated October 24, 2017. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm091151.htm. Accessed April 11, 2019.
457. US Food and Drug Administration. Avioq HIV-1 Microelisa System; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm185249.htm. Accessed April 11, 2019.
458. US Food and Drug Administration. ABBOTT PRISM HIV O Plus; page last updated February 27, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm182928.htm. Accessed April 11, 2019.
459. US Food and Drug Administration. ADVIA Centaur HIV 1/O/2 Enhanced ReadyPack Reagents; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091217.htm. Accessed April 11, 2019.
460. US Food and Drug Administration. OraQuick In-Home HIV Test; page last updated March 27, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm310436.htm. Accessed April 11, 2019.
461. US Food and Drug Administration. Home Access HIV-1 Test System; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091475.htm. Accessed April 11, 2019.
462. US Food and Drug Administration. Cambridge Biotech HIV-1 Western Blot Kit; content current as of April 16, 2019. https://www.fda.gov/vaccines-blood-biologics/blood-donor-screening/cambridge-biotech-hiv-1-western-blot-kit. Accessed June 19, 2019.
463. US Food and Drug Administration. OraSure HIV-1 Western Blot Kit; page last updated March 7, 2018. https://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/ucm091471.htm. Accessed April 11, 2019.

121 The Immunology of Human Immunodeficiency Virus Infection

Susan Moir, Mark Connors, and Anthony S. Fauci[a]

The interactions between the human immunodeficiency virus (HIV) and the human immune system are extraordinarily complex, as evidenced by the highly variable rates of disease progression observed in HIV-infected individuals. Indeed, even individuals who were infected from a common source may experience widely divergent clinical outcomes.[1,2] HIV subverts the immune system by infecting $CD4^+$ T cells that normally orchestrate immune responses and by activating the immune system and inducing a replication-permissive microenvironment that the virus uses to its own advantage. The discoveries that in addition to the CD4 molecule, the primary receptor for HIV, certain chemokine receptors function as essential HIV coreceptors for HIV entry into target cells, whereas other receptors facilitate entry or propagation have expanded the scope of host factors that play a role in the pathogenesis of HIV-induced disease.

The lack of recognizable and consistent correlates of protective immunity in HIV infection continues to hamper vaccine development and immunotherapeutic approaches to HIV disease. Robust HIV-specific immune responses, both humoral and cell mediated, are mounted by most infected individuals. Nonetheless, the vast majority of untreated HIV-infected individuals experience inexorable disease progression, resulting in profound immunodeficiency, despite the presence of these robust antiviral immune responses. In this regard, qualitative in addition to quantitative aspects of virus-specific immune responses clearly are important in the containment of viral replication.

The progress that has been made to date in understanding the pathogenesis of HIV infection is unparalleled. The availability of effective combination antiretroviral therapy (ART) has had extraordinary clinical benefits for patients, has diminished the transmissibility of virus from an infected to an uninfected individual, and has also provided important insights into the immunologic and virologic factors associated with the control of HIV infection and disease progression. Studies on cohorts of well-defined HIV-infected individuals, including long-term nonprogressors/elite controllers (LTNP/ECs) and those initiating ART early after infection, have also provided important insights into HIV immunopathogenesis. However, fundamental questions remain regarding the nature and scope of the precise pathogenic mechanisms of HIV disease. It is clear that HIV induces dysfunction of almost every element of the immune system and that the pathogenesis of HIV disease is multifactorial.[3–5] In this regard, a great deal is known about how HIV hijacks the immune system by targeting lymphoid tissues and perturbing the immunoregulatory balance; however, any attempt to manipulate these enormously complex and intertwined immunologic parameters for the purpose of inhibiting HIV replication without untoward effects is a daunting task. Nonetheless, progress in the understanding of how viral replication can be restricted with immunotherapeutic interventions is providing new treatment and potentially curative opportunities.[6–11]

HIV ENTRY AND DISSEMINATION

HIV Receptors and Entry Into Cells

CD4 was identified as the major cellular receptor for HIV fusion and entry in 1984.[12,13] Additional factors for viral entry were suspected, yet remained elusive for many years until the recognition of HIV coreceptors. In late 1995 and early 1996, a series of papers was published that altered our understanding of how HIV enters a target cell. The first report, by Cocchi and coworkers,[14] identified the CC chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES (*r*egulated on *a*ctivation, *n*ormal *T*-cell *e*xpressed and *s*ecreted), also known as CCL3, CCL4, and CCL5, respectively, as major components of $CD8^+$ T-cell–derived HIV suppressor factors. They observed that these chemokines, in combination, could inhibit the infection of activated $CD4^+$ T cells by certain strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV). Subsequently, a chemokine receptor, CXCR4, together with CD4, was required for entry of X4 viruses into target cells but was not used by R5 strains.[15]

In a separate line of research, Paxton and coworkers studied a population of individuals who had been exposed repeatedly to HIV-infected partners but who remained uninfected.[16] They identified two individuals whose $CD4^+$ T cells were refractory to infection with R5 strains of HIV, but were easily infectable with X4 strains. In addition, cells from these individuals produced high levels of MIP-1α, MIP-1β, and RANTES, the same chemokines previously identified as suppressors of HIV infection. Shortly thereafter, the chemokine receptor CCR5 was identified and shown in a series of papers to be a coreceptor for cellular entry of R5 strains (Fig. 121.1).[17–21] Subsequently, a mutant allele of the *CCR5* gene was described that contained a 32-bp deletion resulting in a truncated nonfunctional coreceptor for HIV entry.[22,23] Because CCR5-utilizing HIV is almost universally responsible for primary infection, individuals homozygous for the *CCR5* mutation are almost completely protected from HIV-1 infection.[24] Heterozygosity for the *CCR5* mutation results in reduced expression of CCR5 on the cell surface. Although heterozygosity for *CCR5* does not appear to afford protection against HIV-1 infection, it may result in slowed progression of disease in HIV-infected individuals.[24] Conversely, several polymorphisms in the *CCR5* gene and its ligands have been associated with more rapid disease progression.[25] Since the report of a single case of long-term control of HIV-1 in the absence of ART by genetic targeting of CCR5,[26] deletion of the *CCR5* gene through either ablation followed by stem cell transplantation from a *CCR5*-deficient donor or gene therapy to block or delete *CCR5* has been proposed as one of several potential strategies for eradicating HIV from infected individuals.[9] Nonetheless, the reproducibility and practicality of such an approach currently remains in doubt, especially for the tens of millions individuals currently living with HIV in resource-poor countries.

Over the past two decades, many other receptors have been suggested to play a role in HIV entry into target cells. There is a general consensus that CD4 and a chemokine coreceptor, usually either CCR5 or CXCR4, are essential for viral entry into target cells, whereas other receptors may serve merely to facilitate infection or transmission of HIV. In this regard, several pattern recognition receptors (PRRs), including dendritic cell (DC)–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) and related calcium-dependent lectins are regarded as important attachment receptors for HIV and may play an important role in early events of transmission (see next section).[27,28] Attachment factors can also enhance cell-to-cell spread via the formation of virologic synapses (see Fig. 121.1). The integrin $\alpha_4\beta_7$ has been shown to bind the HIV envelope glycoprotein gp120 and serve as an attachment receptor for HIV.[29] Most recently, treatment with an antibody that targets $\alpha_4\beta_7$ has been shown to prevent and restrict SIV replication in a nonhuman primate (NHP) model.[30] The interaction between $\alpha_4\beta_7$ and gp120 may be especially important for replication in the gut-associated lymphoid

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

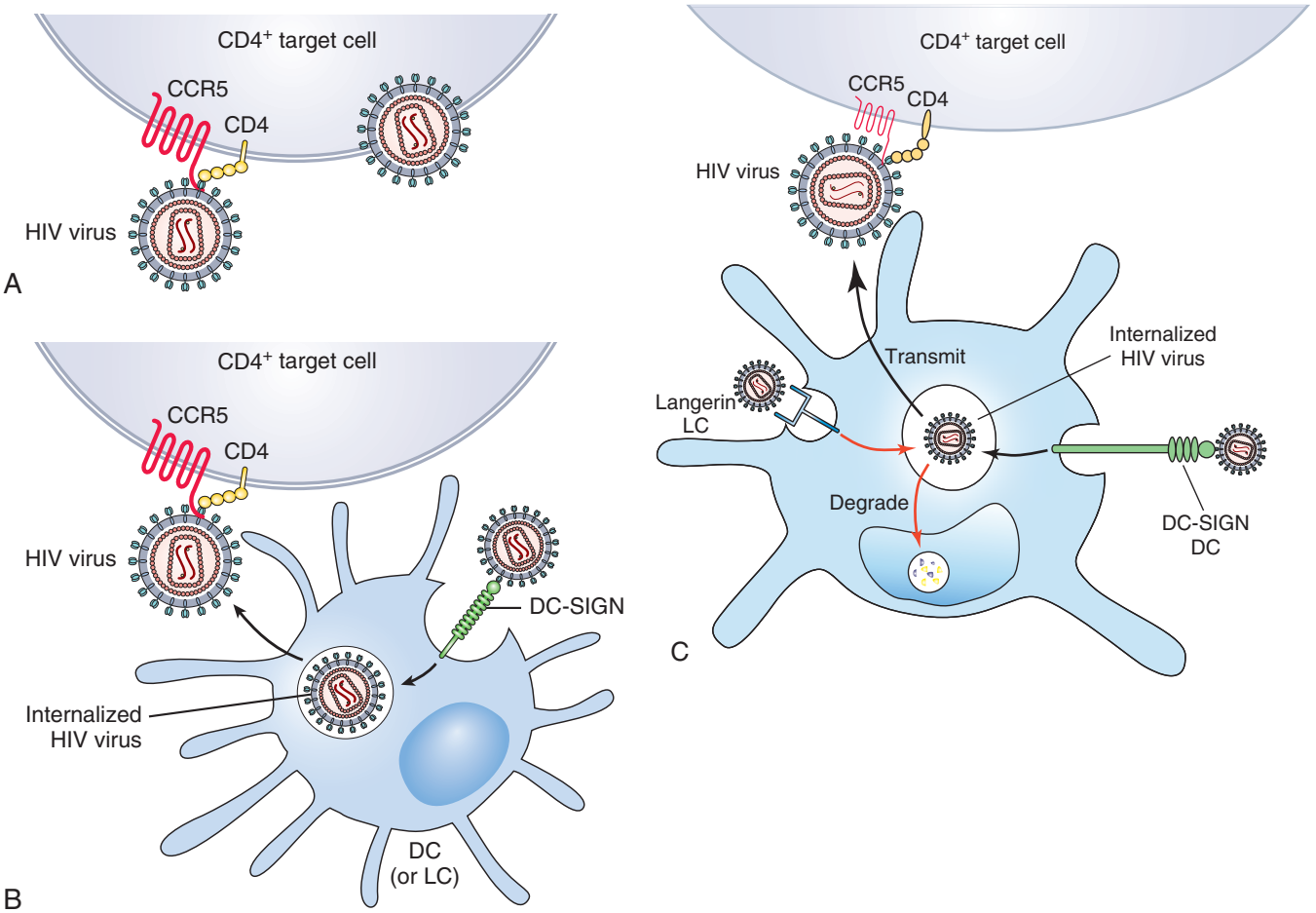

FIG. 121.1 Model of virus-cell infection and cell-to-cell transmission of human immunodeficiency virus *(HIV).* (A) CCR5-utilizing strains of HIV (R5) infect $CD4^+$ target cells by binding to both CD4 and the chemokine receptor CCR5, followed by fusion and entry into the cell. (B) Cell-to-cell spreading of virus can occur by transinfection, whereby a DC or LC captures virus through a C-type lectin receptor such as DC-SIGN or langerin, a process thought to occur both at the site of transmission and in lymphoid tissues. After capture, the DC internalizes the virus into a cellular vesicle and transfers it to a target $CD4^+$ T cell, whereas the LC degrades the virus in a TRIM5α/langerin-dependent process. However, HIV degradation in the LC may be reversed by inflammation and coinfections. (C) Passage of virus from one infected cell to another uninfected target cell also occurs, and both processes can be enhanced by interactions between various ligands and host attachment receptors; for example, the binding of HIV envelope gp120 to the integrin $\alpha_4\beta_7$ leads to binding of LFA-1 to ICAM-1 through a cascade of activation steps. These interactions coalesce to form virologic synapses that can enhance virus spreading by physical proximity and possibly by excluding neutralizing antibodies. *DC,* Dendritic cell; *DC-SIGN,* dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin; *LC,* Langerhans cell.

tissues (GALT), where this integrin is preferentially expressed on resident $CD4^+$ T cells.[28] In addition, the binding of gp120 to $\alpha_4\beta_7$ leads to activation of another integrin, lymphocyte function–associated antigen 1 (LFA-1), which also enhances the replication efficiency of HIV. This receptor may also play a role in the facilitation of HIV transmission across genital mucosal surfaces.[28]

Dissemination of HIV Infection

It remains unclear which cell type is actually the first to become infected after exposure to HIV. The most frequent route of exposure occurs through sexual transmission (Fig. 121.2), with less frequent routes including blood and mother-to-child transmission.[31] However, regardless of the route of entry, rapid migration to regional lymph nodes is followed by dissemination via the bloodstream to various lymphoid tissues (see Fig. 121.2). Massive depletion of memory $CD4^+$ T cells in the GALT has been shown to occur very early after SIV and HIV infections,[32–35] although there is some debate as to whether the depletion is a result of direct infection, bystander effects, or both.[36]

In early studies of macaques exposed to SIV intravaginally, myeloid dendritic cells (mDCs) in the vaginal mucosa were identified as the first cells to contain SIV DNA at approximately 2 days after exposure.[37] Improvements in in situ hybridization and imaging techniques have provided better insight into the early events of virus transmission, particularly in NHP models, although there still remains debate regarding which cells are infected first. The answer may depend on the type of mucosal transmission, whether vaginal, rectal, or penile, with important variables that include target cell availability and attributes of the mucosal epithelial barrier.[38,39] Nonetheless, DCs, especially the specialized DCs called Langerhans cells (LCs), in addition to macrophages, interstitial DCs, and resting $CD4^+$ T cells are all potentially among the first cells to capture and/or replicate HIV.[28,40] Various studies of heterosexual transmission in NHPs have also shown that both cell-free and cell-associated virus in semen, including virus associated with macrophages and to a lesser extent with $CD4^+$ T cells, can penetrate the vaginal epithelium and rapidly disseminate to lymphoid tissues.[41] Given the difficulties of studying early events of HIV infection in humans, various organ cultures, including intact human cervical tissue explants, have been used as models of HIV transmission. Exposure of cervical explants to HIV has demonstrated that intraepithelial $CD4^+$ T cells and LCs rapidly bind virus.[42,43] HIV target cells, including macrophages and $CD4^+$ T cells, and stromal DCs and LCs are also present in the male genital tract and are especially abundant in the foreskin.[31] The very strong protective effect of circumcision on HIV transmission is suggestive of a role for cells of the foreskin in susceptibility to HIV infection.[44] These areas of research have also advanced considerably over the past several years, at least in part driven by a growing interest in preventing

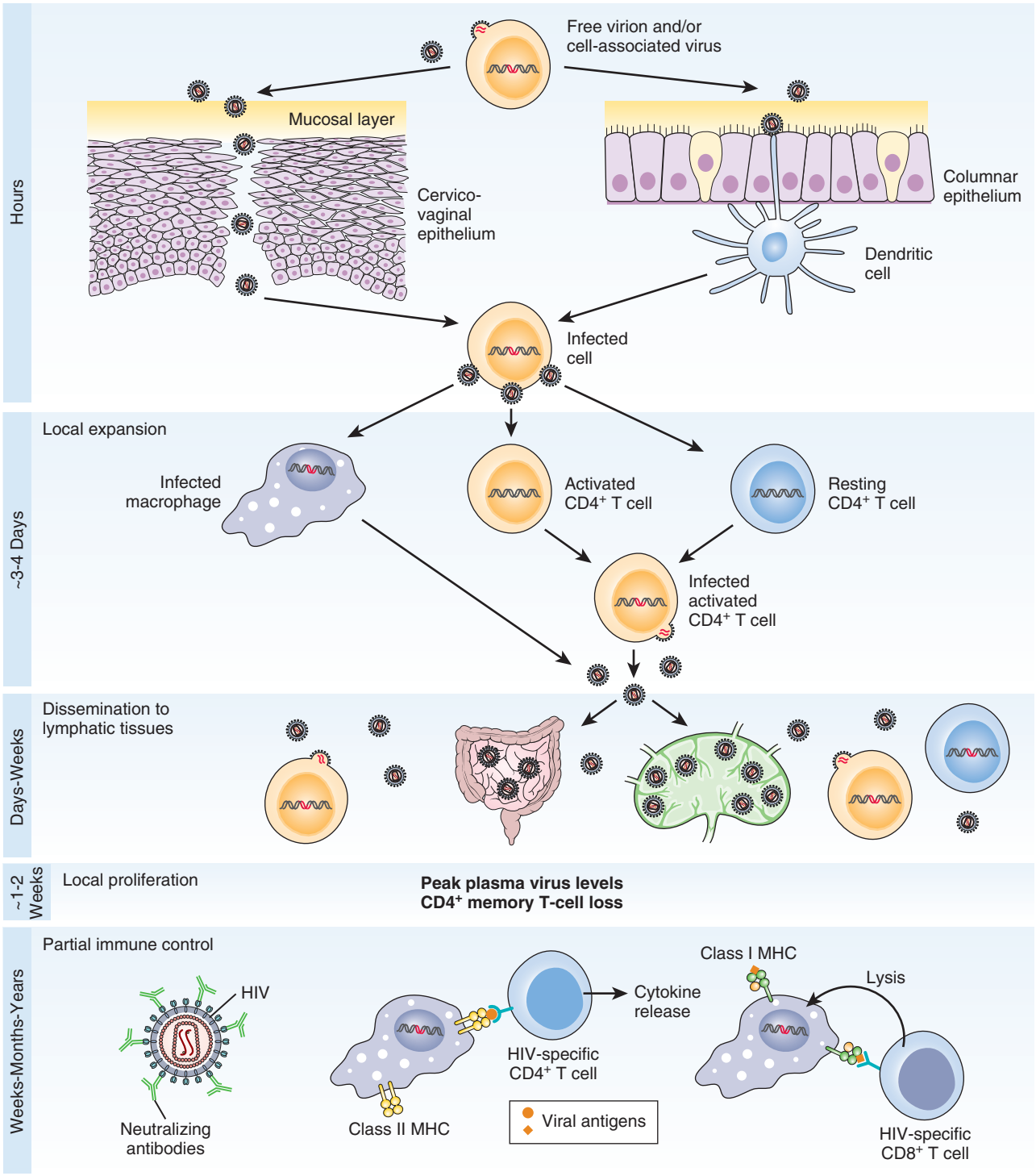

FIG. 121.2 Initial infection and dissemination of human immunodeficiency virus *(HIV)* infection. The sequence of events after vaginal or rectal transmission includes the following: (1) crossing the mucosal barrier; (2) interaction of virus with host cells, such as dendritic cells, that transport HIV to the paracortical regions of draining lymphoid tissues, leading to infection of $CD4^+$ T cells and systemic dissemination of HIV infection in various lymphoid tissues; macrophages can also be infected in this manner, although they are not felt to efficiently produce virus for wide dissemination; and (3) induction of HIV-specific immune responses, beginning with $CD8^+$ T cells and following with antibodies that provide incomplete control of viremia. *MHC,* Major histocompatibility complex. *(Modified from Haase AT. Perils at mucosal front lines for HIV and SIV and their hosts.* Nat Rev Immunol. *2005;5:783–792; with permission.)*

transmission with topical microbicides and various inhibitors that can directly target HIV, including inhibitors of CCR5 binding to HIV or neutralizing antibodies.[45] There has also been considerable interest in identifying the earliest events of HIV transmission, given the evidence that the majority of mucosal infections are established by a single or a few viruses; furthermore, factors such as microbreaches, hormones, and inflammatory responses can affect transmission.[31,39] A better understanding of these events is thought to be essential for advancing HIV vaccine strategies and other strategies aimed at reducing the rates of HIV transmission.[31]

DCs and LCs are capable of retaining infectious virus on their surface or within endosomal compartments for extended periods of time, thus

allowing HIV to remain shielded before encountering a target cell for productive replication. It is somewhat unclear how retention of infectious virus is mediated, although several lectin and nonlectin receptors expressed on DCs and LCs have been proposed.[27] Given that some of the proposed HIV capture molecules such as DC-SIGN are expressed on DCs but not on LCs, it is likely that this process can be achieved by means of a variety of different mechanisms. For example, whereas degradation is the predominant outcome when HIV is endocytosed into LCs by the C-type lectin langerin (see Fig. 121.1) and the host restriction factor TRIM5α (tripartite motif-containing protein 5α) is recruited,[46] this antiviral effect may be abrogated by coinfections and inflammatory responses.[27] Collectively, the preponderance of evidence indicates that LC–T-cell or DC–T-cell conjugates enhance HIV replication via what has been termed a *virologic synapse* (see Fig. 121.1). Furthermore, whereas LCs are thought to be largely sessile, DCs are uniquely poised to carry HIV from tissues in which the initial rounds of viral replication occur to the regional lymph nodes, where $CD4^+$ T cells become infected after contact with DCs. This leads to subsequent rounds of virus replication and spread in the absence of HIV-specific immune responses. Thus lymphoid tissue plays a key role in the initiation and dissemination of HIV infection.

HIV-SPECIFIC IMMUNE RESPONSES

Although the immune response to HIV has been extensively studied, a number of fundamental issues remain unresolved. Among the most important of these is that it remains unclear how HIV avoids immunologic control in most infected individuals. It is striking that unlike the situation with most other viral infections, the human host does not consistently mount an immune response that is able ultimately to eliminate the virus from the body or even suppress it. NHP models have been useful for the study of retrovirus-specific immunity, immunotherapies, and vaccines. Through passive transfer studies or studies involving depletion of $CD8^+$ T cells in experimental animals, insight into the various facets of virus-specific immune responses have been extended from correlative studies in humans. It is known that passive transfer of virus-specific antibodies can protect against retroviral infection, and cellular immune responses can control viral replication in NHP models; in addition, there are indications for similar outcomes in humans. Furthermore, insight into the breadth and magnitude of HIV-specific immune responses in HIV-infected individuals is beginning to inform new strategies for the development of effective prophylactic or therapeutic vaccines.

Humoral Immune Responses

In most individuals who acquire HIV, the virus rapidly establishes a persistent infection and evades the antibody response of the host. Although antibody responses capable of binding multiple HIV encoded proteins can be detected weeks to months after infection, the response to the surface glycoprotein (Env) is the most important with regard to effector functions against virions or infected cells.[47] The constant fragments (Fc) of immunoglobulin G (IgG) antibodies bound to Env expressed on infected cells can in turn bind to receptors on effector cells (FcγRs). These cells can mediate antibody-dependent cellular phagocytosis (ADCP) in the case of monocytes, macrophages, or DCs or antibody-dependent cellular cytotoxicity (ADCC) in the case of perforin-bearing cells such as natural killer (NK) cells or monocytes.[48] Other antibody effector functions include those that directly lyse virions such as the binding of IgG constant regions (Fc) to C1q, to initiate the classical complement cascade. By far the most extensively studied antiviral function, possibly because of its dominant role in protection against infection, is neutralization through blockade of viral entry to target cells by binding to Env. Any single antibody may have one or several of these effector functions, such as HIV-specific neutralizing antibodies that also mediate ADCC.

There are several features of the Env protein that pose challenges for mounting broad and potent antibody responses to diverse HIV isolates. The Env subunits gp120 and gp41 noncovalently associate and trimerize into the functional viral spike that is very heavily glycosylated. These glycosylated surfaces are poorly immunogenic and provide the virus with what has been termed a *glycan shield.*[49] Some conserved epitopes such as those at the apex of the trimer or the CD4 binding site can be partially occluded by glycosylation, which would limit antibody access. Perhaps the greatest challenge to developing broad and potent recognition of HIV is the level of genetic variation. The sequence diversity of Env is high within geographically defined populations and even within a single infected patient. Mutations that occur during the process of reverse transcription and a prolonged duration of infection generate highly diverse viral sequences that coexist in the plasma.[50,51] The level of diversity of HIV is considerably higher than that of most human RNA viruses and is likely a major contributor to the paucity of cross-neutralizing antibodies in the majority of HIV-infected individuals. Even when compared with influenza, which is also an RNA virus that undergoes high rates of mutation, the HIV variability in one individual is often greater than that of the influenza virus circulating in the community for an entire season.[52] HIV viral sequences may vary between 10% to 16% in the plasma of a given chronically infected individual.[51]

Despite these challenges the prevalence of sera with broad neutralizing activity is not rare in chronically infected people. Depending on the neutralization criteria that are used in defining breadth of sera, antibodies with broad neutralizing potential have been shown to arise in approximately 20% to 50% of HIV-infected individuals.[53–56] Sera from these patients are active against difficult-to-neutralize primary isolates and across highly diverse clades of viruses to which the patient has not been exposed. However, such antibodies may take 1 to 2 years to develop in infected individuals.[57,58] Although antibodies that bind Env can be detected in the plasma within weeks of HIV infection,[59] these early antibodies are largely nonneutralizing and do not protect against superinfection.[60,61] Relatively high levels of antibodies that have neutralizing potential appear several weeks later, although their breadth is limited and their ability to eliminate the circulating virus is inadequate[49,62] (see Fig. 121.2). This is at least partly the result of the selective pressure exerted by the antibody response that drives HIV genetic variants to arise rapidly. It is thought that these diverse circulating envelope sequences may represent a constantly evolving target that contributes to the ability of HIV to evade the humoral immune response. As Env changes, the humoral immune response coevolves with regard to shifting specificities, mode of recognition, and affinity maturation (Fig. 121.3). The end result in some patients is the development of antibodies capable of neutralizing highly diverse isolates by targeting accessible conserved epitopes.

Over the past decade, study of the sera and cells from such patients has resulted in extraordinary advances in our understanding of the nature of a broadly neutralizing antibody (bNAb) response. These advances have been largely driven by the formation of well-defined cohorts of patients with broad neutralizing sera[63–68], Env-specific peripheral blood B-cell isolation through microculture or fluorescent probe sorting, and isolation of Ig genes and reexpression as monoclonal antibodies.[69,70] These developments led to an explosion in the number of HIV-specific monoclonal antibodies isolated, resulting in a deconvolution of the specificities responsible for the activity of broadly neutralizing sera, and an understanding of the structure, function, and evolution of these antibodies. The targets of these bNAbs fall into five major groups: the CD4-binding site, a second variable loop (V2) glycan at the trimer apex, a site at the base of V3 centered on the Asn332-glycan on gp120, the membrane-proximal external region on gp41, and epitopes that bridge the interface between gp120 and gp41 (Fig. 121.4).[47] Many of these antibodies are atypical compared with monoclonal antibodies for other viruses. They may have very high levels of somatic hypermutation; often have insertions and deletions, long heavy-chain complementarity–determining 3 regions, or restricted germline use; or may be polyreactive. In some cases patients who ultimately developed broadly neutralizing sera had participated in longitudinal study protocols involving frequent sampling of serum and peripheral blood mononuclear cells (PBMCs) since acute infection.[71–73] Use of these samples combined with next-generation sequencing has permitted detailed studies of how bNAbs arise in some individuals. At present the challenge for the field is how to best use this information to develop immunotherapies or passive or active prophylaxis.

Experiments using passive administration of bNAbs in animals have provided an important proof of concept that they have potent

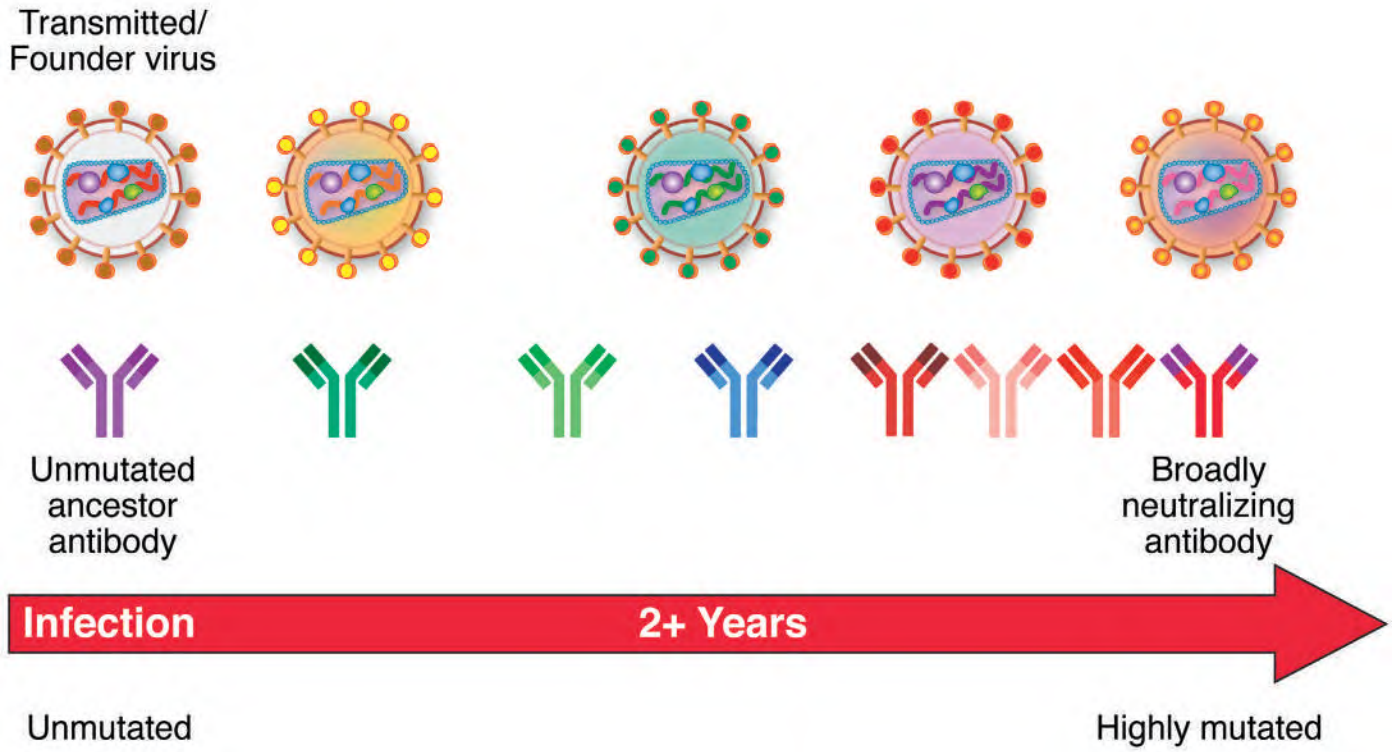

FIG. 121.3 Coevolution of human immunodeficiency virus (HIV) and the antibody response. In response to HIV infection, the antibodies expressed by naïve B cells that bind the HIV envelope undergo somatic hypermutation and increase their affinity. This selects for viral variants that are not recognized. In response, new antibody specificities may then be expanded or existing specificities may further mutate. This process continues over 1 to 2 years, ultimately resulting in the generation of broadly neutralizing antibodies in some patients.

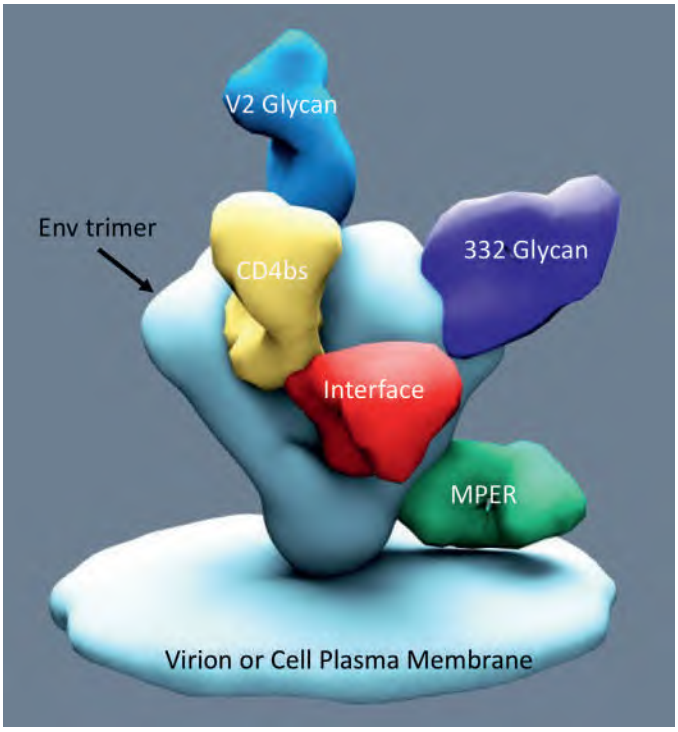

FIG. 121.4 Conserved sites of vulnerability on human immunodeficiency virus envelope glycoprotein. The figure depicts the binding of examples of broadly neutralizing antibodies, here labeled with their respective binding sites. *bs,* Binding site; *MPER,* membrane proximal external region. *(Image courtesy A. Ward; with permission.)*

immunotherapeutic effects. BNAbs can dramatically lower viremia in chimeric simian-human immunodeficiency virus (SHIV)–infected rhesus macaques or HIV-infected humanized mice. Although mutational escape is commonly observed during monotherapy, it is less often observed during administration of antibody combinations. In addition, passive antibody administration to macaques during acute or chronic infection with some SHIVs has resulted in sustained suppression of viremia after the antibody is cleared, suggesting that passive antibodies have the potential to modulate the T-cell response.[74,75] The potential role of neutralizing antibodies as immunotherapies, alone or in combinations with antiretrovirals, should become clearer after completion of several clinical trials using these strategies that are underway in humans.

There is also a large body of data that suggest that antibodies can likely provide complete protection from HIV infection. Numerous bNAbs have been shown to provide sterilizing immunity against mucosal challenges in SHIV-rhesus macaque challenge models.[47] The levels required to protect these animals are well within the range of titers detected in many HIV-infected patients, suggesting that they are likely achievable in uninfected vaccinees. Although stimulating bNAbs through vaccination is a desirable goal, generating such a response has proven extremely challenging. Thus far, most vaccines in humans or experimental animals have generated antibody responses capable of neutralizing only very easy to neutralize viruses or responses specific to only the immunizing strain. As noted earlier, these antibodies take months to years to develop and are in many ways atypical. However, it is not completely clear whether prolonged development and high levels of somatic hypermutation are requirements for generation of these antibodies or simply reflect chronic replication and mutation in an immunocompromised host. Nonetheless, immunization strategies that might recapitulate the pathways to the generation of bNabs are currently the subject of intense interest. These strategies include immunogens designed to better engage naïve B cells, partial Env deglycosylation, or immunogens

derived from the study of Env and bNAb ontogeny to be used in progressive vaccination with sequential immunogens, to initiate and then broaden the antibody response. Although the induction of a bNAb response is an extremely difficult goal to achieve, weakly or nonneutralizing antibodies can provide some, albeit more modest, level of protection, as reviewed by Excler and colleagues.[76] In addition, considerable optimism has been generated by a vaccine trial in humans demonstrating 31% overall efficacy in preventing infection in participants, primarily those at heterosexual risk of HIV infection in Thailand.[77] Participants were vaccinated with a combination of an attenuated poxvirus encoding HIV gene products, followed by an HIV envelope protein boost. Although the precise mechanism of action remains incompletely understood, the vaccine did not induce neutralizing antibodies against circulating strains, and protection from infection may have operated through an antibody-mediated cellular cytotoxicity mechanism. The role of such responses may become more apparent after the completion of several protocols that build on this strategy. In addition to these studies, efforts are underway to use antibodies in passive prophylaxis.[78] These approaches include vectored prophylaxis, by means of delivery of DNA or mRNA encoding bNAbs, or passive antibody therapy by means of intravenous or intramuscular injection. In some cases bNAbs have been engineered to include multiple specificities in a single antibody or to dramatically extend half-life.[79–81] A large multinational prophylaxis trial of passive administration of the bNAb VRC01 is currently in progress. It appears likely that the aforementioned passive prophylactic or immunotherapeutic clinical trials will provide considerable insights regarding potential selection of resistant isolates, and the levels of antibodies necessary for control of, or protection against, HIV infection.

Cellular Immune Responses

Cytotoxic T Lymphocytes

Major histocompatibility complex (MHC) class I–restricted, HIV-specific, $CD8^+$ T-lymphocyte responses are found in the peripheral blood within the first few months of HIV infection and are detected during the chronic phases of infection in most HIV-infected individuals.[82] During the chronic phase of infection, $CD8^+$ T cells specific for each of the known HIV-1 gene products are found in the peripheral blood with bulk cytotoxicity assays, limiting dilution assays for cytolysis, and interferon-γ (IFN-γ) secretion.[83] Several lines of evidence suggest that HIV-specific $CD8^+$ T cells play an important role in restriction of virus replication. First, the temporal association of the peak of the virus-specific cytotoxic response with the decline of viremia during acute HIV infection of humans or SIV infection of macaques is thought to represent the effect of virus-specific $CD8^+$ T cells in restricting retroviral replication in humans.[82,84,85] Second, indirect evidence of an important role of $CD8^+$ T cells in restriction of HIV replication in humans comes from strong associations between restriction of virus replication and certain MHC class I alleles and functional links with epitopes presented by these alleles.[86–90] Third, $CD8^+$ T-cell depletion by exogenous monoclonal antibodies has been shown to abrogate restriction of virus replication in SHIV-infected and SIV-infected monkeys.[91–94] Last, the HIV-specific $CD8^+$ T cells of patients that control HIV replication for many years have been shown to be functionally superior to those without immunologic control of virus replication (see "Long-Term Nonprogressors/Elite Controllers").[83] Based on the aforementioned evidence, it is now generally accepted that $CD8^+$ T cells are an important component of immune system–mediated restriction of HIV replication. However, although HIV-specific $CD8^+$ T cells likely exert some level of control over HIV replication, this restriction is generally incomplete, because viral RNA levels reach 10^3 to 10^6 copies/mL plasma in the vast majority of HIV-infected individuals during the chronic phase of infection in the absence of ART.

Several viral factors unique to HIV likely contribute to the ability of HIV to evade the cellular immune response. Mutations produced during the reverse transcription process, combined with high levels of replication over a prolonged period, result in a highly diverse population of viruses that circulate in a given patient. It is thought that this extraordinary level of viral diversity results from the development of mutant viruses that escape immune recognition. Selection of "escape" mutations has been documented in HIV-infected humans and SIV-infected macaques. Longitudinal studies of viral sequences and $CD8^+$ T-cell responses to known motifs have shown the appearance of mutations that no longer bind to the MHC class I molecule.[95] However, it should be noted that the interplay of host and viral factors is extraordinarily complex, given the large number of viral epitopes targeted by the immune response, the timing of gene expression during the viral replication cycle, and the complement of MHC class I alleles. The host $CD8^+$ T-cell response is limited by the ability of the MHC class I alleles to bind to various viral epitopes, and viral replication is limited by the degree to which an escape mutation impairs viral replicative capacity, or "fitness." In addition, the $CD8^+$ T-cell response to each of the proteins within the autologous virus is very high in frequency and very broad.[96,97] Escape mutations may be found within a single epitope, but it is likely that other conserved epitopes within the same gene remain as targets.[96] Strong associations between escape mutations, or viral fitness, and virus load have not been found.[98,99] Finally, putative escape mutations are also found in viral sequences in the plasma of LTNP/ECs at the same frequency as MHC-matched progressors.[96,97] Taken together, these data suggest that although escape mutations clearly can occur, they do not appear to be the critical driving force in the loss of immunologic control in the vast majority of HIV-infected patients.

In addition to the high levels of diversity generated during HIV infection, other viral factors may contribute to the poor ability of the immune system to control HIV replication. HIV Nef, Tat, and Vpu are each capable of downmodulating surface expression of MHC class I molecules necessary for the recognition of infected cells, as reviewed by Wonderlich and coworkers.[100] The effect of Nef is to diminish cell surface expression of human leukocyte antigen-A (HLA-A) and HLA-B molecules, leaving HLA-C and HLA-E unaffected.[101] It has been proposed that these effects may permit infected cells to avoid lysis by HLA-A– or HLA-B–restricted T cells, which dominate the cellular immune response, yet also avoid lysis by NK cells, which are inhibited by the presence of HLA-C or HLA-E. However, there is not uniform agreement that this is a predominant mechanism by which HIV avoids immunologic control. LTNP/ECs (see later), who can maintain immunologic control for many years, typically have intact MHC and HIV *nef* sequences.[89,102] Several investigators have demonstrated the ability of autologous HIV-infected cells to stimulate cytokine secretion or cytolysis by class I–restricted HIV-specific $CD8^+$ T cells. In addition, the high frequencies of HIV-specific $CD8^+$ T cells found in the peripheral blood of most HIV-infected patients rapidly fall to very low levels after initiation of ART.[103] This indicates that in the absence of therapy, HIV-infected cells stimulate and maintain high frequencies of HIV-specific $CD8^+$ T cells. Although infected cells are recognized, viral replication is not fully suppressed. At present, the relative role of MHC downregulation in the loss of restriction of HIV replication remains unclear.

Another parameter of the $CD8^+$ T-cell response that may provide enhanced restriction of virus replication is the breadth or number of HIV peptides to which a patient may respond. However, when the total $CD8^+$ T-cell response was measured during acute or chronic infection, no relationship was found between the breadth of the response and levels of plasma HIV viremia.[97,104,105] Several reports have described a narrowly focused $CD8^+$ T-cell response in LTNP/ECs or in individuals treated with ART during acute infection and who have restricted virus replication.[89,106,107] Because HIV-specific $CD8^+$ T cells of most chronically infected patients persist at high frequencies and respond to a broad array of HIV peptides, it does not appear likely that inadequate breadth of the response is a major contributor to poor restriction of virus replication during chronic infection.

Several lines of evidence have suggested that the inability of $CD8^+$ T cells to control HIV may lie not in the quantity of these cells but rather in the qualitative properties of the response. Some data have suggested that disrupted HIV-specific $CD8^+$ T-cell function in progressors is associated with increased expression of programmed death (PD)–1 molecules[108] or decreased CD127 (interleukin [IL]-7Rα chain)[109,110] expression, but other studies have noted that these parameters are restored during ART, suggesting that they may be a consequence rather than a cause of the loss of control of viral replication.[111,112] The HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater frequencies of "polyfunctional" cells, named for their ability to degranulate and produce several

cytokines, including IL-2.[113] HIV-infected LTNP/ECs have also been differentiated by increased proliferative capacity of HIV-specific $CD8^+$ T cells linked to enhanced expression of perforin.[114] These functions have been found to be necessary for the killing of autologous HIV-infected $CD4^+$ T cells.[115] It is thought that the relative absence of these functions in progressors may represent a mechanism whereby HIV avoids immunologic control.[115,116]

Recent studies in the SIV/SHIV Rhesus macaque infection model have provided further evidence that the $CD8^+$ T-cell response may be used to suppress, or even cure lentiviral infection.[94,117] In a series of studies, rhesus macaques were immunized with a rhesus cytomegalovirus (CMV) recombinant vaccine encoding HIV gene products that are targets of the cellular immune response. The vaccine caused a chronic CMV infection that induced a persistent SIV-specific $CD8^+$ T-cell response. In those animals that were infected, a profound level of suppression of the pathogenic SIV challenge virus was observed. In 50% of infected animals, the pathogenic challenge virus was reduced below detection threshold levels and thought to have been cleared.[117] Interesting to note, the data suggest that nonclassical MHC class II– and MHC class I-E–restricted $CD8^+$ T-cell responses broadly targeting an average of 35 epitopes mediate this control.[118,119] In a more recent study, macaques treated with potent and broadly neutralizing anti–HIV-1 antibodies during acute SHIV infection unexpectedly facilitated emergence of $CD8^+$ T-cell immunity able to durably suppress virus replication.[74] Thus, in addition to an envelope-specific humoral immune response to an HIV vaccine, the $CD8^+$ T-cell response may provide an important second line of defense against breakthrough infections that might lead to long-term control of the virus. Although highly speculative, based on the results in the rhesus macaque model, it is theoretically possible that the $CD8^+$ T-cell response to vaccination might result in virus clearance in some instances.

Soluble $CD8^+$ T-Cell–Secreted Factors

In addition to cytolysis, other mechanisms of $CD8^+$ T-cell–mediated antiviral activities have been described. $CD8^+$ T cells of HIV-infected patients secrete soluble factors that can inhibit viral replication in the absence of cell killing. This noncytolytic antiviral activity was initially discovered in vitro after use of $CD8^+$ T cells from HIV-infected patients.[120] Years later, the suppressive activity against R5 viruses was shown to be mediated in large part by the CC chemokines MIP-1α, MIP-1β, and RANTES.[14] These molecules are natural ligands for CCR5, a coreceptor for R5 strains of HIV-1, and inhibit viral replication primarily by blocking virus entry. In addition to the CC chemokines, poorly characterized soluble factor(s) act after viral entry to suppress HIV transcription in infected cells. These factor(s) have been termed *$CD8^+$ T-cell antiviral factors* (CAF).[121] Although not required for this suppressive activity, maximal suppression of HIV replication is observed under conditions wherein cell contact is maintained and cells are HLA matched.[122,123] This suppressive activity has been shown to be greater in PBMCs of HIV-infected patients than in uninfected control subjects. A novel anti-HIV chemokine produced by activated $CD8^+$ T cells, XCL1/lymphotactin, has been shown to inhibit a broad spectrum of HIV-1 isolates, irrespective of their coreceptor-usage phenotype.[124] At present, it remains incompletely understood whether suppressive factors are secreted in an antigen-specific manner.

$CD4^+$ T-Cell Responses

It has been demonstrated in several animal models of viral pathogenesis that virus-specific $CD4^+$ T cells are critical for the induction or maintenance of an effective $CD8^+$ T-cell response that mediates restriction of virus replication. Many viral infections of humans or experimental animals typically result in induction of $CD4^+$ T-cell responses that can be demonstrated by proliferation to virus antigens in vitro long after elimination or control of infection, which is a reflection of the persistence of these virus-specific memory $CD4^+$ T cells. Unlike most other infections of humans, HIV infection is characterized by the absence or extremely low level of HIV-specific $CD4^+$ T-cell proliferative responses in the vast majority of untreated patients. Of note, $CD4^+$ T cells from untreated HIV-infected individuals also have defective proliferative responses to non-HIV antigens.[125] In this regard, strong HIV-specific $CD4^+$ T-cell responses are found in relatively rare LTNP/ECs who restrict HIV replication in the absence of ART.[126–128] In addition, proliferative responses to HIV antigens have been found in patients who were treated early during acute infection and who then had restricted HIV replication when ART was withdrawn.[128] Because HIV infects $CD4^+$ T cells, it was believed that the early loss of HIV-specific proliferative responses may be the result of infection and deletion of HIV-specific $CD4^+$ T cells in the lymphoid tissues on encountering the virus.[129] However, several lines of evidence indicate that HIV-specific $CD4^+$ T cells persist in patients with progressive disease. Many reports have documented the persistence of HIV-specific $CD4^+$ T cells in most patients by intracellular cytokine staining after stimulation with HIV antigens.[130–132] HIV-specific $CD4^+$ T cells also persist at the expected frequency and with the expected phenotype and functions in progressors when compared with responses to other viral antigens.[133] IL-2 production and proliferation of HIV-specific $CD4^+$ T cells was abrogated during an interruption of antiviral therapy, but restored in patients receiving ART, suggesting that diminished proliferation of these cells may be partly a result of the presence of high levels of HIV antigen. This effect is similar to that observed during viremia with other human viruses. Thus there is now general agreement that HIV-specific $CD4^+$ T cells persist at the expected frequencies during the chronic phase of infection in patients with progressive disease, although there is some controversy over whether disruptions of function persist. In addition, the role of depletion or disruption of the functions of HIV-specific $CD4^+$ T cells during acute infection or end-stage disease remains to be defined.

Host Genetic Factors

Among the most reproducible and powerful host factors to affect HIV disease is the impact of HLA alleles on viral load and disease progression. Since its initial description, the association between HLA-B*57/58 and B*27 with slower disease progression and B*35 with more rapid disease progression has been repeatedly observed, including in four genome-wide association studies.[95] Associations between these "protective alleles" and reduced viral load is not simply due to their enrichment in LTNP/ECs, but has been observed even in progressor cohorts.[134] Having a protective allele is neither necessary nor sufficient for immunologic control of HIV, given that approximately 20% of LTNP/ECs lack such alleles and they are found in progressors at the same frequency as in the general population.[107] In addition, an association has been observed between reduced viral load and a micropolymorphism in HLA-C,[135,136] and an association with reduced viral load has been made in patients who carry a subset of B alleles and a killer inhibitory receptor.[137] Unlike other protective alleles, this combination is believed to function through the action of NK cells expressing the 3DL1 inhibitory receptor. Although the mechanisms of each of these HLA effects remain incompletely understood, important clues continue to come from in vitro studies of $CD8^+$ T-cell and NK-cell function.

As noted earlier (see "HIV Entry and Dissemination"), the chemokine receptor genotype has an impact on rates of progression of disease. For example, individuals who carry one copy of the mutant CCR5-Δ32 allele have an increased chance of experiencing a slow rate of disease progression compared with individuals who are homozygous for wild-type alleles.[138] In addition, some CCR2 alleles have been associated with slower rates of disease progression. Despite the association between *CCR5* genotype and slower progression of HIV disease, this factor does not appear to be a dominant influence in the determination of the state of long-term nonprogression; neither CCR5-Δ32 nor CCR2 alleles have been found to be enriched in LTNP/ECs who control HIV replication.[89,102]

Long-Term Nonprogressors/Elite Controllers

Important clues regarding mechanisms underlying a successful immune response to HIV-1 have come from the study of rare examples of naturally occurring immunologic control of HIV replication. Although rates of disease progression vary widely among HIV-infected individuals, the median time between infection and development of acquired immunodeficiency syndrome (AIDS) is approximately 10 years. It has become clear that a small percentage of untreated HIV-infected individuals show no evidence of disease progression, despite prolonged infection.[139,140]

In addition to LTNP/ECs, other individuals who remain HIV seronegative, despite multiple exposures to HIV, have been identified, suggesting that elements of protection exist but may not be readily detected with standard measures of the immune response. This group is likely heterogeneous with regard to the mechanisms of resistance to HIV infection, as reviewed by Lederman and coworkers.[141] Studies of LTNP/ECs and highly exposed seronegative patients have advanced our understanding of the pathogenesis of HIV disease and have increased optimism that some forms of immunologic protection from infection or control of viral replication may be harnessed in prophylactic or therapeutic vaccines.

Definitions of LTNP/ECs have varied.[142] One early definition that was commonly used included documented HIV infection for more than 7 years, $CD4^+$ T-cell count higher than 500 cells/μL without significant decline over time, no symptoms of HIV disease, and no history of ART. Because definitions of nonprogressors were created empirically, it is not surprising that these individuals constitute a heterogeneous group. Many who were originally defined by these clinical criteria have now gone on to develop progressive disease. However, there remains a small subgroup of untreated nonprogressors who have now been infected for 20 years and maintain normal $CD4^+$ T-cell counts and plasma viral RNA levels less than 50 copies/mL of plasma. Mechanisms that may determine a course for LTNP/ECs during HIV infection include host genetic factors, effective immunologic control of virus replication, and/or infection with an attenuated strain of HIV (Table 121.1). Although patients with normal $CD4^+$ T-cell counts and low levels of plasma virus are a heterogeneous group, a small subset of patients with truly nonprogressive HIV infection and control of virus replication in the absence of ART are likely to hold important clues to the basis of an effective immune response to HIV.

Host Immune Response Factors

A considerable amount of evidence now suggests that HIV-specific $CD8^+$ T cells play a major role in the maintenance of low viral load in most LTNP/ECs. First, the HLA-B*57 allele is found in approximately 65% of patients in several different cohorts.[142] In addition, the HIV-specific $CD8^+$ T-cell response in these patients is highly focused on B57-restricted peptides, suggesting that the B57 molecule likely plays a direct role in restriction of virus replication in these individuals.[87,89] There are reports documenting viruses sufficiently pathogenic to cause progression to be transmitted to a second patient, who then becomes an LTNP/EC. In this case, both individuals carried the B*5703 allele.[143] Thus the recipient was able to mediate immunologic control despite B*57-associated escape mutations in the virus. In addition, there are cases of virus strains that cause progression in one partner being transmitted by superinfection to a nonprogressor, and that virus being controlled.[144,145] In addition, "elite controller" rhesus macaques have been described that are enriched for the Mamu B*08 and B*17 alleles.[146,147]

TABLE 121.1 Possible Mechanisms of Long-Term Nonprogression/Elite Control With HIV Infection

Host Genetic Factors
HLA type
Heterozygosity for 32-bp deletion in chemokine receptor CCR5
Mannose-binding lectin alleles
Tumor necrosis factor c2 microsatellite alleles
Gc vitamin D–binding factor alleles
Host Immune Response Factors
Effective CTL responses: "polyfunctionality," proliferation, perforin production, cytotoxicity
Secretion of CD8 antiviral factor
Secretion of chemokines that block HIV entry coreceptors CCR5 (e.g., MIP-1α, MIP-1β, and RANTES) and CXCR4 (e.g., SDF-1)
Maintenance of functional lymphoid tissue architecture
Virologic Factors
Infection with attenuated strains of HIV

CTL, Cytotoxic T lymphocyte; *HLA,* human leukocyte antigen; *MIP,* macrophage inflammatory protein; *RANTES,* regulated on activation, normal T-cell expressed and secreted; *SDF,* stromal cell-derived factor.

In some cases, epitopes restricted by these alleles have motifs similar to those that bind HLA-B27 or HLA-B57 in humans. Furthermore, transient $CD8^+$ T-cell depletion of SIV-infected elite controller macaques resulted in a dramatic increase in viral replication.[91] Taken together, these data argue strongly that immunologic control in most LTNP/ECs is not a consequence of infection with attenuated viruses but rather is mediated by HIV-specific $CD8^+$ T cells.

The most recent data regarding immunologic control in LTNP/ECs suggest that the superior ability of these patients to control HIV compared with progressors is mediated not by the quantity of HIV-specific $CD8^+$ T cells but rather by their qualitative function.[83] A number of studies using quantitative techniques that examined the response to a broad array of HIV gene products have not found higher frequencies or breadth of HIV-specific $CD8^+$ T cells in LTNP/ECs compared with progressors. The HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater frequencies of polyfunctional cells, named for their ability to degranulate and produce several cytokines, including IL-2.[112,113] However, these cells make up an extremely small subset of the total HIV-specific $CD8^+$ T-cell response, and many LTNP/ECs demonstrate few or no such cells.

By far, the property that most clearly distinguishes the HIV-specific immune response of LTNP/ECs from other patients is the ability to suppress HIV replication and/or kill HIV-infected cells in vitro. For some time, it had been known that HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater proliferative capacity and ability to upregulate perforin on stimulation compared with progressors.[114] More recently, the $CD8^+$ T cells of LTNP/ECs were shown to have a greater capacity to restrict HIV replication in vitro.[116,148,149] In addition, increases in perforin and granzyme B production were shown to result in a dramatically increased ability of $CD8^+$ T cells of LTNP/ECs to kill autologous HIV-infected $CD4^+$ T cells compared with progressors.[112,115] Although causality must ultimately be demonstrated, the abilities to proliferate, produce perforin, and kill HIV-infected $CD4^+$ T cells appear to be qualities within the HIV-specific $CD8^+$ T-cell response that are clearly associated with immunologic control in LTNP/ECs.

The relationship between humoral immune responses to HIV and disease progression remains incompletely understood. LTNP/ECs, as a group, do not have high levels of neutralizing antibodies.[63,139,140,150] In many cases, they may have low levels of binding or neutralizing antibodies believed in part to be due to lower levels of antigen driving the humoral immune response. In studies that examined clinical parameters that might be associated with bNAbs, the only correlation found was a positive correlation with viral load.[63,64] Some studies have demonstrated that sera that can neutralize a broad panel of primary isolates can be found in patients that have relatively high $CD4^+$ T-cell counts, but are viremic.[63–68] Although such patients tend to have slow rates of disease progression, they are a heterogeneous group. In these patients, antibodies may not be causing slower disease progression, but rather the association may be a consequence of the need for both an intact immune system and the presence of viral antigen to have such a response. At present, it does not appear likely in most cases that maintenance of neutralizing antibodies plays a major role in determining the state of nonprogression.

Virologic Factors

Infection with attenuated strains of HIV may account for nonprogression in a small subset of individuals. The most extensively characterized association of attenuated viral strains and nonprogression is that of an Australian cohort of nonprogressors who were infected by transfusion from a single nonprogressor donor.[151] Viruses from these individuals contained deletions in the *nef* gene and in the U3 region of the long terminal repeat (LTR). Other anecdotal reports have implicated other defective HIV genes with nonprogression, although such cases appear to be the exception rather than the rule.[152] Many of the patients described in the original Australian cohort ultimately developed progressive disease.

RESERVOIRS OF HIV INFECTION

There is unequivocal evidence from multiple lines of investigation that HIV persists in almost all infected individuals receiving effective ART. The most powerful demonstration of the inability of ART to eradicate HIV infection comes from in vivo studies of individuals who began ART during the chronic stage of HIV infection, achieved and maintained

suppression of plasma HIV RNA for several years, and subsequently interrupted therapy. Interruption of ART resulted in a rapid rebound of plasma viremia in 95% of patients.[153] More recent studies have largely confirmed these findings, although there is evidence that a proportion of individuals who initiated ART early control their plasma viremia for variable periods of time after discontinuation of therapy.[11] Although the reasons for this difference are unclear, a delineation of factors involved, including origin of rebounding plasma HIV, effect of time to establish long-lived reservoirs, and role of the immune system, are currently being investigated. A number of studies have suggested that replication-competent virus persists in the $CD4^+$ T-cell compartment in infected individuals receiving effective ART and that early establishment of HIV reservoirs in peripheral lymphoid tissues, including the GALT, are the main impediments to eradicating HIV.[154]

It was demonstrated by using highly sensitive assays with a limit of detection of a single copy of HIV RNA per milliliter of plasma that persistent low levels of plasma viremia from an unknown origin remain in the vast majority of individuals with undetectable plasma HIV when standard assays are used.[155,156] In addition to important HIV reservoir sites in the GALT and peripheral lymphoid tissues, putative reservoir sites include the reproductive tract, bone marrow, and central nervous system (CNS).[156–158] In addition to the high HIV burden in $CD4^+$ T cells, other cells that can potentially contribute to the viral reservoir include DCs and macrophages, in addition to microglial cells, specialized macrophages that reside in the CNS.[157,158] Genetic variability has been demonstrated in HIV isolated simultaneously from the plasma and several reservoir sites, including the reproductive tract and the CNS, indicating that there may be compartmentalization of HIV in different sites.[158] Such compartmentalization may provide a sanctuary for HIV that may be relatively impenetrable by antiretroviral drugs; however, it is unclear whether these sanctuary sites contribute significantly to the persistence of HIV in the presence of ART.

Latent Reservoirs of HIV

The pool of latently infected cells in the resting $CD4^+$ T-cell compartment has been one of the most extensively studied persistent reservoirs to date and is considered to be a major impediment to HIV eradication.[159–161] HIV may enter resting $CD4^+$ T cells, at which point a varying degree of reverse transcription of the HIV genome may occur in these cells (Fig. 121.5).[162,163] This period of preintegration latency may last hours to days; in the absence of an activation signal, unintegrated proviral DNA loses its capacity to initiate a productive infection. If these cells become activated, however, reverse transcription proceeds to completion, followed by nuclear translocation and integration of proviral DNA into cellular DNA.[162,163] It has been clearly demonstrated that a pool of resting $CD4^+$ T cells that carry replication-competent HIV persists in essentially all infected patients who were receiving ART and in whom plasma viremia was suppressed below levels of detectability.[159–161] In addition, this HIV reservoir is established during the earliest stages of HIV infection. The initiation of ART as early as 10 days after infection with

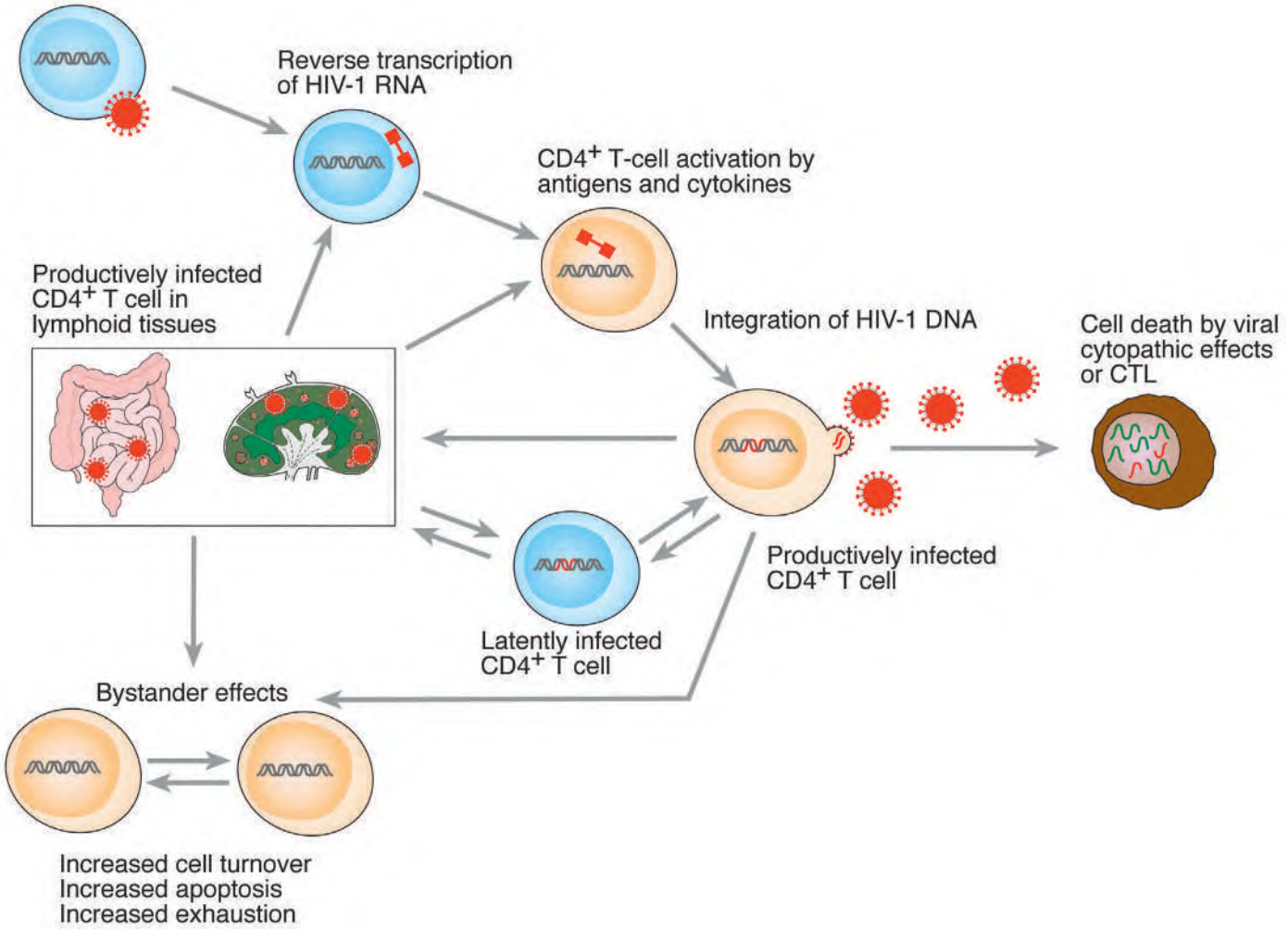

FIG. 121.5 Direct and indirect effects of human immunodeficiency virus *(HIV)* infection. HIV preferentially replicates in activated $CD4^+$ T cells, although virus entry can occur before activation. Productive HIV replication proceeds as follows: entry into cell, reverse transcription of HIV RNA, integration into host genome, and formation of new virions. Consequences of productive HIV infection in $CD4^+$ T cells include cell death by cytolysis and other mechanisms, spreading of virus to new targets, and establishment of long-lived latently infected reservoirs. The availability of high numbers of HIV target cells in lymphoid tissues leads to high levels of viral replication in most untreated individuals, and in turn leads to bystander effects that reflect chronic immune activation. *CTL,* Cytotoxic T lymphocyte.

HIV does not prevent the establishment of the resting CD4+ T-cell reservoir of HIV.[164] Furthermore, several studies have shown that despite effective ART, HIV persists in the resting CD4+ T-cell reservoir for years,[154] including in the recently characterized stem cell memory T cells, which have been shown to be long-lived, quiescent, and relatively insensitive to HIV cytopathic effects.[165] It has been estimated that it would take decades of continuous ART, using the currently available drugs, to eradicate HIV, and these estimates assume 100% effectiveness of ART and that the CD4+ T-cell latent reservoir is the only viral reservoir present in infected individuals. These sobering data and the unlikelihood of certain of the assumptions have led many to conclude that the latent in addition to other viral reservoirs present major impediments to eradication of HIV.

Lymphoid Tissues

Secondary lymphoid tissues, especially those of lymph nodes and GALT, represent major sites of HIV replication early, after, and throughout all stages of infection. In the absence of ART, HIV infection is characterized by a dichotomy in viral load between peripheral blood and lymphoid tissue, in which the frequency of infected cells in lymphoid tissues far exceeds that in peripheral blood.[154] Early findings on tissue sections excised from axillary lymph nodes have demonstrated that up to 25% of CD4+ T cells present in the germinal centers (GCs) harbor HIV DNA.[166] More recent analyses performed on tissues isolated from GALT have demonstrated massive depletion of CD4+ T cells in the lamina propria within several weeks after infection.[34,35] Depletion of CD4+ T cells in lymphoid tissues (and in peripheral blood owing to a direct link with lymphoid tissues) has also been shown to persist throughout all stages of HIV infection in untreated individuals.[156,158] Collectively, these data emphasize the role of secondary lymphoid tissues as critical reservoirs for HIV in vivo.

The significant role of secondary lymphoid tissues in ongoing HIV replication during all stages of disease in the absence of ART suggests that these compartments may also play a significant role in the maintenance of viral reservoirs in the presence of ART. Initiation of ART leads to a rapid decrease in lymph node viral burden, and within 24 weeks the levels of HIV RNA in the GCs decrease significantly.[167] However, even with prolonged ART, it is almost universally possible to detect HIV RNA within B-cell follicles of lymph nodes, where follicular dendritic cells (FDCs) have been proposed as long-lived repositories for intact HIV virions. Similar to DCs, FDCs have been shown to retain infectious HIV within endosomal compartments that can recycle to the surface and transmit virus to target CD4+ T cells.[168] The GALT also harbor HIV that is not completely cleared with ART; levels of HIV DNA in CD4+ T cells remain severalfold higher in GALT compared with the peripheral blood in those whose levels of plasma HIV RNA had remained below the limit of detection for several years. There are also indications that macrophages in mucosal, adipose, and pulmonary tissues may be a long-lived source of infectious HIV, although most of these observations have been made in animal models and need further evaluation in humans.[158,169] Finally, there is also evidence that virus in myeloid cells is acquired by phagocytosis of infected CD4+ T cells.[170]

Strategies for HIV Eradication

Over the past several years, there has been a flurry of discussion regarding the possibility that HIV can be eradicated from HIV-infected individuals.[b] Such a pursuit has accelerated with evidence that one such individual, commonly referred to as the "Berlin patient," has been cured of HIV.[26] His case is unique and unlikely to be clinically applicable, given that the approach taken was to treat an underlying leukemia with chemotherapy and to perform a stem cell transplantation from a donor who was homozygous for the HIV resistance gene *CCR5-Δ32*. The post-transplantation therapy also included chronic immunosuppressive therapy for the prevention of transplant rejection. Despite numerous attempts since 2009, similar attempts to eradicate HIV involving bone marrow or stem cell transplantation or gene therapy have failed to replicate the outcome of the Berlin patient.[9,11] Alternative cure strategies are also being pursued, including the use of agents that could reverse HIV latency and/or use modalities involving gene therapy or molecules engineered for virus-specific killing.[9] Finally, there are also discussions of using immune-based interventions as part of a combination of approaches to control HIV replication in infected individuals through the induction of an immune response that could prevent viral rebound in the absence of ART. In this case, the more appropriate term would be sustained ART-free virologic remission, whereby the virus, although still present in the body, would be kept in check by the individual's own immune system.[9,154]

[b]References 9, 11, 155, 156, 169, 171, 172.

IMMUNE DYSFUNCTION CAUSED BY HIV INFECTION

A wide array of immune system deficits is associated with HIV infection. Abnormalities in the function of all limbs of the immune system, including T and B lymphocytes, antigen-presenting cells, and NK cells, have been described. Immunodeficiency may become so profound in the late stages of disease that HIV-specific antibody and cytolytic T-lymphocyte responses diminish in the face of high levels of ongoing viral replication.

Lymphoid Tissues

The dysfunctions induced by HIV can be best appreciated in the context of the microenvironment in which the virus replicates. Most CD4+ T cells, which comprise the main targets for HIV replication, reside in secondary lymphoid tissues, such as lymph nodes and GALT, in which immune responses are generated. Early studies conducted before the availability of effective ART demonstrated that the clinically asymptomatic phase of HIV infection is characterized by lymphoid hyperplasia, whereas advanced HIV infection is marked by a striking disruption and atrophy of lymphoid tissue architecture.[173] Follicular involution, hypervascularity, and fibrosis are some of the histopathologic changes evident in lymph nodes from patients with advanced HIV disease. FDCs are eliminated during disease progression and the resulting disturbances in follicular structure have important implications regarding the pathogenesis of HIV-related immunodeficiency. The ability to mount immune responses against new antigens and the ability to maintain memory responses are dependent on the maintenance of an intact follicular architecture, which is severely impaired when the FDC network is destroyed.[168] Fibroblastic reticular cells (FRCs), another constituent of lymph nodes that provide the structural backbone and framework for the generation and maintenance of immune responses, are also progressively affected by the lack of containment of HIV replication.[168]

In studies on the effect of HIV infection on lymph node architecture, the inflammation caused by persistent viral replication contributes to lymphatic tissue damage and limits the potential for immunologic reconstitution after ART. Deposition of collagen occurs mainly in the paracortex region of lymph nodes, which is populated mainly by T cells, and as such is thought to be associated with the loss of total CD4+ T cells in general, and naïve CD4+ T cells in particular.[174] Early initiation of ART may help limit the HIV-related damage and enable normalization of CD4+ T-cell populations. A distinct subset of lymphoid tissue memory CD4+ T cells, called T follicular helper (Tfh) cells, are present in GCs and play a critical role in their formation and maintenance.[175] Tfh cells provide and receive survival and differentiation signals by interacting with cognate B cells during the GC reaction. In lymph nodes of chronically infected HIV-viremic individuals, there is an accumulation of Tfh cells in GCs, which has been correlated with hypergammaglobulinemia and frequencies of GC B cells.[176] However, it is unclear whether these Tfh cells can provide adequate help to GC B cells, given that antibody responses to HIV and other pathogens are impaired in HIV-infected individuals and that Tfh cells are major targets for active HIV replication.[177]

In GALT, massive depletion of CD4+ memory T cells, especially Th17+ CD4+ T cells, appears to occur within weeks after infection. Although possible explanations for the profound level of destruction at mucosal surfaces were first reported within the controlled setting of SIV infection,[32,33] findings of extensive depletion of CD4+ T cells in GALT during the acute phase of HIV infection had been reported before the SIV studies.[34] Later studies established that Th17 CD4+ T cells were preferentially lost in the GALT of HIV-infected individuals

and after pathogenic SIV infection.[178] The consequences of massive depletion of CD4+ T cells in GALT on the course of HIV disease remain somewhat speculative and mainly based on animal models; however, it has been proposed that the depletion compounds immune activation, a hallmark of HIV disease (see Fig. 121.5), through the disruption of mucosal integrity and leakage of inflammatory products.[39] Despite these uncertainties, and as with studies on the effect of early ART on lymph node architecture, early treatment appears to help restore the intestinal CD4+ T-cell pool.[179]

CD4+ T Cells

CD4+ T-cell dysfunction, both quantitative and qualitative, is a major hallmark of HIV disease. The opportunistic infections observed with advancing disease are primarily caused by defects in CD4+ T-cell number and function that result directly or indirectly from HIV infection, although debate remains regarding the pathogenic mechanisms of CD4+ T-cell depletion. Direct effects of HIV on CD4+ T-cell function include direct infection of these cells, with resultant cell death that leads to loss of absolute cell numbers. Indirect effects of HIV infection result in decreased CD4+ T-cell production and differentiation, altered activation and migration, dysregulation of effector functions, and other defects in T-cell helper function.[180] Furthermore, there is evidence that differences in phenotypic and functional attributes of HIV-specific and opportunistic pathogen–specific CD4+ T cells contribute to HIV disease progression and susceptibility to opportunistic infections.[181] For example, CD4+ T-cell responses against tuberculosis (TB) are lost earlier than those directed against CMV in HIV-infected individuals, in part because TB-specific CD4+ T cells tend to be more susceptible to HIV than their CMV-specific counterparts.[182] These differences are also thought to help explain at least in part the progressive yet somewhat selective susceptibility of HIV-infected individuals to opportunistic pathogens.[183]

T cells from HIV-infected individuals manifest various phenotypic and functional abnormalities that begin early after infection and worsen with disease progression.[183] Functionally, CD4+ T cells are assessed according to their capacity to expand and differentiate into subsets that produce characteristic cytokines and chemokines (Th1, Th2, and Th17), and/or characteristic function, as with Tfh cells that promote B-cell affinity maturation and T-regulatory cells (Tregs) that regulate effector responses. Defects in early HIV infection include depletion in the GALT of Th17 cells, which mainly secrete IL-17, IL-22, and IL-21 and are responsible for host responses against extracellular bacteria and fungi at mucosal sites.[39] Given the importance of Th17 responses in protection of fungal infections, loss of Th17 cells may explain the early and common manifestations of mucosal candidiasis in HIV infection.[184] Defects associated with chronic HIV disease and ongoing viral replication include decreases in response to and production of IL-2, particularly with IL-2 as part of a polyfunctional immune response; this latter defect is most clearly associated with poor outcome.[185] Although single IFN-γ–producing CD4+ T cells are not affected until late in disease, these cells have poor proliferative capacity compared with the polyfunctional populations and predominate throughout the chronic phase in most untreated individuals.

Functional attributes of a given T-cell population can generally be linked to a distinct phenotypic profile, although there is more plasticity among helper T cells than previously thought.[185,186] A number of surface markers are typically used to define various stages of CD4+ T-cell differentiation, from naïve to central, effector, and terminally differentiated memory populations. Effector memory CD4+ T cells, which are primed for immediate effector response, including secretion of IFN-γ and cytotoxicity, are relatively short-lived and have a low capacity to self-renew.[187] Central memory CD4+ T cells, which are better preserved in HIV-infected individuals who spontaneously control viral replication than in those who do not, have a high expansion capacity and are relatively long-lived as a result of autocrine production of IL-2.[188] In addition, HIV disease progression has been associated with skewing toward more differentiated and senescent CD4+ T cells,[189] including loss of CD4+ T cells expressing CD28 (i.e., the major costimulatory receptor of the B7-CD28 family, which is needed for normal activation of T cells and expressed on central but not effector memory T cells).[187] Furthermore, there is an increase in the frequency of CD4+ T cells expressing high levels of inhibitory members of the B7-CD28 family, namely PD-1, TIGIT, and CTLA-4, in HIV-infected individuals with persistent viremia.[7,108,110,190] This phenomenon has been termed T-cell exhaustion, a dysfunctional manifestation of HIV persistence that includes impairment of cytokine production and proliferative potential. Such defects can be reversed ex vivo by blocking the immunoregulatory molecules, referred to as checkpoint inhibitors. Inhibition of immunologic checkpoints has been increasingly used to treat various cancers by removing the blocks to immune control of the neoplasm. However, the reversibility of exhaustion in chronic viral infections in humans remains unknown.[8]

A variety of additional mechanisms probably also contribute to the observed functional defects in T cells. Preferential infection by HIV of CD4+ memory T cells,[191] and in particular HIV-specific CD4+ T cells,[192] and those of the Th17 and Tfh lineages,[187] helps explain loss of an effective response against the virus itself and other pathogens. In this regard, disruptions and reductions in the CD4+ T-cell receptor (TCR) repertoire is observed with progressive HIV disease, and particularly in association with aging; these disruptions are not restricted to a particular subset of CD4+ T cells but are thought to help explain the impaired response to pathogens in individuals with advancing HIV disease and age.[5,193] Likely mechanisms involved in these disruptions, which are more frequently encountered as people with HIV age and live longer, include thymic involution and shrinkage of the naïve T-cell pool, in addition to the loss of T-cell populations with self-renewal potential and the accumulation of those with exhausted and senescent properties.[194] Although early initiation of ART appears to stabilize and even improve the TCR repertoire, other promising modalities, such as cytokine-based therapies with IL-7, have shown potential; however, more studies are needed to address repertoire restoration.[179]

Mechanisms of CD4+ T-Cell Depletion

Even though CD4+ T cells were shown more than 30 years ago to be the cells most deleteriously affected in HIV-infected individuals, there remains considerable debate regarding the relative contribution of various mechanisms for the depletion of CD4+ T cells during the course of HIV infection. As with many other areas of HIV immunopathogenesis, evaluations of patients who control viremia without treatment for prolonged periods of time, those who begin effective ART during acute or chronic phases of infection, and those who interrupt treatment have provided fundamental insights into the understanding of the potential contributions of increased destruction, decreased production, and redistribution as mechanisms for CD4+ T-cell depletion in HIV-infected individuals.

Increased Destruction

Direct infection. The observations that CD4+ T cells are the principal targets of HIV infection in vivo[12,13] and that HIV infection of CD4+ T cells in vitro causes cytopathogenicity have led to a reasonable assumption that direct infection of CD4+ T cells in vivo results in their depletion (see Fig. 121.5). However, quantitative studies of the frequency of HIV-infected cells in vivo suggest that single-cell killing by direct infection with HIV may not be the predominant mechanism of CD4+ T-cell depletion. In this regard, the proportion of productively infected peripheral blood CD4+ T cells in HIV-infected individuals rarely exceeds 1 in 100, even in advanced disease.[195] Viral burden and levels of virus expression are far greater in lymphoid tissues, including GALT and lymph nodes, than in peripheral blood.[34,35,166,173] Furthermore, qualitative analyses showing a higher susceptibility of certain CD4+ T-cell subsets, and in particular those that are HIV specific,[192] has led to a reevaluation of the mechanisms of HIV pathogenesis. Tfh cells in lymph nodes,[177] and Th17 cells in mucosal tissues,[39] have been shown to be particularly more susceptible to HIV infection than other CD4+ T cells. Although these distinctions may not be enough to account solely for the progressive, albeit slow, loss of CD4+ T cells that occurs over the course of HIV disease, their critical role in immune responses and homeostasis certainly helps explain the incapacity of the immune system to contain HIV.

Death by apoptosis and pyroptosis. Apoptosis is the morphologic description of a form of programmed cell death critical to physiologic homeostasis in almost every organ system; pyroptosis is similar in form but is associated with inflammatory responses to microbes.[196,197] Apoptosis

is mediated by a series of effectors (e.g., caspases) and regulators and proceeds by one of two major pathways: the extracellular pathway, triggered by the binding of a ligand to a death receptor; and the intracellular pathway, triggered by mitochondrial events after stress signals from within the cell.[197] Mechanisms of apoptosis associated with HIV infection include cytolysis of infected cells through activation of granzymes, death of infected cells by virus-induced cytopathogenicity, death of bystander cells from proapoptotic viral proteins released from infected cells, and death resulting from HIV-induced immune activation and proinflammatory cytokines such as TNF-α.[198] Pyroptosis is induced after abortive HIV infection of $CD4^+$ T cells,[199] whereby the presence of HIV after cell-to-cell spread is sensed, inducing the formation of an inflammasome that ultimately triggers cell death via caspase-1 and release of the proinflammatory cytokine IL-1β.[198]

It has been suggested that aberrant intracellular signals transduced by HIV proteins, including gp120 and Tat, might prime $CD4^+$ T cells for apoptosis. However, whereas this mode of depletion of $CD4^+$ T cells by induction of apoptosis in HIV-infected cells has been shown in numerous in vitro models, the vast majority of cell death that occurs in vivo during the chronic phase of infection results from activation-induced and inflammation-induced bystander mechanisms associated with apoptosis and pyroptosis.[197,198] The massive depletion of $CD4^+$ T cells in GALT that occurs during acute SIV and HIV infection is likely to involve multiple mechanisms.[200] Fas-mediated apoptosis is also the primary mechanism of apoptosis resulting from HIV-induced activation of $CD4^+$ T cells, in addition to $CD8^+$ T cells, B cells, and NK cells (see Fig. 121.5).[201,202] Also, both Fas- and TNF-related apoptosis-inducing ligand (TRAIL)-mediated pathways of apoptosis have been linked to the induction of IFN-induced genes, a major mechanism of HIV-mediated immune dysregulation during the chronic phase of infection in viremic individuals that has been shown to affect all major lymphocyte populations.[196,202,203] In addition to activation-induced cell death mediated by ligation of death receptors, there is also intrinsic apoptosis induced by activation that results in decreased expression of prosurvival members of the Bcl-2 family including Bcl-2 and Bcl-X_L. Both these prosurvival proteins are downregulated in activated $CD4^+$ and $CD8^+$ T cells isolated from HIV-infected individuals.[201] Studies on the immunotherapeutic potential of cytokines such as IL-7 and IL-15 have suggested that part of their mechanism of action is to increase survival of T cells by increasing intrinsic levels of Bcl-2 and other prosurvival members of this family.[204] However, the increased survival may also promote HIV persistence.[205]

Lymphocyte turnover. Mathematical models of lymphocyte turnover derived through analysis of immediate changes in circulating $CD4^+$ T-cell counts in individuals, after the initiation of ART, have led to estimates that approximately 2×10^9 $CD4^+$ T cells are destroyed and replenished daily.[206] These seminal findings initially led to the suggestion that increased turnover, which is defined by increased proliferation and cell death, of $CD4^+$ T cells results from homeostatic compensation caused by HIV-mediated killing of $CD4^+$ T cells. However, later studies using a variety of techniques to measure lymphocyte proliferation, including Ki-67 antigen, 5-bromo-2′-deoxyuridine (BrdU), and $^2H_2$-glucose to evaluate the effects of HIV on cell turnover have revealed a more complicated view. Several investigators have demonstrated that the increase in cell turnover during ongoing viral replication not only affects $CD4^+$ T cells but also is observed in cells that are not direct targets of HIV and SIV, namely $CD8^+$ T cells, B cells, and NK cells.[207–209] These observations led to the conclusion that increased cell turnover is mainly caused by HIV-induced immune activation and increased homeostatic proliferation in response to lymphopenia. Consistent with this view is the observation that $CD4^+$ T-cell counts are maintained in natural SIV infections in which chronically high levels of virus replication occur in the absence of immune activation.[210] A similar analysis has been made for HIV-2, wherein $CD4^+$ T-cell numbers are maintained and disease progression is slow compared with HIV-1, although levels of virus replication are also lower in HIV-2 infection.[180] One lingering question with regard to the view that increased cell turnover is driven by increased immune activation is how to explain why $CD4^+$ T cells are gradually depleted while $CD8^+$ T cells are maintained until late-stage disease. Although there is no clear answer to this conundrum, several possibilities have been proposed, including a greater susceptibility of $CD4^+$ than $CD8^+$ T cells to the detrimental effects of immune activation and/or a lower regenerative capacity for $CD4^+$ compared with $CD8^+$ T cells. There are also indications that differences in homeostatic regulation between $CD4^+$ and $CD8^+$ T-cell compartments may help explain why the CD4:CD8 ratio is skewed in HIV infection and does not always recover with ART.[211–213]

Decreased Production

Decreased production of $CD4^+$ T cells could occur as a result of reduced thymic output. Proposed mechanisms for reduced thymic output in HIV infection include disruption of the thymic microenvironment, direct HIV infection and depletion of $CD4^+$ thymocytes, reduction in thymocyte proliferation, and destruction of thymocytes by apoptosis.[194] Douek and coworkers first demonstrated that recent thymic emigrants in HIV-infected individuals are significantly decreased as a consequence of HIV infection.[214] The percentage of circulating and lymph node naïve $CD4^+$ T cells carrying signal-joint T-cell receptor excision circle (TREC) gene products, a marker of recent thymic emigration, was significantly reduced in HIV-infected individuals compared with age-matched control subjects. Initiation of effective ART resulted in a significant increase in signal-joint TRECs in $CD4^+CD45RO^-CD27^+$ naïve T cells in the periphery; the latter finding suggested that the thymus remains functional in these individuals, who were past adolescence, and may contribute to immune reconstitution. Advances in approaches for measuring the various forms of TREC and new cell-surface markers for tracking thymic emigrants, including CD31, have been used to confirm decreased thymic output in HIV disease.[215] These findings are also supported by observations of increased thymic output after initiation of ART in HIV-infected individuals, especially in individuals who initiate therapy early after infection.[5] An enhanced capacity for immune reconstitution when ART is initiated early after HIV infection may help explain the more rapid recovery of $CD4^+$ T cells in these individuals.[216,217]

The disruption of normal hematopoiesis may contribute to the depletion of $CD4^+$ T cells during HIV infection. Although there is limited evidence for direct infection of $CD34^+$ progenitor cells and a role for the bone marrow in HIV persistence, abnormalities in bone marrow architecture and stromal auxiliary cells are observed in HIV-infected individuals. Such defects may thus compromise the bone marrow's ability to serve as the primary source of lymphocyte precursors, a process that is worsened by the effects of aging.[193]

Redistribution. Although the role of redistribution in the loss of $CD4^+$ T cells is unclear and difficult to ascertain, data from both HIV and SIV infections indicate that there is significant trafficking of $CD4^+$ T cells from the peripheral blood to lymphoid tissue in acute and chronic infection. The trafficking of lymphocytes is mediated, in part, through the expression of homing receptors, which guide cells from the peripheral blood into lymphoid tissues via extravasation that involves cross-linking of their ligands on endothelial venule cells. In this regard, several investigators have suggested that redistribution of $CD4^+$ T cells back from lymphoid tissue to the peripheral blood contributes significantly to the increase in $CD4^+$ T cells after the initiation of ART.[218]

$CD8^+$ T Cells

Dysregulation of $CD8^+$ T-cell numbers and function is evident throughout the course of HIV disease. After acute primary infection, $CD8^+$ T-cell counts usually rebound to supranormal levels and may remain elevated for prolonged periods. Increases in $CD8^+$ T cells during all but the late stages of disease may partly reflect the effects of chronic immune activation that affect both $CD4^+$ and $CD8^+$ T cells, albeit with distinct responses in terms of rates of proliferation, death, and regeneration. Although many explanations for the inverse ratio of CD4 to CD8 remain speculative, there is general agreement that the inversion is not simply a reflection of $CD4^+$ T cells being direct targets for HIV.[211–213] As with $CD4^+$ T cells, $CD8^+$ T cells of HIV-viremic individuals manifest an increased cell turnover and decreased levels of TREC. However, there are some indications that compared with $CD4^+$ T cells, $CD8^+$ T cells are less dependent on thymic function and an intact lymphoid tissue architecture for homeostasis and have a lower deficit to overcome after the acute phase of massive cell depletion in GALT.[174,213] In addition, changes in

lymphoid tissue architecture that occur with progressive HIV disease, including increased fibrosis and loss of FRC networks, are thought to cause an increased relative proportion of $CD8^+$ T cells in the circulation, thus accounting at least in part for the low CD4:CD8 ratio in the blood.[219]

Furthermore, whereas both activation and death rates are increased for $CD4^+$ T cells, there is likely a net increase in activation for $CD8^+$ T cells that may be explained by differences in how $CD4^+$ and $CD8^+$ T cells become activated in HIV-infected individuals.[220] In this regard, the degree of T-cell activation, as measured by the expression of the activation marker CD38 on $CD8^+$ but not on $CD4^+$ T cells, remains one of the strongest predictors of time to HIV disease progression. Even in patients receiving ART, the level of activation on $CD8^+$ T cells is inversely correlated to gains in $CD4^+$ T-cell counts.[221]

During HIV disease progression, $CD8^+$ T cells also undergo alterations that reflect a skewing within the $CD8^+$ T-cell compartment. Increased frequencies of $CD8^+$ T cells with reduced expression of CD28 and increased expression of CD57 are observed in HIV disease, possibly reflecting expansion of immunosenescent cells that normally occurs in the elderly.[193] Data from numerous studies have suggested that impaired HIV-specific $CD8^+$ T-cell activity in HIV-viremic individuals is at least in part associated with a skewed effector memory $CD8^+$ T-cell population that displays low polyfunctionality, proliferative capacity, and effector function.[220,222] Furthermore, HIV-induced T-cell exhaustion, which includes upregulation of the inhibitory receptors, including PD-1, LAG-3, TIGIT, and Tim-3, among others, has been described both for HIV-specific $CD8^+$ T cells and in the general $CD8^+$ T-cell population.[223] Several transcriptional factors that control T-cell effector function and differentiation, including BATF,[224] T-bet, and Eomes, are also modulated in exhausted $CD8^+$ T cells and may control the extent of dysregulation,[223] even after successful reduction of viremia by ART.[225] Other $CD8^+$ T-cell functions may be impaired during HIV disease progression, such as loss of noncytolytic, non–MHC-restricted $CD8^+$ T-cell–derived HIV suppression.[226]

B Cells

Dysregulation of B cells and an intrinsic loss of function are likely responsible, in part, for the increase in certain bacterial infections seen in advanced HIV disease in adults, and for the morbidity and mortality associated with bacterial infections in HIV-infected children who cannot mount an adequate humoral response to common bacterial pathogens.[183] The number of circulating B cells is decreased in HIV-infected individuals; this likely reflects a combination of effects associated with HIV viremia, including the expansion of apoptosis-prone subsets and redistribution of cells into lymphoid tissues.[227] One of the hallmarks of chronic HIV viremia is hypergammaglobulinemia,[228] a consequence of generalized immune activation and the many inflammatory cytokines that favor terminal differentiation of B cells.[229] The increase in immunoglobulins occurs for all classes and subclasses of antibody, although IgG1 is the most affected, and the increase is largely polyclonal.[230] The association between hypergammaglobulinemia and ongoing HIV replication was first demonstrated in longitudinal studies in which initiation of ART led to the normalization of serum levels of both immunoglobulins and the corresponding antibody-secreting cells in the blood (plasmablasts or plasma cells).[231] In lymphoid tissues, as mentioned earlier (see "Immune Dysfunction Caused by HIV Infection"), more recent studies have shown strong correlations among HIV-induced hyperplasia, hypergammaglobulinemia, and frequencies of Tfh and GC B cells, the latter accounting for up to 50% of cells in biopsy specimens.[232] Chronic HIV plasma viremia has also been associated with the appearance of various subpopulations of B cells in the peripheral blood that express reduced levels of CD21, some of which are responsible for the increased secretion of immunoglobulins and the decreased response to B-cell stimuli, an abnormality that was first demonstrated in 1983. [228] Several of these phenotypic and functional aberrations can be reversed by reduction of HIV plasma viremia by ART, suggesting that control of viremia may contribute to the restoration of the humoral arm of the immune system, especially when ART is initiated during primary infection.[230]

Several defects in B cells from HIV-infected individuals have been attributed to the expansion of subsets that are rare in the peripheral blood of healthy individuals.[230] In addition to the plasmablasts, described earlier, that are associated with hypergammaglobulinemia, there is also an expansion of immature transitional B cells and tissue-like memory B cells bearing features of exhaustion. The expansion of immature transitional B cells in the peripheral blood of HIV-infected individuals is associated with increased serum levels of IL-7 and $CD4^+$ T-cell lymphopenia, with the strongest effects observed in advanced HIV disease and in individuals treated with exogenous IL-7.[233] In contrast, the expansion of tissue-like memory B cells, defined by reduced expression of CD21 and CD27, the marker of classic B-cell memory, in the peripheral blood of HIV-infected individuals is associated with chronic HIV viremia.[234] Tissue-like memory B cells show signs of HIV-associated exhaustion, manifesting with the following: increased expression of multiple inhibitory receptors and altered expression of homing receptors, similar to exhausted virus-specific T cells; decreased replication history and somatic hypermutation required for an effective antibody response; and decreased proliferative responses. Furthermore, the HIV-specific B-cell response is enriched within this exhausted B-cell compartment, indicating that B-cell exhaustion may contribute to the poor antibody response against HIV observed in HIV disease. Finally, correlates of B-cell dysfunction observed in HIV-infected viremic individuals also included an increased frequency of B-cell neoplasms, which is likely a reflection of transformation arising from increased HIV-induced B-cell turnover in the face of impaired T-cell immunoregulation.[235]

Classic memory B cells are defined by the expression of CD27 on B cells that are resting and long-lived. Of note, the decrease in the frequency of resting memory B cells in HIV-infected individuals occurs early after infection and is not reversed by ART, although there are some indications that early initiation of ART helps normalize the B-cell compartment.[236] These observations likely explain, at least in part, the reduced memory B-cell responses that have been reported against various T-cell–dependent and T-cell–independent immunogens and against HIV itself. In this regard, several recently developed methods for probing, expanding, and isolating HIV-specific B cells have become available and are providing new insights into the antibody response against HIV. As discussed earlier (see "Humoral Immune Responses") potent and bNAbs arise after several years of infection in a relatively small percentage of untreated individuals for reasons that remain unclear but likely involve sustained antigenic stimulation from persistent HIV replication. It is hoped that a better understanding of the HIV-specific antibody and B-cell responses in HIV-infected individuals will lead to better strategies for an effective antibody-based vaccine.

Natural Killer Cells

NK cells provide innate immune defense against virus-infected cells, certain tumor cells, and allogeneic cells. Activation of NK cells is governed by a balance of signals received from the triggering of cytotoxic activating and inhibitory receptors, the latter of which recognize MHC class I molecules and prevent cytolysis.[237] NK cells can be divided into three subsets based on the expression of CD56; most NK cells in the peripheral blood of healthy individuals are composed of $CD56^{dim}$ NK cells, compared with a minority that are $CD56^{bright}$. This latter subset tends to have a more regulatory function, secreting high levels of cytokines such as IFN-γ and TNF-α, whereas the former subset has a more cytotoxic function. The third subset, $CD56^{neg}$ NK cells, are almost absent in healthy individuals, yet become overexpressed in HIV-infected individuals, especially those who experience chronic viremia.[238] A reduction in the ability of NK cells to lyse target cells is observed throughout the course of HIV disease, and this defect increases with disease progression. Loss of function is associated with the increased frequency of $CD56^{neg}$ NK cells. These functional defects are associated with increased frequencies of $CD56^{neg}$ NK cells and are accompanied by alterations in expression of receptors that regulate NK-cell activity, namely the upregulation of certain inhibitory receptors and the downregulation of several activating receptors.[239]

There is little evidence that HIV productively infects NK cells in vivo; however, there is evidence that many HIV proteins, including envelope and accessory proteins, perturb NK cells through evasion properties that reduce the ability of NK cells to kill infected target cells.[239] NK cells are also the primary mediators of the ADCC response, an antibody constant (Fc) region-mediated effector function that may be responsible for some of the nonneutralizing antibody-mediated protective effects

observed in the RV144 HIV vaccine trial.[240] In HIV-infected individuals, there are indications that ADCC is involved in LTNP/EC control, although there is also evidence of HIV-mediated evasion.[239] Several studies, reviewed by Carrington and coworkers,[241] have also found an association between genotype of certain NK cell–related HLA molecules and control of HIV viremia and disease progression, suggesting a role for NK cells in the control of HIV disease. In this regard, mutations in HIV accessory genes of chronically infected individuals likely have been shown to arise in response to pressure from killer-cell immunoglobulin-like receptors (KIR), providing additional evidence that HIV evolves to evade NK cell–mediated responses against the virus.[242]

Monocytes and Macrophages

Cells of the monocyte-macrophage lineage play key roles in the immunopathogenesis of HIV disease. These cells are likely involved in facilitating the transmission and establishment of HIV infection, as described earlier (see "Dissemination of HIV Infection"), although their role in maintaining the viral reservoir in ART-treated individuals is less certain.[243] Dysfunction of these cells also occurs in HIV-infected individuals, especially with regard to impaired host defense against intracellular pathogens and their contribution to the immune-activating effects associated with ongoing HIV replication (see "Role of Immune Activation in the Pathogenesis of HIV Infection").[244]

Monocytic cells express CD4 and numerous HIV coreceptors on their surface, including CCR5 and CXCR4, and can theoretically serve as targets of HIV infection for both R5- and X4-tropic strains. However, unlike infection of $CD4^+$ T cells, HIV coreceptor usage is more restricted to CCR5 in the monocyte-macrophage lineage.[243] Differences in receptor-binding affinities and mechanisms of cell-cell spread and host factors likely explain the more restricted replication of HIV in this lineage despite the expression of appropriate HIV receptors.[243,244] Whereas circulating monocytes are rarely found to be infected unless differentiated into macrophages ex vivo, the presence of HIV in vivo can be detected in tissue macrophages, including resident microglial cells in the brain, and pulmonary alveolar macrophages.[243,244] Lymphoid tissue macrophages were identified as prolific producers of HIV in the setting of advanced HIV disease two decades ago, [245] and more recently as a source of potent pro-inflammatory factors.[246]

In pre-ART years, HIV-induced CNS disease was common and involved infection and activation of microglia and perivascular macrophages in the CNS. The process resulted in neurologic impairment, including encephalopathy, neuropathy, astrocytosis, and cerebral vasculitis. The inflammatory processes linked to HIV-associated dementia have been associated with several factors, including chemokines and cytokines that are produced by activated macrophages.[243,244] Increased levels of lipopolysaccharide (LPS), a highly inflammatory bacterial product that preferentially binds to macrophages through interactions with CD14 and Toll-like receptors, are observed in the serum of HIV-infected individuals. The binding of LPS to monocytes may promote disruption of the blood-brain barrier, further enhancing the migration of activated monocytes into the CNS and thus contributing to HIV-associated dementia.[247] Even in individuals whose HIV viremia is controlled by ART, there is evidence for residual neurocognitive disorders that are likely to be associated with residual macrophage activation.[244]

Dendritic Cells

DCs are among the first cells to encounter HIV after mucosal exposure and are probably responsible for transporting the virus to lymphoid organs, thus facilitating infection of $CD4^+$ T cells and viral dissemination (see "Dissemination of HIV Infection"). Whereas DCs express several different chemokine receptors that can be used as HIV coreceptors for entry,[248] there is limited evidence that DCs become productively infected in vivo. Instead, DCs are more likely to be involved in capturing HIV, forming conjugates with $CD4^+$ T cells by way of long flexible filopodia, and contributing to the formation of infectious viral synapses.[249] This sequence of events is thought to be highly efficient at promoting the transmission of HIV virions from DCs to $CD4^+$ T cells. Several C-type lectin receptors, including DC-SIGN and langerin, which are differentially expressed on subsets of DCs, play an important role in the capture of HIV virions by DCs.[27]

Early studies provided conflicting data on the dysfunction of DCs during HIV infection. Interpretation of these studies was complicated by the use of different DC purification techniques, existence of multiple poorly defined DC subsets, and differential culture conditions used by investigators. The delineation of DCs into subsets that reflect origin, location, and function—namely mDCs and plasmacytoid dendritic cells (pDCs)—has helped clarify their dysregulation in HIV infection. The mDCs are further classified by tissue location and circulation, and although their importance in antigen presentation is well established, their sensing properties and how HIV evades them have been more recently described.[196] Viral infections are sensed by cells of the innate immune system through PRRs such as TLRs. Type I IFNs, including IFN-α, produced primarily by pDCs, are one of the first sensing mechanisms induced by viral infections, including HIV. In this regard, recent findings suggest that type I IFNs can block SIV infection, but that in chronic infection, they have a detrimental effect (see "Cytokines and HIV Disease: Dysregulation of Cytokine Production").[250] HIV infection can also lead to a decrease in the number of circulating pDCs through mechanisms that are not well delineated but may involve direct infection with HIV or direct interactions with the HIV envelope, redistribution to lymphoid tissues, and apoptosis.[40,251] Several mechanisms of immune dysfunction in HIV disease have also been attributed to pDCs, including secretion of factors that induce Treg cells, which then suppress the antiviral response, and induction of apoptosis-mediating ligands on pDCs.[252] Finally, there are also indications that the increase in Tregs and reciprocal depletion of Th17 cells in the gut of HIV-infected individuals is in part due to inflammatory factors secreted by chronically activated mDCs and pDCs.[253]

ROLE OF IMMUNE ACTIVATION IN THE PATHOGENESIS OF HIV INFECTION

The end result of HIV infection is profound immunodeficiency; however, paradoxically, HIV infection is associated with hyperactivation of the immune system throughout most of the course of disease.[228] HIV subverts the immune system by inducing immune activation, using this milieu toward its own replicative advantage, and causing widespread damage to the immune system. Whereas it is well accepted that the replicative cycle of HIV infection is most efficiently achieved in activated cells,[162,163] most of the damage caused by HIV is not the result of direct infection of $CD4^+$ T cells but, rather, the widespread bystander activation, dysregulation, and cell death of $CD4^+$ T cells and other cells of the immune system.[213] In this regard, numerous studies have demonstrated that the level of $CD8^+$ T-cell activation, as measured through expression of CD38 and HLA-DR, is a better correlate of HIV disease progression than HIV viremia.[4] More recently, alterations in expression and secretion of factors or biomarkers, especially those associated with the myeloid lineage such as soluble CD14 and D-dimer, among others,[244] have also been proposed as indicators of immune activation induced by both active viral replication and by the residual effects of viremia in individuals receiving effective ART.[179] The persistence of immune activation in individuals receiving ART is thought to explain, at least in part, their increased risk of comorbidities, such as cardiovascular and liver diseases, type 2 diabetes, osteoporosis, and malignancies.[254,255]

With regard to T cells, and to a lesser extent B cells and NK cells, the evidence for HIV-induced immune activation is severalfold. There is a high frequency of cells expressing markers of activation and cell cycling that is accompanied by homeostatic dysregulation and cell death during ongoing HIV replication.[187,213] Many of these manifestations are attenuated or reversed by effective ART. One of the most widely accepted indirect pieces of evidence that immune activation plays a major role in HIV immunopathogenesis is the nonpathogenic outcome of SIV infection in its natural host. In the case of naturally SIV-infected sooty mangabeys, high levels of SIV viremia occur in the absence of generalized immune activation and progressive $CD4^+$ T-cell depletion.[210] In further contrast to HIV infection, which almost invariably leads to AIDS if left untreated, SIV infection in natural hosts almost never leads to disease progression. Over the years, the differences between pathogenic HIV infection in humans and nonpathogenic SIV infection in nonhuman primates, such as sooty mangabeys, have been extensively investigated.

The most consistent feature of these comparisons remains the lack of generalized immune activation in natural SIV infection, with a muting of the innate immune response, including systemic inflammation and a strong type I IFN response after the acute phase, being the most important difference observed between naturally controlled SIV and uncontrolled HIV infections.[169,210]

The underlying causes of HIV-induced immune activation, increased cell turnover, and eventual depletion of $CD4^+$ T cells remain a matter of debate. There is a growing body of evidence, mainly from NHP models,[169] that the early innate immune proinflammatory response that occurs at mucosal sites leads to recruitment and activation of adaptive immune cells, including HIV target cells, which then allow the virus to disseminate to secondary lymphoid tissues for rapid amplification before an effective adaptive immune response. A number of studies have shown that when this initial phase of viral replication is blunted,[30,74] immunologic and/or virologic control ensues and disease progression can be averted. In the absence of early intervention, the early massive depletion of $CD4^+$ T cells in mucosal and other lymphoid tissues leads to a chronic state of increased proliferation, activation, and cell death. The eventual systemic depletion of $CD4^+$ T cells that characterizes AIDS is also explained by a pronounced loss of naïve and central memory $CD4^+$ T cells, both of which are strictly regulated and may be more prone to regenerative failure than other lymphocytes.[187]

There are important consequences of generalized immune activation that likely contribute to the inefficiency of the HIV-specific immune response and to the eventual impairment of the regenerative potential of the entire immune system. The increase in immune activation and inflammation that arises from persistent HIV replication is accompanied by an expansion of exhausted T cells and B cells and by increased levels of immunoregulatory factors such as IL-10.[256] Exhaustion is defined by decreased proliferative and effector function, alterations in homing receptor expression patterns consistent with migration to sites of inflammation, and increased expression of inhibitory receptors,[190] such as PD-1, 2B4, LAG-3, CD160, and Tim-3 on $CD8^+$ T cells, and PD-1 and CTLA-4 on $CD4^+$ T cells.[108,110] A reversal of HIV-specific exhausted T cells has been demonstrated with PD-1 and CTLA-4 antagonists, many of which are used as checkpoint blockers in treatment of certain cancers.[8]

The effect of generalized immune activation on the immune system also includes gradual destruction of lymph node architecture and changes in the composition of lymphoid tissues that impede immune function. The changes in lymph node architecture that occur with HIV infection and persistent viremia include progressive damage to follicular structures, from hyperplasia to lysis, atrophy, and involution; each stage has increasing deleterious effects, both on the generation of effective immune responses and on T-cell homeostasis.[168] Collagen deposition and fibrosis have also been shown to contribute to defects in normal T-cell homeostasis, and similar effects have been proposed for B cells.[168,218]

CYTOKINES AND HIV DISEASE: DYSREGULATION OF CYTOKINE PRODUCTION

One important facet of chronic immune activation induced by HIV infection is the increased production of proinflammatory cytokines and chemokines. Early after infection, high levels of TNF-α, IP-10, IFN-γ, and IL-6 are found in serum. Several cytokines have been shown to upregulate HIV expression in infected cells.[257] High in vivo levels of cytokines, which are unique to acute HIV at least in potency and breadth,[258] are commonly referred to as a cytokine storm.[254] Levels of other proinflammatory and immunoregulatory cytokines increase over the course of HIV disease, including IL-1β, IL-10, and transforming growth factor-β (TGF-β), the last of which is particularly evident in lymphoid tissues and is thought to contribute to the deposition of collagen and ensuing damage to the stromal FRC network on which lymphocytes migrate.[168,218] The production of proinflammatory cytokines and chemokines is thought to originate, at least in part, from innate immune cells such as inflammatory monocytes and neutrophils as a result of increased expression of PRRs such as Toll-like receptors.[5] In this regard, the triggering of Toll-like receptors, as part of the innate immune responses against HIV or because of mucosal leaching of microbial products such as LPS, is thought to lead to secretion of proinflammatory cytokines by macrophages and DCs.[254]

The role of pDCs in shaping the cytokine milieu in HIV-infected individuals is considered potentially very important, although difficult to ascertain because of the low frequency of pDCs in the circulation. Although numbers of pDCs may be decreased in the peripheral blood of HIV-infected individuals, their induction and secretion of copious amounts of IFN-α is most likely to occur as part of the host innate immune response to HIV. In this regard, the induction of type I IFNs as part of the early response to HIV can be highly beneficial, as shown in a recent NHP model.[250] However, it is the chronic effect of these pathways that is thought to be detrimental, and numerous studies have shown that ongoing HIV replication is associated with the induction of numerous IFN-induced genes in all major lymphocyte populations.[196] Among these IFN-induced genes are ones coding for death receptors and ligands that have been associated with increased susceptibility to apoptosis of lymphocytes isolated from HIV-viremic individuals. These include Fas and FasL and the ligand TRAIL and accompanying death receptors DR4 and DR5. These latter receptors have also been associated with T-cell exhaustion,[259] as has TNF most recently, which is one of the cytokines strongly induced by HIV and now being proposed as a critical link between inflammation and T-cell dysregulation in chronic viral infections.[260]

Another disruption in cytokine networks observed in HIV disease is a progressive loss of the ability to produce immunoregulatory cytokines, such as IL-2, that reflects changes in $CD4^+$ and $CD8^+$ T-cell effector function (see "Cellular Immune Responses"). Whereas functional impairment was once attributed to loss of Th1 cytokines (secretion of IL-2 and IFN-γ) and possible dominance of Th2-like responses (i.e., secretion of IL-4, IL-5, and IL-10) during progression of HIV disease, many of these paradigms have been complicated by plasticity between subsets and the identification of other $CD4^+$ T-cell subsets. These include Th17 cells, which reside primarily in skin and mucosal tissues, produce IL-17 and IL-22, and play important roles in antibacterial and antifungal immunity.[261] In mucosal tissue dysregulation associated with HIV-induced immune activation, the secretion of indoleamine 2,3-dioxygenase, an immunomodulatory enzyme, by DCs leads to suppression of Th17 cells while inducing the development of Tregs.[262] Although the role of Tregs in HIV disease remains controversial, there does appear to be a consensus that the balance of Tregs and Th17 is disrupted in HIV-infected individuals as a result of persistent immune activation and that these changes are, in part, driven by increases in Treg suppressive cytokines, such as TGF-β and IL-10.[261]

Role of Cytokines as Therapeutic Agents in HIV Infection

The use of cytokine-based therapies aimed at immune reconstitution and immune modulation in HIV disease has expanded over the past decade, particularly with the knowledge that ART alone is unable to restore $CD4^+$ T-cell numbers and function completely. However, each approach has faced challenges, and the prospects of cytokine-based therapeutic interventions remain unclear.

Most cytokine-based therapies have been aimed at T-cell deficiencies in the common gamma chain (γc) family of cytokines. Among this family, IL-2 initially received the most attention because it was first considered as a therapeutic agent in 1983.[263] Several years of trials and detailed analyses have determined that intermittent administration of IL-2, although associated with a sustained expansion of naïve and memory regulatory $CD4^+$ T cells and diminution in levels of activation and apoptosis, does not appear to provide sustained clinical benefits.[264] Two additional members of the γc family of cytokines—IL-7 and IL-15—are being considered as therapeutic agents to improve T-cell function in HIV-infected individuals.

IL-7 is primarily produced by stromal and epithelial cells in the thymus, lymph nodes, and intestinal epithelium and binds to IL-7Rα (CD127) complexed with common γc.[211] IL-7 is considered a major regulator of thymocyte development and of the survival and homeostasis of naïve and memory T cells. In HIV-infected individuals, high levels of IL-7 are detected in serum, especially in advanced $CD4^+$ T-cell lymphopenia.[265] These increased levels of IL-7 are thought to result from lower clearance due to decreased presence of cells expressing

CD127.[211] Nonetheless, several clinical studies in humans have also demonstrated that administration of IL-7 leads to improvement of T-cell homeostasis, repertoire, and function without inducing increased Tregs as had been shown to occur with IL-2 administration.[8] There are also indications that IL-7 is less toxic than IL-2 and may also improve reconstitution of lymphoid tissues. However, concerns remain, including that HIV may cause $CD4^+$ T cells to be refractory to IL-7 signals and that IL-7 may lead to increased HIV replication.[266] IL-15, also a member of the γc family, is produced by antigen-presenting cells, including monocyte-macrophages and DCs, and binds to the IL-2Rβ complexed with common γc. In both HIV-infected and HIV-negative individuals, IL-15 induces many of the same responses as IL-2. However, in contrast with IL-2 and IL-7, IL-15 may be most effective at driving the expansion and tissue emigration of $CD4^+$ and $CD8^+$ effector memory T cells. However, these findings were described in an SIV model, and it remains to be seen how IL-15 will modulate T cells in HIV-infected individuals. There are also concerns that IL-15 may be difficult to implement as part of a clinical regimen of immune reconstitution, given that it can accelerate disease progression when administered during the acute phase of infection, and also accelerate lymphoid tissue damage and fibrosis.[211] Finally, IL-21, also a member of the γc family, is produced primarily by Tfh and Th17 cells and has been proposed to enhance HIV-specific cell-mediated and humoral responses based on findings in animal models and LTNP/ECs.[204]

CONCLUSIONS

HIV is the quintessential opportunist, as illustrated by its ability to subvert activation of the immune system to its own replicative advantage. The virus can disarm multiple components of the host immune response by direct and indirect mechanisms. Further understanding of the interactions between the virus and host that lead to dysfunction and depletion of multiple components of the immune system should aid in the development of preventive and therapeutic strategies. Whereas effective ART currently remains the best approach for delaying HIV disease progression, several therapeutic interventions are being considered, including those intended to boost the immune system, such as vaccines, or to reverse HIV-induced cellular exhaustion, such as checkpoint inhibitors. Other immune-based therapies, including bNAb-based interventions with and without ART, are being considered. There are also indications that therapeutics aimed at dampening HIV-induced immune activation may be beneficial, especially early during the course of infection. Finally, agents aimed at purging HIV from latent and other viral reservoirs are also being evaluated, although such modalities aimed at eradicating HIV from infected individuals have been unsuccessful thus far. However, regardless of the approach, there is growing agreement that virologic remission involving some degree of induced immunologic control may be a more attainable goal than achieving complete eradication.

Key References

The complete reference list is available online at Expert Consult.

12. Dalgleish AG, Beverley PCL, Clapham PR, et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature*. 1984;312:763–767.
13. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature*. 1984;312:767–768.
14. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by $CD8^+$ T cells. *Science*. 1995;270:1811–1815.
22. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*. 1996;86:367–377.
26. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med*. 2009;360:692–698.
29. Arthos J, Cicala C, Martinelli E, et al. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol*. 2008;9:301–309.
30. Byrareddy SN, Arthos J, Cicala C, et al. Sustained virologic control in SIV^+ macaques after antiretroviral and alpha4beta7 antibody therapy. *Science*. 2016;354:197–202.
32. Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory $CD4^+$ T cells depletes gut lamina propria $CD4^+$ T cells. *Nature*. 2005;434:1148–1152.
33. Mattapallil JJ, Douek DC, Hill B, et al. Massive infection and loss of memory $CD4^+$ T cells in multiple tissues during acute SIV infection. *Nature*. 2005;434:1093–1097.
42. Hladik F, Sakchalathorn P, Ballweber L, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity*. 2007;26:257–270.
46. Ribeiro CM, Sarrami-Forooshani R, Setiawan LC, et al. Receptor usage dictates HIV-1 restriction by human TRIM5alpha in dendritic cell subsets. *Nature*. 2016;540:448–452.
47. Burton DR, Mascola JR. Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat Immunol*. 2015;16:571–576.
74. Nishimura Y, Gautam R, Chun TW, et al. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature*. 2017;543:559–563.
77. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med*. 2009;361:2209–2220.
83. Migueles SA, Connors M. Success and failure of the cellular immune response against HIV-1. *Nat Immunol*. 2015;16:563–570.
94. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature*. 2011;473:523–527.
115. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of $CD8^+$ T cells is required for HIV-infected cell elimination associated with immune control. *Immunity*. 2008;29:1009–1021.
117. Hansen SG, Piatak M Jr, Ventura AB, et al. Immune clearance of highly pathogenic SIV infection. *Nature*. 2013;502:100–104.
125. Lane HC, Depper JM, Greene WC, et al. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome: evidence for a selective defect in soluble antigen recognition. *N Engl J Med*. 1985;313:79–84.
136. Fellay J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science*. 2007;317:944–947.
154. Chun TW, Moir S, Fauci AS. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat Immunol*. 2015;16:584–589.
159. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA*. 1997;94:13193–13197.
160. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997;278:1295–1300.
161. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291–1295.
162. Zack JA, Arrigo SJ, Weitsman SR, et al. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*. 1990;61:213–222.
166. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature*. 1993;362:359–362.
167. Cavert W, Notermans DW, Staskus K, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. *Science*. 1997;276:960–964.
173. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature*. 1993;362:355–359.
181. Saharia KK, Koup RA. T cell susceptibility to HIV influences outcome of opportunistic infections. *Cell*. 2013;155:505–514.
192. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific $CD4^+$ T cells. *Nature*. 2002;417:95–98.
195. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997;387:183–188.
199. Doitsh G, Galloway NL, Geng X, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*. 2014;505:509–514.
200. Barouch DH, Ghneim K, Bosche WJ, et al. Rapid Inflammasome Activation following Mucosal SIV Infection of Rhesus Monkeys. *Cell*. 2016;165:656–667.
206. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*. 1995;373:123–126.
207. Mohri H, Perelson AS, Tung K, et al. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J Exp Med*. 2001;194:1277–1287.
208. Kovacs JA, Lempicki RA, Sidorov IA, et al. Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *J Exp Med*. 2001;194:1731–1741.
214. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature*. 1998;396:690–695.
216. Le T, Wright EJ, Smith DM, et al. Enhanced $CD4^+$ T-cell recovery with earlier HIV-1 antiretroviral therapy. *N Engl J Med*. 2013;368:218–230.
217. Group ISS, Lundgren JD, Babiker AG, et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection. *N Engl J Med*. 2015;373:795–807.
222. Champagne P, Ogg GS, King AS, et al. Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature*. 2001;410:106–111.
228. Lane HC, Masur H, Edgard LC, et al. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N Engl J Med*. 1983;309:453.
231. Morris L, Binley JM, Clas BA, et al. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J Exp Med*. 1998;188:233–245.
234. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med*. 2008;205:1797–1805.
240. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med*. 2012;366:1275–1286.
245. Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. *Science*. 1997;276:1857–1861.
248. Granelli-Piperno A, Moser B, Pope M, et al. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J Exp Med*. 1996;184:2433–2438.
250. Sandler NG, Bosinger SE, Estes JD, et al. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature*. 2014;511:601–605.
258. Cohen MS, Shaw GM, McMichael AJ, et al. Acute HIV-1 infection. *N Engl J Med*. 2011;364:1943–1954.
259. Wherry EJ. T cell exhaustion. *Nat Immunol*. 2011;12:492–499.
260. Beyer M, Abdullah Z, Chemnitz JM, et al. Tumor-necrosis factor impairs $CD4(^+)$ T cell-mediated immunological control in chronic viral infection. *Nat Immunol*. 2016;17:593–603.

References

1. Palmer LD, Weng N, Levine BL, et al. Telomere length, telomerase activity, and replicative potential in HIV infection: analysis of CD4+ and CD8+ T cells from HIV-discordant monozygotic twins. *J Exp Med*. 1997;185:1381–1386.
2. Castro-Nallar E, Perez-Losada M, Burton GF, et al. The evolution of HIV: inferences using phylogenetics. *Mol Phylogenet Evol*. 2012;62:777–792.
3. Lederman MM, Funderburg NT, Sekaly RP, et al. Residual immune dysregulation syndrome in treated HIV infection. *Adv Immunol*. 2013;119:51–83.
4. Paiardini M, Muller-Trutwin M. HIV-associated chronic immune activation. *Immunol Rev*. 2013;254:78–101.
5. Ripa M, Chiappetta S, Tambussi G. Immunosenescence and hurdles in the clinical management of older HIV-patients. *Virulence*. 2017;8:508–528.
6. Ferrari G, Pollara J, Tomaras GD, et al. Humoral and innate antiviral immunity as tools to clear persistent HIV infection. *J Infect Dis*. 2017;215(suppl_3):S152–S159.
7. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol*. 2017.
8. Pantaleo G, Levy Y. Therapeutic vaccines and immunological intervention in HIV infection: a paradigm change. *Curr Opin HIV AIDS*. 2016;11:576–584.
9. Deeks SG, Lewin SR, Ross AL, et al. International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nat Med*. 2016;22:839–850.
10. Jones RB, Walker BD. HIV-specific CD8(+) T cells and HIV eradication. *J Clin Invest*. 2016;126:455–463.
11. Perreau M, Banga R, Pantaleo G. Targeted immune Interventions for an HIV-1 cure. *Trends Mol Med*. 2017;23:945–961.
12. Dalgleish AG, Beverley PCL, Clapham PR, et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature*. 1984;312:763–767.
13. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature*. 1984;312:767–768.
14. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science*. 1995;270:1811–1815.
15. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science*. 1996;272:872–877.
16. Paxton WA, Martin SR, Tse D, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nat Med*. 1996;2:412–417.
17. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature*. 1996;381:661–666.
18. Dragic T, Litwin V, Allaway GP, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC- CKR-5. *Nature*. 1996;381:667–673.
19. Alkhatib G, Combadiere C, Broder CC, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science*. 1996;272:1955–1958.
20. Choe H, Farzan M, Sun Y, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell*. 1996;85:1135–1148.
21. Doranz BJ, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta- chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell*. 1996;85:1149–1158.
22. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*. 1996;86:367–377.
23. Samson M, Libert F, Doranz BJ, et al. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature*. 1996;382:722–725.
24. Naranbhai V, Carrington M. Host genetic variation and HIV disease: from mapping to mechanism. *Immunogenetics*. 2017;69:489–498.
25. Ahuja SK, Kulkarni H, Catano G, et al. CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1-infected individuals. *Nat Med*. 2008;14:413–420.
26. Hutter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med*. 2009;360:692–698.
27. Hertoghs N, Geijtenbeek TB, Ribeiro CM. Interplay between HIV-1 innate sensing and restriction in mucosal dendritic cells: balancing defense and viral transmission. *Curr Opin Virol*. 2017;22:112–119.
28. Law KM, Satija N, Esposito AM, et al. Cell-to-Cell spread of HIV and viral pathogenesis. *Adv Virus Res*. 2016;95:43–85.
29. Arthos J, Cicala C, Martinelli E, et al. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol*. 2008;9:301–309.
30. Byrareddy SN, Arthos J, Cicala C, et al. Sustained virologic control in SIV+ macaques after antiretroviral and alpha4beta7 antibody therapy. *Science*. 2016;354:197–202.
31. Shaw GM, Hunter E. HIV transmission. *Cold Spring Harb Perspect Med*. 2012;2.
32. Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature*. 2005;434:1148–1152.
33. Mattapallil JJ, Douek DC, Hill B, et al. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature*. 2005;434:1093–1097.
34. Brenchley JM, Schacker TW, Ruff LE, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*. 2004;200:749–759.
35. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med*. 2004;200:761–770.
36. Geldmacher C, Koup RA. Pathogen-specific T cell depletion and reactivation of opportunistic pathogens in HIV infection. *Trends Immunol*. 2012;33:207–214.
37. Spina CA, Kwoh TJ, Chowers MY, et al. The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. *J Exp Med*. 1994;179:115–123.
38. Fackler OT, Murooka TT, Imle A, et al. Adding new dimensions: towards an integrative understanding of HIV-1 spread. *Nat Rev Microbiol*. 2014;12:563–574.
39. Burgener A, McGowan I, Klatt NR. HIV and mucosal barrier interactions: consequences for transmission and pathogenesis. *Curr Opin Immunol*. 2015;36:22–30.
40. Ahmed Z, Kawamura T, Shimada S, et al. The role of human dendritic cells in HIV-1 infection. *J Invest Dermatol*. 2015;135:1225–1233.
41. Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. *Annu Rev Med*. 2011;62:127–139.
42. Hladik F, Sakchalathorn P, Ballweber L, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity*. 2007;26:257–270.
43. Hu Q, Frank I, Williams V, et al. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J Exp Med*. 2004;199:1065–1075.
44. Tobian AA, Kacker S, Quinn TC. Male circumcision: a globally relevant but under-utilized method for the prevention of HIV and other sexually transmitted infections. *Annu Rev Med*. 2014;65:293–306.
45. Malik T, Chauhan G, Rath G, et al. "Fusion and binding inhibition" key target for HIV-1 treatment and pre-exposure prophylaxis: targets, drug delivery and nanotechnology approaches. *Drug Deliv*. 2017;24:608–621.
46. Ribeiro CM, Sarrami-Forooshani R, Setiawan LC, et al. Receptor usage dictates HIV-1 restriction by human TRIM5alpha in dendritic cell subsets. *Nature*. 2016;540:448–452.
47. Burton DR, Mascola JR. Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat Immunol*. 2015;16:571–576.
48. Forthal DN, Moog C. Fc receptor-mediated antiviral antibodies. *Curr Opin HIV AIDS*. 2009;4:388–393.
49. Wei X, Decker JM, Wang S, et al. Antibody neutralization and escape by HIV-1. *Nature*. 2003;422:307–312.
50. Taylor BS, Hammer SM. The challenge of HIV-1 subtype diversity. *N Engl J Med*. 2008;359:1965–1966.
51. Buonaguro L, Tornesello ML, Buonaguro FM. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. *J Virol*. 2007;81:10209–10219.
52. Julien JP, Lee PS, Wilson IA. Structural insights into key sites of vulnerability on HIV-1 Env and influenza HA. *Immunol Rev*. 2012;250:180–198.
53. Hraber P, Seaman MS, Bailer RT, et al. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. *AIDS*. 2014;28:163–169.
54. Tomaras GD, Binley JM, Gray ES, et al. Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1-infected individuals. *J Virol*. 2011;85:11502–11519.
55. Binley JM, Lybarger EA, Crooks ET, et al. Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. *J Virol*. 2008;82:11651–11668.
56. Doria-Rose NA. HIV neutralizing antibodies: clinical correlates and implications for vaccines. *J Infect Dis*. 2010;201:981–983.
57. Mikell I, Sather DN, Kalams SA, et al. Characteristics of the earliest cross-neutralizing antibody response to HIV-1. *PLoS Pathog*. 2011;7:e1001251.
58. Gray ES, Madiga MC, Hermanus T, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. *J Virol*. 2011;85:4828–4840.
59. Liao HX, Chen X, Munshaw S, et al. Initial antibodies binding to HIV-1 gp41 in acutely infected subjects are polyreactive and highly mutated. *J Exp Med*. 2011;208:2237–2249.
60. Bonsignori M, Alam SM, Liao HX, et al. HIV-1 antibodies from infection and vaccination: insights for guiding vaccine design. *Trends Microbiol*. 2012;20:532–539.
61. Blish CA, Dogan OC, Derby NR, et al. Human immunodeficiency virus type 1 superinfection occurs despite relatively robust neutralizing antibody responses. *J Virol*. 2008;82:12094–12103.
62. Richman DD, Wrin T, Little SJ, et al. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci USA*. 2003;100:4144–4149.
63. Doria-Rose NA, Klein RM, Daniels MG, et al. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. *J Virol*. 2010;84:1631–1636.
64. Sather DN, Armann J, Ching LK, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J Virol*. 2009;83:757–769.
65. Walker LM, Simek MD, Priddy F, et al. A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. *PLoS Pathog*. 2010;6:e1001028.
66. Simek MD, Rida W, Priddy FH, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol*. 2009;83:7337–7348.
67. Moore PL, Gray ES, Sheward D, et al. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. *J Virol*. 2011;85:3128–3141.
68. Gray ES, Taylor N, Wycuff D, et al. Antibody specificities associated with neutralization breadth in plasma from human immunodeficiency virus type 1 subtype C-infected blood donors. *J Virol*. 2009;83:8925–8937.
69. Tiller T, Meffre E, Yurasov S, et al. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J Immunol Methods*. 2008;329:112–124.
70. Scheid JF, Mouquet H, Feldhahn N, et al. A method for identification of HIV gp140 binding memory B cells in human blood. *J Immunol Methods*. 2009;343:65–67.
71. Liao HX, Lynch R, Zhou T, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature*. 2013;496:469–476.
72. Doria-Rose NA, Schramm CA, Gorman J, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature*. 2014;509:55–62.
73. Moore PL, Gray ES, Wibmer CK, et al. Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. *Nat Med*. 2012;18:1688–1692.
74. Nishimura Y, Gautam R, Chun TW, et al. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature*. 2017;543:559–563.
75. Barouch DH, Whitney JB, Moldt B, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature*. 2013;503:224–228.
76. Excler JL, Ake J, Robb ML, et al. Nonneutralizing functional antibodies: a new "old" paradigm for HIV vaccines. *Clin Vaccine Immunol*. 2014;21:1023–1036.
77. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med*. 2009;361:2209–2220.
78. Morris L, Mkhize NN. Prospects for passive immunity to prevent HIV infection. *PLoS Med*. 2017;14:e1002436.
79. Huang Y, Yu J, Lanzi A, et al. Engineered bispecific antibodies with exquisite HIV-1-neutralizing activity. *Cell*. 2016;165:1621–1631.
80. Xu L, Pegu A, Rao E, et al. Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques. *Science*. 2017;358:85–90.

81. Ko SY, Pegu A, Rudicell RS, et al. Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature*. 2014;514:642–645.
82. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune response with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J Virol*. 1994;68:4650–4655.
83. Migueles SA, Connors M. Success and failure of the cellular immune response against HIV-1. *Nat Immunol*. 2015;16:563–570.
84. Reimann KA, Tenner-Racz K, Racz P. Immunopathogenic events in acute infection of Rhesus monkeys with simian immunodeficiency virus of Macaques. *J Virol*. 1994;68:2362–2370.
85. Ndhlovu ZM, Kamya P, Mewalal N, et al. Magnitude and kinetics of CD8$^+$ T cell activation during hyperacute HIV infection impact viral set point. *Immunity*. 2015;43:591–604.
86. Flores-Villanueva PO, Yunis EJ, Delgado JC, et al. Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. *Proc Natl Acad Sci USA*. 2001;98:5140–5145.
87. Goulder PJ, Bunce M, Krausa P, et al. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. *AIDS Res Hum Retroviruses*. 1996;12:1691–1698.
88. Kaslow RA, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat Med*. 1996;2:405–411.
89. Migueles SA, Sabbaghian MS, Shupert WL, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci USA*. 2000;97:2709–2714.
90. Chikata T, Van Tran G, Murakoshi H, et al. HLA class I-mediated HIV-1 control in Vietnamese infected with HIV-1 subtype A/E. *J Virol*. 2017.
91. Friedrich TC, Valentine LE, Yant LJ, et al. Subdominant CD8$^+$ T-cell responses are involved in durable control of AIDS virus replication. *J Virol*. 2007;81:3465–3476.
92. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8$^+$ lymphocytes. *Science*. 1999;283:857–860.
93. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med*. 1999;189:991–998.
94. Hansen SG, Ford JC, Lewis MS, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature*. 2011;473:523–527.
95. Goulder PJ, Walker BD. HIV and HLA class I: an evolving relationship. *Immunity*. 2012;37:426–440.
96. Migueles SA, Laborico AC, Imamichi H, et al. The differential ability of HLA B*5701$^+$ long-term nonprogressors and progressors to restrict human immunodeficiency virus replication is not caused by loss of recognition of autologous viral gag sequences. *J Virol*. 2003;77:6889–6898.
97. Draenert R, Verrill CL, Tang Y, et al. Persistent recognition of autologous virus by high-avidity CD8 T cells in chronic, progressive human immunodeficiency virus type 1 infection. *J Virol*. 2004;78:630–641.
98. Brumme ZL, Tao I, Szeto S, et al. Human leukocyte antigen-specific polymorphisms in HIV-1 Gag and their association with viral load in chronic untreated infection. *AIDS*. 2008;22:1277–1286.
99. Salgado M, Swanson MD, Pohlmeyer CW, et al. HLA-B*57 elite suppressor and chronic progressor HIV-1 isolates replicate vigorously and cause CD4$^+$ T cell depletion in humanized BLT mice. *J Virol*. 2014;88:3340–3352.
100. Wonderlich ER, Leonard JA, Collins KL. HIV immune evasion disruption of antigen presentation by the HIV Nef protein. *Adv Virus Res*. 2011;80:103–127.
101. Cohen GB, Gandhi RT, Davis DM, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity*. 1999;10:661–671.
102. Pereyra F, Addo MM, Kaufmann DE, et al. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. *J Infect Dis*. 2008;197:563–571.
103. Gray CM, Lawrence J, Schapiro JM, et al. Frequency of Class I HLA-restricted anti-HIV CD8$^+$ T Cells in Individuals Receiving Highly Active Antiretroviral Therapy (HAART). *J Immunol*. 1999;162:1780–1788.
104. Cao J, McNevin J, Holte S, et al. Comprehensive analysis of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-secreting CD8$^+$ T cells in primary HIV-1 infection. *J Virol*. 2003;77:6867–6878.
105. Addo MM, Yu XG, Rathod A, et al. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. *J Virol*. 2003;77:2081–2092.
106. Altfeld M, Rosenberg ES, Shankarappa R, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med*. 2001;193:169–180.
107. Migueles SA, Mendoza D, Zimmerman MG, et al. CD8(+) T-cell Cytotoxic Capacity Associated with Human Immunodeficiency virus-1 Control Can Be Mediated through Various Epitopes and Human Leukocyte Antigen Types. *EBioMedicine*. 2015;2:46–58.
108. Trautmann L, Janbazian L, Chomont N, et al. Upregulation of PD-1 expression on HIV-specific CD8$^+$ T cells leads to reversible immune dysfunction. *Nat Med*. 2006;12:1198–1202.
109. Paiardini M, Cervasi B, Albrecht H, et al. Loss of CD127 expression defines an expansion of effector CD8$^+$ T cells in HIV-infected individuals. *J Immunol*. 2005;174:2900–2909.
110. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006;443:350–354.
111. Rehr M, Cahenzli J, Haas A, et al. Emergence of polyfunctional CD8$^+$ T cells after prolonged suppression of human immunodeficiency virus replication by antiretroviral therapy. *J Virol*. 2008;82:3391–3404.
112. Migueles SA, Weeks KA, Nou E, et al. Defective human immunodeficiency virus-specific CD8$^+$ T-cell polyfunctionality, proliferation, and cytotoxicity are not restored by antiretroviral therapy. *J Virol*. 2009;83:11876–11889.
113. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8$^+$ T cells. *Blood*. 2006;107:4781–4789.
114. Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8$^+$ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol*. 2002;3:1061–1068.
115. Migueles SA, Osborne CM, Royce C, et al. Lytic granule loading of CD8$^+$ T cells is required for HIV-infected cell elimination associated with immune control. *Immunity*. 2008;29:1009–1021.
116. Saez-Cirion A, Lacabaratz C, Lambotte O, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci USA*. 2007;104:6776–6781.
117. Hansen SG, Piatak M Jr, Ventura AB, et al. Immune clearance of highly pathogenic SIV infection. *Nature*. 2013;502:100–104.
118. Hansen SG, Wu HL, Burwitz BJ, et al. Broadly targeted CD8(+) T cell responses restricted by major histocompatibility complex E. *Science*. 2016;351:714–720.
119. Hansen SG, Sacha JB, Hughes CM, et al. Cytomegalovirus vectors violate CD8$^+$ T cell epitope recognition paradigms. *Science*. 2013;340:1237874.
120. Walker CM, Moody DJ, Stites DP, et al. CD8$^+$ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science*. 1986;234:1563–1566.
121. Levy JA. The search for the CD8$^+$ cell anti-HIV factor (CAF). *Trends Immunol*. 2003;24:628–632.
122. Chun TW, Justement JS, Moir S, et al. Suppression of HIV replication in the resting CD4$^+$ T cell reservoir by autologous CD8$^+$ T cells: implications for the development of therapeutic strategies. *Proc Natl Acad Sci USA*. 2001;98:253–258.
123. Yang OO, Kalams SA, Trocha A, et al. Suppression of human immunodeficiency virus type 1 replication by CD8$^+$ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol*. 1997;71:3120–3128.
124. Guzzo C, Fox J, Lin Y, et al. The CD8-derived chemokine XCL1/lymphotactin is a conformation-dependent, broad-spectrum inhibitor of HIV-1. *PLoS Pathog*. 2013;9:e1003852.
125. Lane HC, Depper JM, Greene WC, et al. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome: evidence for a selective defect in soluble antigen recognition. *N Engl J Med*. 1985;313:79–84.
126. Iyasere C, Tilton JC, Johnson AJ, et al. Diminished proliferation of human immunodeficiency virus-specific CD4$^+$ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. *J Virol*. 2003;77:10900–10909.
127. Valentine FT, Paolino A, Saito A, et al. Lymphocyte-proliferative responses to HIV antigens as a potential measure of immunological reconstitution in HIV disease. *AIDS Res Hum Retroviruses*. 1998;14(suppl 2):S161–S166.
128. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4$^+$ T cell responses associated with control of viremia. *Science*. 1997;278:1447–1450.
129. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4$^+$ T cells. *Nature*. 2002;417:95–98.
130. Palmer BE, Boritz E, Blyveis N, et al. Discordance between frequency of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-producing CD4(+) T cells and HIV-1- specific Lymphoproliferation in HIV-1-infected subjects with active viral replication. *J Virol*. 2002;76:5925–5936.
131. Betts MR, Ambrozak DR, Douek DC, et al. Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. *J Virol*. 2001;75:11983–11991.
132. Pitcher CJ, Quittner C, Peterson DM, et al. HIV-1-specific CD4$^+$ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. *Nat Med*. 1999;5:518–525.
133. Tilton JC, Luskin MR, Johnson AJ, et al. Changes in paracrine interleukin-2 requirement, CCR7 expression, frequency, and cytokine secretion of human immunodeficiency virus-specific CD4$^+$ T cells are a consequence of antigen load. *J Virol*. 2007;81:2713–2725.
134. Prentice HA, Porter TR, Price MA, et al. HLA-B*57 versus HLA-B*81 in HIV-1 Infection: Slow and Steady Wins the Race? *J Virol*. 2013;87:4043–4051.
135. Apps R, Qi Y, Carlson JM, et al. Influence of HLA-C expression level on HIV control. *Science*. 2013;340:87–91.
136. Fellay J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science*. 2007;317:944–947.
137. Carrington M, Martin MP, van Bergen J. KIR-HLA intercourse in HIV disease. *Trends Microbiol*. 2008;16:620–627.
138. O'Brien SJ, Moore JP. The effect of genetic variation in chemokines and their receptors on HIV transmission and progression to AIDS. *Immunol Rev*. 2000;177:99–111.
139. Pantaleo G, Menzo S, Vaccarezza M, et al. Studies in subjects with long-term nonprogressive human immunodeficiency virus infection. *N Engl J Med*. 1995;332:209–216.
140. Cao Y, Qin L, Zhang L, et al. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. *N Engl J Med*. 1995;332:201–208.
141. Lederman MM, Alter G, Daskalakis DC, et al. Determinants of protection among HIV-exposed seronegative persons: an overview. *J Infect Dis*. 2010;202(suppl 3):S333–S338.
142. Migueles SA, Connors M. Long-term nonprogressive disease among untreated HIV-infected individuals: clinical implications of understanding immune control of HIV. *JAMA*. 2010;304:194–201.
143. Bailey JR, O'Connell K, Yang HC, et al. Transmission of human immunodeficiency virus type 1 from a patient who developed AIDS to an elite suppressor. *J Virol*. 2008;82:7395–7410.
144. Buckheit RW 3rd, Allen TG, Alme A, et al. Host factors dictate control of viral replication in two HIV-1 controller/chronic progressor transmission pairs. *Nat Commun*. 2012;3:716.
145. Rachinger A, Navis M, van Assen S, et al. Recovery of viremic control after superinfection with pathogenic HIV type 1 in a long-term elite controller of HIV type 1 infection. *Clin Infect Dis*. 2008;47:e86–e89.
146. Loffredo JT, Maxwell J, Qi Y, et al. Mamu-B*08-positive macaques control simian immunodeficiency virus replication. *J Virol*. 2007;81:8827–8832.
147. Yant LJ, Friedrich TC, Johnson RC, et al. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. *J Virol*. 2006;80:5074–5077.
148. Freel SA, Lamoreaux L, Chattopadhyay PK, et al. Phenotypic and functional profile of HIV-inhibitory CD8 T cells elicited by natural infection and heterologous prime/boost vaccination. *J Virol*. 2010;84:4998–5006.
149. Spentzou A, Bergin P, Gill D, et al. Viral inhibition assay: a CD8 T cell neutralization assay for use in clinical trials of HIV-1 vaccine candidates. *J Infect Dis*. 2010;201:720–729.
150. Montefiori DC, Pantaleo G, Fink LM, et al. Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. *J Infect Dis*. 1996;173:60–67.
151. Learmont J, Tindall B, Evans L, et al. Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. *Lancet*. 1992;340:863–867.

152. Miura T, Brockman MA, Brumme CJ, et al. Genetic characterization of human immunodeficiency virus type 1 in elite controllers: lack of gross genetic defects or common amino acid changes. *J Virol*. 2008;82:8422–8430.
153. Davey RT Jr, Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc Natl Acad Sci USA*. 1999;96:15109–15114.
154. Chun TW, Moir S, Fauci AS. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat Immunol*. 2015;16:584–589.
155. Boritz EA, Douek DC. Perspectives on Human Immunodeficiency Virus (HIV) Cure: HIV Persistence in Tissue. *J Infect Dis*. 2017;215(suppl_3):S128–S133.
156. Henrich TJ, Deeks SG, Pillai SK. Measuring the size of the latent human immunodeficiency virus reservoir: the present and future of evaluating eradication strategies. *J Infect Dis*. 2017;215(suppl_3):S134–S141.
157. Barton K, Winckelmann A, Palmer S. HIV-1 reservoirs during suppressive therapy. *Trends Microbiol*. 2016;24:345–355.
158. Wong JK, Yukl SA. Tissue reservoirs of HIV. *Curr Opin HIV AIDS*. 2016;11:362–370.
159. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci USA*. 1997;94:13193–13197.
160. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997;278:1295–1300.
161. Wong JK, Hezareh M, Gunthard HF, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291–1295.
162. Zack JA, Arrigo SJ, Weitsman SR, et al. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*. 1990;61:213–222.
163. Bukrinsky MI, Stanwick TL, Dempsey MP, et al. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science*. 1991;18:423–427.
164. Chun TW, Engel D, Berrey MM, et al. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci USA*. 1998;95:8869–8873.
165. Buzon MJ, Sun H, Li C, et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. *Nat Med*. 2014;20:139–142.
166. Embretson J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature*. 1993;362:359–362.
167. Cavert W, Notermans DW, Staskus K, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. *Science*. 1997;276:960–964.
168. Dimopoulos Y, Moysi E, Petrovas C. The lymph node in HIV pathogenesis. *Curr HIV/AIDS Rep*. 2017.
169. Tomalka J, Ghneim K, Bhattacharyya S, et al. The sooner the better: innate immunity as a path toward the HIV cure. *Curr Opin Virol*. 2016;19:85–91.
170. Calantone N, Wu F, Klase Z, et al. Tissue myeloid cells in SIV-infected primates acquire viral DNA through phagocytosis of infected T cells. *Immunity*. 2014;41:493–502.
171. Deeks SG, Odorizzi PM, Sekaly RP. The interferon paradox: can inhibiting an antiviral mechanism advance an HIV cure? *J Clin Invest*. 2017;127:103–105.
172. Clayton KL, Garcia JV, Clements JE, et al. HIV infection of macrophages: implications for pathogenesis and cure. *Pathog Immun*. 2017;2:179–192.
173. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature*. 1993;362:355–359.
174. Zeng M, Smith AJ, Wietgrefe SW, et al. Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. *J Clin Invest*. 2011;121:998–1008.
175. Qi H. T follicular helper cells in space-time. *Nat Rev Immunol*. 2016;16:612–625.
176. Lindqvist M, van Lunzen J, Soghoian DZ, et al. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. *J Clin Invest*. 2012;122:3271–3280.
177. Perreau M, Savoye AL, De Crignis E, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med*. 2013;210:143–156.
178. Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol*. 2012;10:655–666.
179. Wilson EM, Sereti I. Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. *Immunol Rev*. 2013;254:343–354.
180. Vidya Vijayan KK, Karthigeyan KP, Tripathi SP, et al. Pathophysiology of CD4+ T-Cell depletion in HIV-1 and HIV-2 infections. *Front Immunol*. 2017;8:580.
181. Saharia KK, Koup RA. T cell susceptibility to HIV influences outcome of opportunistic infections. *Cell*. 2013;155:505–514.
182. Geldmacher C, Ngwenyama N, Schuetz A, et al. Preferential infection and depletion of *Mycobacterium tuberculosis*-specific CD4 T cells after HIV-1 infection. *J Exp Med*. 2010;207:2869–2881.
183. Zhang Q, Frange P, Blanche S, et al. Pathogenesis of infections in HIV-infected individuals: insights from primary immunodeficiencies. *Curr Opin Immunol*. 2017;48:122–133.
184. Klein RS, Harris CA, Small CB, et al. Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome. *N Engl J Med*. 1984;311:354–358.
185. Morou A, Palmer BE, Kaufmann DE. Distinctive features of CD4+ T cell dysfunction in chronic viral infections. *Curr Opin HIV AIDS*. 2014;9:446–451.
186. Gattinoni L, Speiser DE, Lichterfeld M, et al. T memory stem cells in health and disease. *Nat Med*. 2017;23:18–27.
187. Okoye AA, Picker LJ. CD4+T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunol Rev*. 2013;254:54–64.
188. Flynn JK, Gorry PR. Stem memory T cells (TSCM)–their role in cancer and HIV immunotherapies. *Clin Transl Immunology*. 2014;3:e20.
189. Younes SA, Yassine-Diab B, Dumont AR, et al. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. *J Exp Med*. 2003;198:1909–1922.
190. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. 2015;15:486–499.
191. Schnittman SM, Lane HC, Greenhouse J, et al. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc Natl Acad Sci USA*. 1990;87:6058–6062.
192. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*. 2002;417:95–98.
193. Appay V, Sauce D. Assessing immune aging in HIV-infected patients. *Virulence*. 2017;8:529–538.
194. Kolte L. Thymic function in HIV-infection. *Dan Med J*. 2013;60:B4622.
195. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997;387:183–188.
196. Doyle T, Goujon C, Malim MH. HIV-1 and interferons: who's interfering with whom? *Nat Rev Microbiol*. 2015;13:403–413.
197. Sampath R, Cummins NW, Badley AD. Casp8p41: the Protean Mediator of Death in CD4 T-cells that Replicate HIV. *J Cell Death*. 2016;9:9–17.
198. Doitsh G, Greene WC. Dissecting how CD4 T cells are lost during HIV infection. *Cell Host Microbe*. 2016;19:280–291.
199. Doitsh G, Galloway NL, Geng X, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*. 2014;505:509–514.
200. Barouch DH, Ghneim K, Bosche WJ, et al. Rapid inflammasome activation following mucosal SIV infection of rhesus monkeys. *Cell*. 2016;165:656–667.
201. Cummins NW, Badley AD. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. *Cell Death Dis*. 2010;1:e99.
202. Sauce D, Elbim C, Appay V. Monitoring cellular immune markers in HIV infection: from activation to exhaustion. *Curr Opin HIV AIDS*. 2013;8:125–131.
203. Cha L, Berry CM, Nolan D, et al. Interferon-alpha, immune activation and immune dysfunction in treated HIV infection. *Clin Transl Immunology*. 2014;3:e10.
204. Toe JG, Pellegrini M, Mak TW. Promoting immunity during chronic infection–the therapeutic potential of common gamma-chain cytokines. *Mol Immunol*. 2013;56:38–47.
205. Chomont N, El-Far M, Ancuta P, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009;15:893–900.
206. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature*. 1995;373:123–126.
207. Mohri H, Perelson AS, Tung K, et al. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J Exp Med*. 2001;194:1277–1287.
208. Kovacs JA, Lempicki RA, Sidorov IA, et al. Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *J Exp Med*. 2001;194:1731–1741.
209. De Boer RJ, Mohri H, Ho DD, et al. Turnover rates of B cells, T cells, and NK cells in simian immunodeficiency virus-infected and uninfected rhesus macaques. *J Immunol*. 2003;170:2479–2487.
210. Garcia-Tellez T, Huot N, Ploquin MJ, et al. Non-human primates in HIV research: achievements, limits and alternatives. *Infect Genet Evol*. 2016;46:324–332.
211. Freeman ML, Shive CL, Nguyen TP, et al. Cytokines and T-cell homeostasis in HIV infection. *J Infect Dis*. 2016;214(suppl 2):S51–S57.
212. Emu B, Moretto WJ, Hoh R, et al. Composition and function of T cell subpopulations are slow to change despite effective antiretroviral treatment of HIV disease. *PLoS ONE*. 2014;9:e85613.
213. Younas M, Psomas C, Reynes J, et al. Immune activation in the course of HIV-1 infection: causes, phenotypes and persistence under therapy. *HIV Med*. 2016;17:89–105.
214. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature*. 1998;396:690–695.
215. Corbeau P, Reynes J. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood*. 2011;117:5582–5590.
216. Le T, Wright EJ, Smith DM, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. *N Engl J Med*. 2013;368:218–230.
217. Group ISS, Lundgren JD, Babiker AG, et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection. *N Engl J Med*. 2015;373:795–807.
218. Zeng M, Haase AT, Schacker TW. Lymphoid tissue structure and HIV-1 infection: life or death for T cells. *Trends Immunol*. 2012;33:306–314.
219. Zeng M, Southern PJ, Reilly CS, et al. Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. *PLoS Pathog*. 2012;8:e1002437.
220. Mudd JC, Lederman MM. CD8 T cell persistence in treated HIV infection. *Curr Opin HIV AIDS*. 2014;9:500–505.
221. Lu W, Mehraj V, Vyboh K, et al. CD4:CD8 ratio as a frontier marker for clinical outcome, immune dysfunction and viral reservoir size in virologically suppressed HIV-positive patients. *J Int AIDS Soc*. 2015;18:20052.
222. Champagne P, Ogg GS, King AS, et al. Skewed maturation of memory HIV-specific CD8 T lymphocytes. *Nature*. 2001;410:106–111.
223. Okoye IS, Houghton M, Tyrrell L, et al. Coinhibitory receptor expression and immune checkpoint blockade: maintaining a balance in CD8+ T cell responses to chronic viral infections and cancer. *Front Immunol*. 2017;8:1215.
224. Quigley M, Pereyra F, Nilsson B, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat Med*. 2010;16:1147–1151.
225. Buggert M, Tauriainen J, Yamamoto T, et al. T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV infection. *PLoS Pathog*. 2014;10:e1004251.
226. Lifson JD, Haigwood NL. Lessons in nonhuman primate models for AIDS vaccine research: from minefields to milestones. *Cold Spring Harb Perspect Med*. 2012;2:a007310.
227. Moir S, Fauci AS. B cells in HIV infection and disease. *Nat Rev Immunol*. 2009;9:235–245.
228. Lane HC, Masur H, Edgard LC, et al. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N Engl J Med*. 1983;309:453.
229. Borrow P, Moody MA. Immunologic characteristics of HIV-infected individuals who make broadly neutralizing antibodies. *Immunol Rev*. 2017;275:62–78.
230. Moir S, Fauci AS. B-cell responses to HIV infection. *Immunol Rev*. 2017;275:33–48.
231. Morris L, Binley JM, Clas BA, et al. HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J Exp Med*. 1998;188:233–245.
232. Noto A, Pantaleo G. B-cell abnormalities and impact on antibody response in HIV infection. *Curr Opin HIV AIDS*. 2017;12:203–208.
233. Lundstrom W, Fewkes NM, Mackall CL. IL-7 in human health and disease. *Semin Immunol*. 2012;24:218–224.
234. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med*. 2008;205:1797–1805.
235. Goncalves PH, Montezuma-Rusca JM, Yarchoan R, et al. Cancer prevention in HIV-infected populations. *Semin Oncol*. 2016;43:173–188.
236. Hu Z, Luo Z, Wan Z, et al. HIV-associated memory B cell perturbations. *Vaccine*. 2015;33:2524–2529.

237. Jost S, Altfeld M. Control of human viral infections by natural killer cells. *Annu Rev Immunol*. 2013.
238. Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56-/CD16^{+} natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc Natl Acad Sci USA*. 2005;102:2886–2891.
239. Scully E, Alter G. NK cells in HIV disease. *Curr HIV/AIDS Rep*. 2016;13:85–94.
240. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med*. 2012;366:1275–1286.
241. Carrington M, Alter G. Innate immune control of HIV. *Cold Spring Harb Perspect Med*. 2012;2:a007070.
242. Alter G, Heckerman D, Schneidewind A, et al. HIV-1 adaptation to NK-cell-mediated immune pressure. *Nature*. 2011;476:96–100.
243. Sattentau QJ, Stevenson M. Macrophages and HIV-1: an unhealthy constellation. *Cell Host Microbe*. 2016;19:304–310.
244. DiNapoli SR, Hirsch VM, Brenchley JM. Macrophages in progressive human immunodeficiency virus/simian immunodeficiency virus infections. *J Virol*. 2016;90:7596–7606.
245. Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. *Science*. 1997;276:1857–1861.
246. Petrovas C, Ferrando-Martinez S, Gerner MY, et al. Follicular CD8 T cells accumulate in HIV infection and can kill infected cells in vitro via bispecific antibodies. *Sci Transl Med*. 2017;9.
247. Hong S, Banks WA. Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications. *Brain Behav Immun*. 2015;45:1–12.
248. Granelli-Piperno A, Moser B, Pope M, et al. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J Exp Med*. 1996;184:2433–2438.
249. Shimauchi T, Piguet V. DC-T cell virological synapses and the skin: novel perspectives in dermatology. *Exp Dermatol*. 2015;24:1–4.
250. Sandler NG, Bosinger SE, Estes JD, et al. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature*. 2014;511:601–605.
251. O'Brien M, Manches O, Bhardwaj N. Plasmacytoid dendritic cells in HIV infection. *Adv Exp Med Biol*. 2013;762:71–107.
252. Miller E, Bhardwaj N. Dendritic cell dysregulation during HIV-1 infection. *Immunol Rev*. 2013;254:170–189.
253. Favre D, Mold J, Hunt PW, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. *Sci Transl Med*. 2010;2:32ra6.
254. Ipp H, Zemlin AE, Erasmus RT, et al. Role of inflammation in HIV-1 disease progression and prognosis. *Crit Rev Clin Lab Sci*. 2014;51:98–111.
255. Lucas S, Nelson AM. HIV and the spectrum of human disease. *J Pathol*. 2015;235:229–241.
256. Said EA, Dupuy FP, Trautmann L, et al. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4^{+} T cell activation during HIV infection. *Nat Med*. 2010;16:452–459.
257. Poli G, Fauci AS. The effect of cytokines and pharmacologic agents on chronic HIV infection. *AIDS Res Hum Retroviruses*. 1992;8:191–197.
258. Cohen MS, Shaw GM, McMichael AJ, et al. Acute HIV-1 Infection. *N Engl J Med*. 2011;364:1943–1954.
259. Wherry EJ. T cell exhaustion. *Nat Immunol*. 2011;12:492–499.
260. Beyer M, Abdullah Z, Chemnitz JM, et al. Tumor-necrosis factor impairs CD4(+) T cell-mediated immunological control in chronic viral infection. *Nat Immunol*. 2016;17:593–603.
261. Wacleche VS, Landay A, Routy JP, et al. The Th17 lineage: from barrier surfaces homeostasis to autoimmunity, cancer, and HIV-1 pathogenesis. *Viruses*. 2017;9.
262. Tincati C, Douek DC, Marchetti G. Gut barrier structure, mucosal immunity and intestinal microbiota in the pathogenesis and treatment of HIV infection. *AIDS Res Ther*. 2016;13:19.
263. Rook AH, Masur H, Lane HC, et al. Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immune deficiency syndrome. *J Clin Invest*. 1983;72:398–403.
264. Group I-ES, Committee SS, Abrams D, et al. Interleukin-2 therapy in patients with HIV infection. *N Engl J Med*. 2009;361:1548–1559.
265. Napolitano LA, Grant RM, Deeks SG, et al. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat Med*. 2001;7:73–79.
266. Vandergeeten C, Fromentin R, Chomont N. The role of cytokines in the establishment, persistence and eradication of the HIV reservoir. *Cytokine Growth Factor Rev*. 2012;23:143–149.

122 General Clinical Manifestations of Human Immunodeficiency Virus Infection (Including Acute Retroviral Syndrome and Oral, Cutaneous, Renal, Ocular, Metabolic, and Cardiac Diseases)

Timothy R. Sterling and Richard E. Chaisson

SHORT VIEW SUMMARY

ACUTE RETROVIRAL SYNDROME
- This syndrome is the initial manifestation of human immunodeficiency virus (HIV) infection.
- One-half to two-thirds of patients present with an acute mononucleosis-like illness (see Table 122.1), often with a truncal exanthem.
- Symptoms generally resolve within 10 to 15 days.

PERSISTENT GENERALIZED LYMPHADENOPATHY
- This disorder occurs in 50% to 70% of people with HIV infection and usually affects the cervical, submandibular, occipital, and axillary regions.
- In patients treated with antiretroviral therapy (ART), previously involuted nodes may enlarge.

METABOLIC AND ENDOCRINE ABNORMALITIES
- Hypogonadism, adrenal insufficiency, constitutional wasting, lipid abnormalities, insulin resistance, lipodystrophy, and osteoporosis have been reported.

ORAL DISEASE
- Oral candidiasis, oral hairy leukoplakia, gingivitis, and periodontitis may occur.

CUTANEOUS DISEASE
- Dermatologic consequences include cutaneous infections (herpes simplex virus, varicella-zoster virus, molluscum contagiosum, scabies, bacillary angiomatosis, and Kaposi sarcoma).

RENAL DISEASE
- Multiple causes of renal disease may affect patients with HIV infection as well as a specific HIV-related nephropathy, which includes proteinuria, mildly elevated serum creatinine concentration reflecting glomerular injury, mesangial proliferation, and tubular degeneration.

CARDIAC DISEASE
- Accelerated atherosclerosis with myocardial infarction is seen in patients with HIV infection who have been treated with ART and appears to be associated with elevations in proinflammatory cytokines and prothrombotic markers.
- Myocarditis and pericarditis are also found.

IMMUNE RECONSTITUTION SYNDROMES
- Immune reconstitution syndromes are seen after ART and can involve *Mycobacterium tuberculosis, Mycobacterium avium* complex, *Cryptococcus neoformans,* cytomegalovirus, hepatitis B, hepatitis C, lymphoma, and other opportunistic diseases.

Human immunodeficiency virus (HIV) infection results in a wide range of clinical consequences from asymptomatic infection despite active viral replication to severe immunodeficiency with life-threatening opportunistic disease. In people infected with HIV, ongoing viral replication produces a steady decline in and eventual ablation of cell-mediated immunity as well as marked immune activation and inflammation, all of which give rise to diverse manifestations of opportunistic disease. Acquired immunodeficiency syndrome (AIDS), in which the infected host can no longer control opportunistic organisms or malignancies that rarely cause illness in immunocompetent individuals, is the most advanced stage of this illness. Major advances in treatment with antiretroviral drugs have dramatically altered the clinical course of HIV infection and converted what was once a highly fatal disease into a manageable chronic condition. For individuals with access to antiretroviral therapy (ART), median life expectancy following infection has increased from 10 to 12 years to 25 to 50 years, depending on age at infection.[1] Despite these impressive improvements in survival, treated chronic HIV infection is associated with immune activation and chronic inflammation that can result in damage to a number of organ systems. Current research is focused on finding a cure (or functional cure) for HIV infection and treating its inflammatory complications to prevent cardiovascular, renal, endocrinologic, and neurologic consequences.

HISTORY

Disease caused by HIV was first described in late 1980 and early 1981 with outbreaks of Kaposi sarcoma and opportunistic infections due to *Pneumocystis jirovecii,* herpes simplex, and cytomegalovirus (CMV) in young men who have sex with men (MSM).[2–7] In 1982 the US Centers for Disease Control and Prevention (CDC) developed a case definition based on clinical, immunologic, and epidemiologic features for what was termed the *acquired immunodeficiency syndrome.*[8] Soon after the initial case reports of AIDS, additional cases were reported in heterosexual injection drug users, immigrants from Haiti,[8–12] people with hemophilia, recipients of blood transfusions, and Africans.[13,14] As the groups of people at risk for AIDS expanded, clinicians noted an increasing spectrum of clinical manifestations of AIDS-associated immunodeficiency such as generalized lymphadenopathy, idiopathic thrombocytopenia, oral candidiasis, and a constitutional wasting syndrome.[15–18] The now archaic term *AIDS-related complex* was coined to describe the signs and symptoms of immunodeficiency recognized with increasing frequency in people at risk for AIDS.[19,20] After HIV was first described in 1983–84,[21–23] serologic tests to identify people infected with HIV were developed that allowed large serologic surveys of at-risk populations to estimate the number of individuals infected with the virus and to delineate the spectrum of HIV-associated diseases.

Over almost 40 years, case definitions and classifications of HIV disease have evolved to reflect a growing understanding of pathogenesis, natural history, and therapeutics. Many of the common manifestations from the earliest days of the epidemic are rarely seen in high-income countries and are becoming less frequent in low-income and middle-income countries.

NATURAL HISTORY OF HIV INFECTION

The clinical spectrum of untreated HIV infection includes primary infection (acute retroviral syndrome) (Table 122.1), asymptomatic infection, early symptomatic infection, and advanced immunodeficiency with opportunistic complications and, ultimately, death. Fig. 122.1 shows a schematic diagram of the key immunologic, viral, and clinical features of HIV infection in untreated individuals. Untreated HIV infection is characterized by active viral replication that results in progressive immunodeficiency and serious clinical consequences. Although many patients may be unaware of their infection, HIV is usually not virologically latent, and chronic viremia, inflammation, and progressive immunodeficiency result in significant impairment and death. Viral load or viremia is monitored by measurement of HIV-1 RNA in plasma, and immunologic status is reflected by the absolute number of $CD4^+$ lymphocytes or the proportion of lymphocytes that express $CD4^+$. Primary HIV infection is characterized by a high concentration of HIV-1 RNA in plasma and suppression of the $CD4^+$ cell count. Plasma viremia declines precipitously with antibody seroconversion and the development of an anti-HIV immune response, usually reaching a steady-state level or setpoint within 6 to 12 months.[24,25] In most untreated asymptomatic patients, the $CD4^+$ count declines gradually over several years. The slope of decline is a function of the plasma viral load. Plasma viremia increases, accompanied by a more rapid decline in $CD4^+$ count, before the onset of symptomatic disease. As the viral load increases and the $CD4^+$ count falls, the risk for opportunistic infections, malignancies, wasting, neurologic complications, and death increases substantially.

There is considerable variation in the progression of HIV disease, with some individuals progressing from infection to AIDS in less than 5 years[26] and so-called long-term nonprogressors remaining asymptomatic without treatment or evidence of immunologic decline for many years.[26,27] Long-term nonprogressors appear to fall into at least two categories. Most have detectable viremia but maintain $CD4^+$ counts that provide adequate protection from the development of opportunistic disease. These individuals generally have gradual loss of $CD4^+$ lymphocytes, however, and eventually progress to advanced immunodeficiency. A much smaller group of individuals (<1%) are so-called elite controllers, who have undetectable HIV viral loads and maintain normal $CD4^+$ counts.[28] This group is of considerable interest because of their ability to remain aviremic. However, studies have shown that such individuals have increased immune activation, have ongoing viral replication in $CD4^+$ cells, and experience higher rates of hospitalization for cardiovascular conditions and other non–AIDS-related illnesses than individuals treated with ART.[29–31] Nonetheless, understanding the mechanisms whereby these patients control HIV is of potential value for the development of HIV vaccines and functional cures for HIV.

Before the availability of effective ART, the rate of progression from initial HIV infection to AIDS among MSM in San Francisco was 9.8 years.[32] Other studies estimated the period from infection to AIDS to be 7 years for transfusion recipients and 10 years for hemophiliacs and injection drug users.[33] In the absence of treatment, survival is short after diagnosis of clinically defined AIDS. Studies of survival of the first patients with AIDS in San Francisco and New York found a median survival of 9 to 12 months, with most patients dead within 2 years.[34,35] Patients with a diagnosis of an opportunistic infection had the most rapid mortality, whereas survival was significantly longer in patients with an initial diagnosis of Kaposi sarcoma. Subsequent studies revealed that survival after diagnosis of AIDS was directly related to the $CD4^+$ count at diagnosis. In most studies before the availability of effective ART, median survival after diagnosis of AIDS was estimated to be between 12 and 18 months.[36] The mean survival time after a $CD4^+$ count of 200 cells/mm^3 was 38 to 40 months.[37,38]

A number of laboratory tests have been correlated with progressive immunodeficiency, the development of AIDS, and mortality. Taken together, however, the $CD4^+$ lymphocyte count and plasma viral load are the best prognostic markers for subsequent disease course in an HIV-infected individual. The $CD4^+$ count, a specific test for cellular immunocompetence, is a sensitive predictor of the development of symptomatic HIV infection and AIDS in the near term because it reflects current immunologic capacity.[39–43] Conversely, the plasma viral load (HIV-1 RNA) is an extremely useful predictor of disease course over a more extended period and is strongly associated with the rate of subsequent $CD4^+$ count decline.[44–52] A more rapid decline in $CD4^+$ count, faster clinical progression, and decreased survival all are associated with a higher baseline viral load. In a study of HIV-infected MSM (homosexual or bisexual men) enrolled in the Multicenter AIDS Cohort Study, the risk for progression to AIDS and death was highly correlated with plasma viral load at study entry, independent of $CD4^+$ count.[47,48] Baseline plasma viral load was a stronger predictor of progression and mortality than $CD4^+$ count. In addition, the average annual decline in the $CD4^+$ count of HIV-infected men varied according to their initial viral load, decreasing by 36 $CD4^+$ cells per year in men with baseline

TABLE 122.1 Symptoms and Signs of Acute Retroviral Syndrome in 209 Patients

SYMPTOM OR SIGN	NO. WITH FINDING	FREQUENCY (%)
Fever	200	96
Adenopathy	154	74
Pharyngitis	146	70
Rash	146	70
Myalgia or arthralgia	112	54
Thrombocytopenia	94	45
Leukopenia	80	38
Diarrhea	67	32
Headache	66	32
Nausea, vomiting	56	27
Elevated aminotransferase levels[a]	38	21
Hepatosplenomegaly	30	14
Thrush	24	12
Neuropathy	13	6
Encephalopathy	12	6

[a]Based on 178 subjects.
Modified from Niu MT, Stein DS, Schnittman SM. Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment intervention in human and animal retrovirus infections. J Infect Dis. *1993;168:1490–1501.*

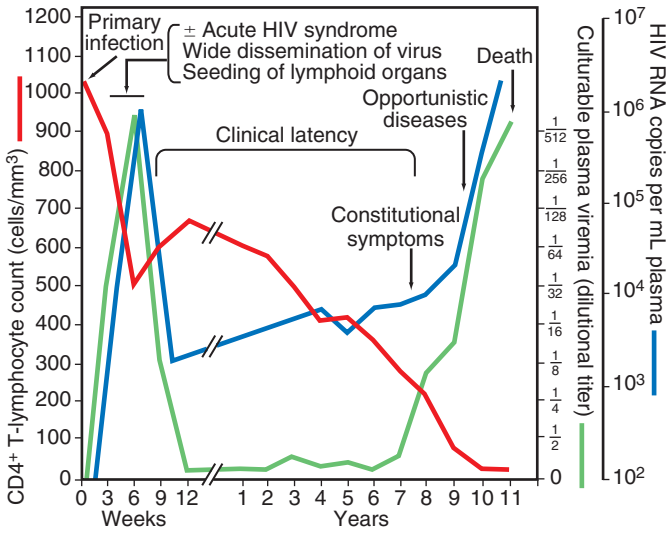

FIG. 122.1 Natural history of HIV infection in the absence of therapy in a hypothetical patient. *(From Fauci AS, Pantaleo G, Stanley S, et al. Immunopathogenic mechanisms of HIV infection.* Ann Intern Med. *1996;124:654–663.)*

TABLE 122.2 Probability of Developing AIDS in 1604 Men in the Multicenter AIDS Cohort Study

BASELINE VIRAL LOAD[C]	BASELINE CD4⁺ COUNT[D]	NO. STUDIED	NO. WITH AIDS	% AIDS AT 3 YEARS	% AIDS AT 6 YEARS	% AIDS AT 9 YEARS
<500	>750	66	3	0	1.7	3.6
	<750	56	13	3.7	9.6	22.3
501–3000	Any	257	90	2.0	16.6	35.4
3001–10,000	>750	93	39	3.2	14.2	59.7
	<750	300	179	8.1	37.7	62.4
10,001–30,000	>750	64	42	9.5	36.7	62.4
	351–750	259	194	16.1	54.9	76.3
	≤350	73	63	40.1	72.9	86.2
>30,000	>500	141	105	32.6	66.8	76.3
	351–500	121	111	47.9	77.7	94.4
	201–350	104	92	64.4	89.3	92.9
	<200	70	67	85.5	97.9	100

[a]1987 Centers for Disease Control and Prevention case definition.
[b]Based on baseline HIV branched-chain DNA viral load and CD4⁺ cell count.
[c]HIV RNA copies/mL of plasma by branched-chain DNA. Viral load determined by reverse-transcriptase polymerase chain reaction approximately twofold greater.
[d]CD4⁺ cells/mm³.
Data from Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4⁺ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med. *1997;126:946–954.*

HIV-1 RNA less than 500 copies/mL and by 77 CD4⁺ cells per year in men with baseline HIV-1 RNA higher than 30,000 copies/mL.[47] Using the viral load and CD4⁺ count together, however, provides the best prognostic estimate of subsequent clinical course (Table 122.2 and Fig. 122.2). Put in the context of HIV pathogenesis, the viral load measures the replicative rate of the infection and its destructive potential for the cellular immune system, and the CD4⁺ count gauges the extent of immune compromise and the present risk for opportunistic disease. Rather than initiate ART on all HIV-infected individuals as early as possible, guidelines for initiating ART have historically been based on CD4⁺ counts and plasma viral load due to ART toxicity with prolonged use. In addition, opportunistic infection prophylaxis has been based on the current CD4⁺ count, which determines the short-term risks for developing specific infections (e.g., *P. jirovecii* pneumonia or disseminated *Mycobacterium avium* complex infection) and death. Frequent monitoring of CD4⁺ counts was previously recommended for patients not on ART to determine when treatment was indicated and for patients on ART to assess their response to therapy. However, as studies have demonstrated the long-term value of treating all HIV-infected individuals regardless of CD4⁺ count, this is now recommended.[53] Use of plasma viral load to monitor treatment response is now preferred, with monitoring of CD4⁺ counts recommended at the start of treatment and periodically for individuals with initially low counts to determine when to discontinue opportunistic infection prophylaxis. No CD4⁺ cell monitoring is considered necessary for individuals with initial counts >500 cells/mm³.

The rate of progression of HIV infection in population-based studies varies depending on age, with older individuals generally having a more rapidly progressive course.[54–58] Patients who experience more severe symptoms during acute retroviral syndrome tend to have higher viral loads after seroconversion and progress more rapidly than patients who seroconvert without symptoms.[59] Women have approximately half-$\log_{10}$ lower HIV-1 RNA than men after seroconversion, but this difference diminishes with time from seroconversion.[60–62] Although HIV-1 RNA is an important predictor of subsequent disease progression in both women and men,[47,63,64] there is no sex difference in HIV disease progression, particularly when women and men have equal access to care.[65–67] There do not appear to be racial differences in HIV-1 RNA levels[68] or the natural history of HIV disease progression.[65,69] Geographic differences in the risk of infections that can occur at higher CD4⁺ counts such as tuberculosis, salmonellosis, and some bacterial pneumonias can result in shorter survival times for individuals in low-income and middle-income countries.

Other laboratory studies that predict the development of AIDS in populations of HIV-infected people include total lymphocyte count less than 1000 cells/mm³, total white blood cell count less than 4000 cells/mm³, and hematocrit less than 40 mL/dL.. Although these tests are readily available and inexpensive, they do not discriminate short-term risks for individuals as well as CD4⁺ counts and are generally not used for clinical management. Other markers of HIV disease progression that have been validated in clinical studies but are not widely used include the HIV p24 antigen, serum β_2-microglobulin, neopterin, acid-labile interferon-α, anti-p24 antibody, and soluble CD8. These so-called surrogate markers are measures of virus presence or host immune responses to HIV. These measures do not provide prognostic information independent of the viral load and have therefore been supplanted by quantitative plasma HIV-1 RNA monitoring in developed countries. Low-cost alternatives to flow cytometric quantification of CD4⁺ lymphocytes for application in resource-poor settings are now

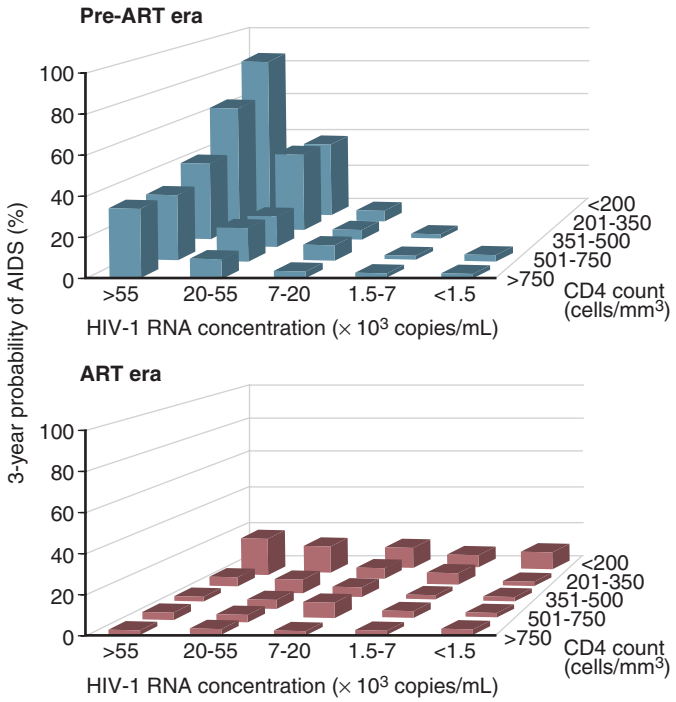

FIG. 122.2 Prognosis according to CD4⁺ cell count and viral load in the pre–antiretroviral therapy (ART) and ART eras. These data were from 12,574 adult patients starting ART with a combination of at least three drugs. Kaplan-Meier estimates of the probability of AIDS at 3 years are shown. *(From Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies.* Lancet. *2002;360:119–129.)*

available and include manual assays that use enzyme-linked immunosorbent assay or bead-based formats.[70]

CLASSIFICATION OF HIV INFECTION

In the early years of the HIV pandemic, surveillance case definitions for AIDS were necessary for tracking the growth of the epidemic; as awareness of the clinical spectrum of HIV infection grew, the number of conditions diagnostic of AIDS increased. In addition, staging systems for HIV were developed by the CDC, World Health Organization (WHO), Walter Reed Army Institute of Research, and others, incorporating clinical and laboratory features into discrete categories with differing prognoses. Distinguishing between HIV infection and AIDS was historically useful for epidemiologic purposes, but the difference is arbitrary and is less meaningful from a clinical perspective in an era of potent ART. At the present time, two classification systems are in wide use. The 2014 CDC Revised Surveillance Case Definition for HIV infection includes four stages based on laboratory testing, $CD4^+$ lymphocyte count and percentage, and clinical evidence including AIDS-defining conditions (Table 122.3).[71]

The CDC classification system for HIV infection recognizes the prognostic significance of the $CD4^+$ count in individuals with HIV infection, but there is considerable variation in risk for opportunistic complications and prognosis in individuals with $CD4^+$ counts <200 cells/mm^3. Individuals with $CD4^+$ counts <50 cells/mm^3, for example, are generally considered to have advanced HIV disease and are at much higher risk for the development of opportunistic infections such as CMV disease or disseminated *Mycobacterium avium* complex infection and death. The CDC classification system was initially developed at a time when the inevitable course of HIV infection was progression toward advanced immunodeficiency and death and when drug therapy was of limited and transient efficacy in stemming the course of the disease. According to the CDC classification system, HIV-infected individuals are classified on the basis of the most advanced stage that they have reached.[71] In the present era, patients treated with ART usually experience marked improvement in cellular immune function and may have a normal or near-normal life expectancy with dramatically lower risk for developing opportunistic disease than they had before receiving treatment.[72] There is no current mechanism for reclassifying patients on the basis of immunologic and clinical improvement resulting from ART, a situation that understandably curtails use of the CDC system.

The WHO classification system (Tables 122.4, 122.5, and 122.6) is used primarily in resource-limited countries. However, there are limitations that make it difficult for this staging system to be uniformly implemented. Many of the classifications require the diagnosis of opportunistic infections that cannot be readily confirmed in most resource-limited settings; clinical criteria for establishing presumptive or definitive diagnoses may be useful. Estimates of weight loss and other constitutional symptoms are also difficult in such settings. The CDC and WHO staging systems are compared in Table 122.7.

CLINICAL MANIFESTATIONS

HIV infection causes disease manifestations of three principal types: (1) an acute viral illness seen in the initial weeks of infection and associated with a high viral load and an intense host immune response; (2) immunologically mediated processes related to host responses to chronic viral infection in the absence of ART (e.g., lymphadenopathy, thrombocytopenia, HIV-related dementia) or persistent inflammation

TABLE 122.3 HIV Infection Staging Systems

STAGE	LABORATORY EVIDENCE[A]	CLINICAL EVIDENCE
Stage 0	Negative or indeterminate HIV test result within 180 days before first confirmed positive HIV test result of any type *or* Sequence of tests that demonstrate the presence of HIV-specific viral markers such as p24 antigen or nucleic acid (RNA or DNA) 0–180 days before or after antibody test that had a negative or indeterminate result	None required (but no AIDS-defining condition)
Stage 1	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of >500 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of ≥29	None required (but no AIDS-defining condition)
Stage 2	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of 14–28	None required (but no AIDS-defining condition)
Stage 3 (AIDS)	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of <200 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of <14[b]	*Or* documentation of AIDS-defining condition (with laboratory confirmation of HIV infection)[b]
Stage unknown[c]	Laboratory confirmation of HIV infection *and* no information on $CD4^+$ T-lymphocyte count or percentage	*And* no information on presence of AIDS-defining conditions

[a]The $CD4^+$ T-lymphocyte percentage is the percentage of total lymphocytes. If the $CD4^+$ T-lymphocyte count and percentage do not correspond to the same HIV infection stage, select the more severe stage.
[b]Documentation of an AIDS-defining condition supersedes a $CD4^+$ T-lymphocyte count of ≥200 cell/μL and a $CD4^+$ T-lymphocyte percentage of total lymphocytes of ≥14.
[c]Although cases with no information on $CD4^+$ T-lymphocyte count or percentage or on the presence of AIDS-defining conditions can be classified as stage unknown, every effort should be made to report $CD4^+$ T-lymphocyte counts or percentages and the presence of AIDS-defining conditions at the time of diagnosis. Additional $CD4^+$ T-lymphocyte counts or percentages and any identified AIDS-defining conditions can be reported as recommended.
Data from Selik RM, Mokotoff ED, Branson B, et al; Centers for Disease Control and Prevention. Revised surveillance case definitions for HIV infection—United States, 2014. MMWR Recomm Rep. *2014;63(RR03):1–10.*

TABLE 122.4 World Health Organization Clinical Staging of Established HIV Infection

HIV-ASSOCIATED SYMPTOMS	WHO CLINICAL STAGE
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe symptoms	4

WHO, World Health Organization.

TABLE 122.5 World Health Organization Immunologic Classification for Established HIV Infection

	AGE-RELATED CD4 VALUES			
HIV-ASSOCIATED IMMUNODEFICIENCY	<11 Months (% $CD4^+$)	12–35 Months (% $CD4^+$)	36–59 Months (% $CD4^+$)	>5 Years (Absolute No./mm^3 or % $CD4^+$)
None or not significant	>35	>30	>25	>500
Mild	30–35	25–30	20–25	350–500
Advanced	25–29	20–24	15–19	200–349
Severe	<25	<20	<15	<200 or <15%

TABLE 122.6 World Health Organization Clinical Staging of HIV/AIDS for Adults and Adolescents With Confirmed HIV Infection

Clinical Stage 1

Asymptomatic
Persistent generalized lymphadenopathy

Clinical Stage 2

Moderate unexplained weight loss (<10% of presumed or measured body weight)[a]
Recurrent respiratory tract infections (e.g., sinusitis, tonsillitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulceration
Papular pruritic eruptions
Seborrheic dermatitis
Fungal nail infections

Clinical Stage 3

Unexplained[a] severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhea for longer than 1 month
Unexplained persistent fever (>37.6°C [99.7°F]), intermittent or constant, for longer than 1 month)
Persistent oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (current)
Severe bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, or bacteremia)
Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
Unexplained anemia (<8 g/dL), neutropenia (<0.5 × 10^9/L), or chronic thrombocytopenia (<50 × 10^9/L)

Clinical Stage 4[b]

HIV wasting syndrome
Pneumocystis jirovecii
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital, or anorectal, for longer than 1 month, or visceral at any site)
Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)
Extrapulmonary tuberculosis
Kaposi sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis including meningitis
Disseminated nontuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis (with diarrhea)
Chronic isosporiasis
Disseminated mycosis (coccidioidomycosis or histoplasmosis)
Recurrent nontyphoidal *Salmonella* bacteremia
Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumors
Invasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

[a]Unexplained refers to when the condition is not explained by other causes.
[b]Some additional specific conditions can also be included in regional classifications (such as reactivation of American trypanosomiasis [meningoencephalitis and/or myocarditis]) in the World Health Organization region of the Americas and disseminated penicilliosis in Asia.

even following ART initiation (e.g., cardiovascular disease, dyslipidemia, hypercoagulability, other metabolic disorders); and (3) opportunistic diseases resulting from impaired host responses as the cellular immune system is damaged or ablated. The major clinical syndromes most frequently seen in HIV-infected individuals fall into the last category. Potent ART has added two new categories of clinical manifestations that may be commonly encountered in patients with HIV infection: (1) immune reconstitution syndromes with exacerbations of previously silent or adequately treated infections, especially mycobacterial infections,[73,74] and (2) a syndrome of lipodystrophy with fat loss and redistribution, elevated serum triglyceride and cholesterol levels, and insulin resistance seen in patients receiving ART, especially with protease inhibitors.[75,76] The clinical features of immune reconstitution syndromes are discussed later in this chapter, and the manifestations of drug toxicity related to the treatment of HIV are discussed in Chapter 128.

Although the clinical manifestations of HIV infection do not vary according to HIV subtype, the incidence of specific opportunistic infections is profoundly influenced by geography and the prevalence of infectious diseases in particular regions. HIV-1 infection increases susceptibility to tuberculosis, and the incidence of tuberculosis in HIV-infected individuals is extremely high in sub-Saharan Africa, where tuberculosis is endemic. In this setting, tuberculosis is the most common opportunistic infection in people with HIV infection and the leading cause of death. Approximately 22% of all deaths from tuberculosis worldwide are associated with HIV disease, mostly in sub-Saharan Africa.[77] Malaria is also endemic in many developing countries and occurs with increased frequency and severity in HIV-infected individuals, particularly during pregnancy.[78] Opportunistic infections such as *P. jirovecii* pneumonia, *M. avium* complex disease, CMV retinitis, non-Hodgkin lymphoma, and HIV encephalopathy, which are relatively common in developed countries, are uncommon in developing countries such as those in West Africa.[79] In regions where it is endemic (e.g., the Mediterranean, Central America, South America, Africa, and Asia), leishmaniasis occurs with increased frequency among HIV-infected individuals. Similarly, *Trypanosoma cruzi* (South America), histoplasmosis (Ohio and Mississippi river valleys), and *Talaromyces marneffei* (Thailand, China, Hong Kong) occur with increased frequency in certain regions.

The incidence of specific opportunistic diseases has been determined for several large cohorts of HIV-infected patients. In a cohort of more than 1200 patients with $CD4^+$ counts less than 300 cells/mm^3 before the availability of ART, the most common opportunistic infection was *Candida* esophagitis (13.3 cases per 100 person-years). *P. jirovecii* pneumonia, disseminated *M. avium* complex, CMV disease, and AIDS dementia complex occurred at rates of 5 to 9 cases per 100 person-years. The relatively lower incidence of *P. jirovecii* pneumonia reflects the use of specific prophylaxis with trimethoprim-sulfamethoxazole or aerosolized pentamidine, which dramatically lowers the risk for this infection even in the absence of ART.[80,81] Less common were toxoplasmosis, cryptococcal meningitis, herpes zoster, the wasting syndrome, and Kaposi sarcoma (2–4 cases per 100 person-years). The least common complications were non-Hodgkin lymphoma, tuberculosis, progressive multifocal leukoencephalopathy, and cryptosporidiosis (1–2 cases per 100 person-years). Similar results have been found in other cohorts of patients in developed countries.[82] Considerably less information is available on the natural history of HIV infection in developing countries, but the spectrum of disease in patients presenting with HIV-related illnesses is different, with tuberculosis, cryptococcosis, bacterial sepsis and pneumonia, herpes zoster, and gastroenteritis predominating.[83]

Early clinical findings may also predict disease progression in HIV-infected individuals who have not developed opportunistic disease. Oral candidiasis and oral hairy leukoplakia are markers of immunosuppression and herald the development of AIDS in many patients.[43,84,85] Generalized lymphadenopathy is a common clinical finding in early HIV infection but does not predict progression to AIDS.[86] The occurrence of an opportunistic disease increases the risk for death independently of the $CD4^+$ cell count[87,88] including death due to non–AIDS-related causes.[89] This may be caused not only by morbidity related to the complication itself but also by an increase in immune activation and inflammatory responses leading to upregulation of HIV replication, with acceleration of HIV disease progression. A number of studies have demonstrated increases in HIV viral load in patients with acute opportunistic infections.[90–93] Although viral load generally decreases after the acute illness, it generally does not return to premorbid levels.

Effect of Antiretroviral Therapy on Natural History of HIV Infection

Even before the era of effective combination ART, it was clear that nonsuppressive ART and prophylaxis against *P. jirovecii* pneumonia had substantially altered the natural history of AIDS, prolonging the median survival of treated patients with AIDS to 2 to 3 years.[94–98] ART and prophylaxis against *P. jirovecii* pneumonia and *M. avium* complex prolonged the time from HIV infection to AIDS, decreased the incidence of opportunistic complications, and improved overall survival.[99–106]

TABLE 122.7 Comparison of World Health Organization and Centers for Disease Control and Prevention Staging Systems

WHO STAGE[B]	WHO T-LYMPHOCYTE COUNT AND PERCENTAGE[C]	CDC STAGE[D]	CDC T-LYMPHOCYTE COUNT AND PERCENTAGE
Stage 1 (HIV infection)	$CD4^+$ T-lymphocyte count of ≥500 cells/mm^3	Stage 1 (HIV infection)	$CD4^+$ T-lymphocyte count of ≥500 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of ≥29
Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 350–499 cells/mm^3	Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of 14–28
Stage 3 (advanced HIV disease)	$CD4^+$ T-lymphocyte count of 200–349 cells/mm^3	Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of 14–28
Stage 4 (AIDS)	$CD4^+$ T-lymphocyte count of <200 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of <15	Stage 3 (AIDS)	$CD4^+$ T-lymphocyte count of <200 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of <14

[a]For reporting purposes only.
[b]Among adults and children ≥5 years of age.
[c]Percentage applicable for stage 4 only.
[d]Among adults and adolescents (≥13 years of age). CDC also includes a fourth stage, stage unknown; laboratory confirmation of HIV infection but no information on $CD4^+$ T-lymphocyte count or percentage and no information on AIDS-defining conditions.
CDC, Centers for Disease Control and Prevention; *WHO,* World Health Organization.

Changes in plasma HIV-1 RNA and $CD4^+$ lymphocyte counts resulting from ART have been shown to be strong predictors of clinical regression of HIV disease and restoration of cellular immunity.[107–109]

The use of combination ART and the introduction of protease inhibitors in the mid-1990s led to dramatic changes in the natural history of treated HIV disease (Figs. 122.3, 122.4, and 122.5).[110–115] In the United States as a whole, deaths attributed to AIDS have decreased by 85% since peaking at approximately 50,000 in 1995 to approximately 6700 in 2014.[111,112,116] Studies among cohorts of HIV-infected individuals in multiple settings demonstrate the beneficial effect of ART on clinical disease progression and death (Fig. 122.6).[117] Global improvements in survival of people with HIV infection have now been documented as a result of the expansion of access to ART to almost 20 million people worldwide through efforts of initiatives such as the US President's Emergency Program for AIDS Relief and the Global Fund for AIDS, TB and Malaria as well as efforts by national governments to scale up treatment of HIV infection. According to UNAIDS, deaths from AIDS decreased by almost half between 2005 and 2016, from 1.9 million to 1.0 million.[118–120] As discussed later on, as deaths due to AIDS have dramatically declined, morbidity and mortality in people with HIV infection is now more frequently caused by chronic diseases not traditionally classified as related to HIV infection. In the HIV Outpatient Study cohort, for example, declines in AIDS-related mortality between 1997 and 2004 were accompanied by a relative increase in deaths due to non–AIDS-related causes such as liver disease, non–AIDS-related cancers, and cardiovascular disease.[114] The Antiretroviral Therapy Cohort Collaboration reported that among more than 1800 deaths in 13 HIV clinical cohorts that occurred between 1996 and 2006, about half were due to AIDS, and the remainder were due to non–AIDS-related cancers, cardiovascular disease, trauma, liver disease, and other causes; the rate of AIDS-related deaths fell steadily with increased time on ART.[121] In the United States in 2014, almost half of deaths in people with HIV were attributed to non–AIDS-related causes. Among non–AIDS-related causes of death in Europe, non–AIDS-defining cancers were the leading cause.[122]

Effective therapy has not only decreased the incidence of new opportunistic infections but has also led to resolution of preexisting conditions.[123] In some cases the immune restoration resulting from ART can alter the clinical presentation of specific opportunistic infections, as in the case of focal mycobacterial lymphadenitis or CMV vitritis.[124] It may also unmask opportunistic infections that were not evident before ART initiation such as tuberculosis.[125,126] It is becoming increasingly clear that the immunologic changes resulting from ART represent at least a partial immune reconstitution, although the recovery of antigen-specific immunity appears to lag behind $CD4^+$ cell count increases.[127–130] The clinical manifestations of immune reconstitution syndromes are discussed at the end of this chapter. The incidence of new opportunistic infections in patients who have had satisfactory virologic and immunologic responses to ART is extremely low, even when primary prophylaxis has been discontinued.[131,132] Moreover, reactivation of previously diagnosed opportunistic infections such as *M. avium* complex infections and CMV retinitis appears to be uncommon in patients with immune recovery who discontinue maintenance therapy.[133,134]

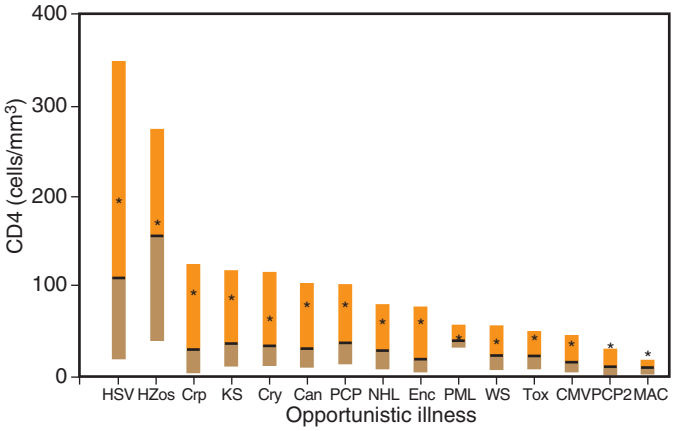

FIG. 122.3 Range of $CD4^+$ lymphocyte counts at the time of diagnosis of opportunistic diseases in HIV-infected patients. Boxes represent the 25th to 75th percentiles, bars represent medians, and asterisks represent means. *Can, Candida* esophagitis; *CMV,* cytomegalovirus; *Crp,* cryptosporidiosis; *Cry,* cryptococcosis; *Enc,* HIV encephalopathy; *HSV,* herpes simplex virus; *HZos,* herpes zoster; *KS,* Kaposi sarcoma; *MAC, Mycobacterium avium* complex; *NHL,* non-Hodgkin's lymphoma; *PCP,* first episodes of *Pneumocystis jirovecii* pneumonia; *PCP2,* recurrent *P. jirovecii* pneumonia; *PML,* progressive multifocal leukoencephalopathy; *Tox,* toxoplasmosis; *WS,* wasting syndrome. *(From Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban cohort.* Ann Intern Med. *1996;124:633–642.)*

Over the past 4 decades, the natural history of HIV infection and our understanding of it have undergone considerable change. The clinical course of HIV disease in patients receiving ART is likely to evolve further in the coming years, with additional manifestations and complications of long-standing infection and use of antiretroviral drugs becoming apparent as larger numbers of patients are treated for longer periods of time.

Acute Retroviral Syndrome

The initial manifestation of HIV infection in one-half to three-quarters of recently infected individuals is a mononucleosis-like illness referred to as acute retroviral syndrome. Whereas the first reports noted a high prevalence of symptoms in acutely infected men,[135,136] other studies have found a wide range of symptomatic acute HIV infections.[137–139] In one study of 378 subjects with acute retroviral syndrome, injection drug users had or reported symptoms less frequently than persons who

acquired HIV through sexual transmission.[140] Most health care workers with occupationally acquired HIV had acute retroviral syndrome after exposure.[141,142] A recent intensive study of blood donors in Thailand reported that 78% of 430 adults diagnosed before HIV antibody seroconversion had symptoms of acute retroviral syndrome.

The clinical features of acute retroviral syndrome are nonspecific and variable.[143–148] The onset of the illness ranges from 1 to 6 weeks after exposure to the virus but peaks at 3 weeks. Table 122.1 lists the signs and symptoms of acute retroviral syndrome reported in 209 cases.[146] Fever, sweats, malaise, myalgias, anorexia, nausea, diarrhea, and a nonexudative pharyngitis are prominent symptoms.[146–153] Many patients report headaches, photophobia, and meningismus. Two-thirds of patients may have a truncal exanthem that may be maculopapular, roseola-like, or urticarial. Findings of skin biopsies are nonspecific, with perivascular

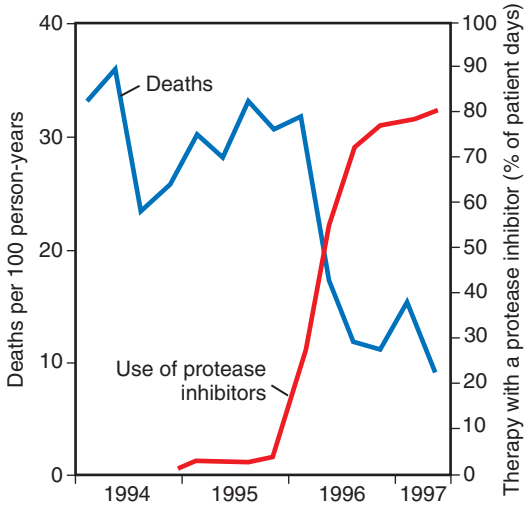

FIG. 122.4 Incidence of death and use of protease inhibitors in HIV-infected patients and CD4⁺ count lower than 100 cells/mm³ in the HIV Outpatient Study. *(From Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.* N Engl J Med. *1998;338:853–860. © 1998 Massachusetts Medical Society.)*

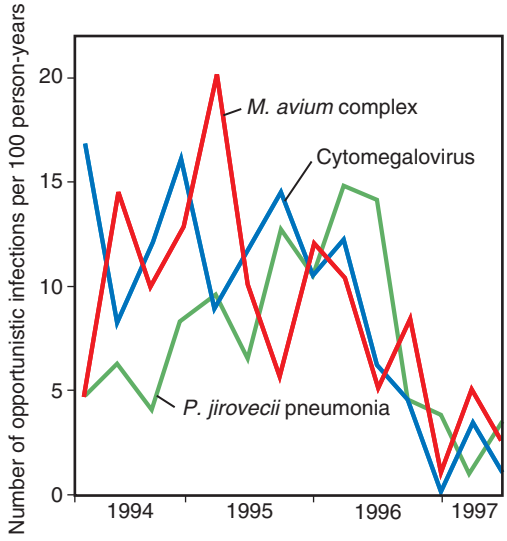

FIG. 122.5 Incidence of selected opportunistic infections in HIV-infected patients and CD4⁺ count lower than 100 cells/mm³ in the HIV Outpatient Study in the era before and after the introduction of protease inhibitors. *(From Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.* N Engl J Med. *1998;338:853-860. © 1998 Massachusetts Medical Society.)*

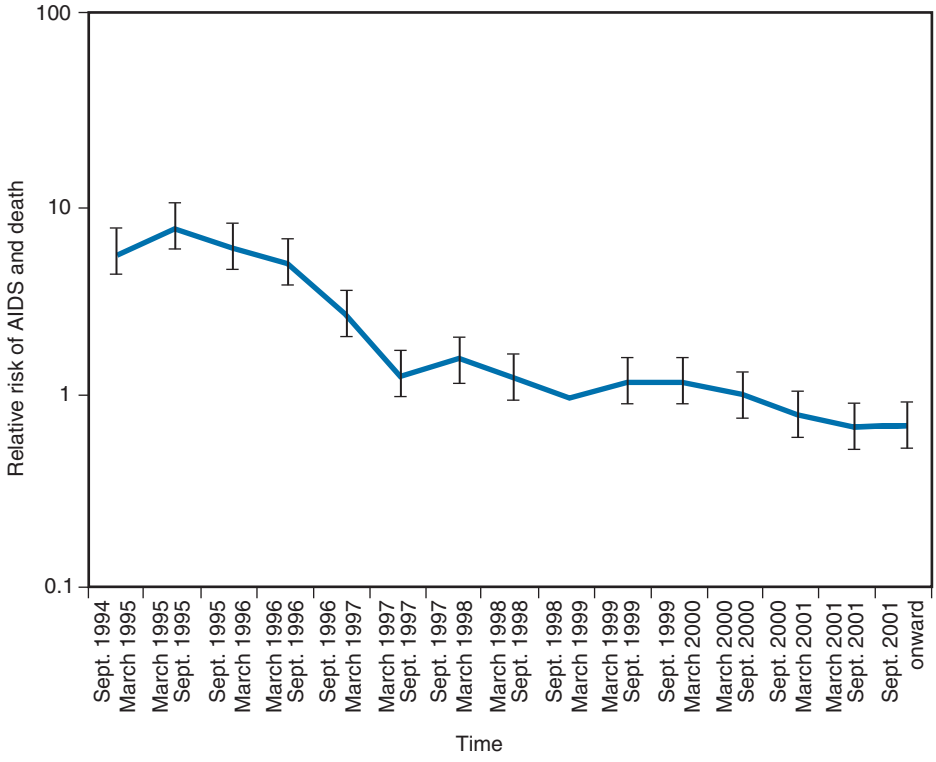

FIG. 122.6 Relative risk of AIDS or death since the introduction of antiretroviral therapy (ART) adjusted for CD4⁺ count at recruitment, age, previous ART treatment, and AIDS status. Data are from 9803 HIV-infected patients seen at 70 treatment centers in Europe, Israel, and Argentina. Vertical bars represent 95% confidence intervals. *(From Mocroft A, Ledergerber B, Katlama C, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study.* Lancet. *2003;362:22–29.)*

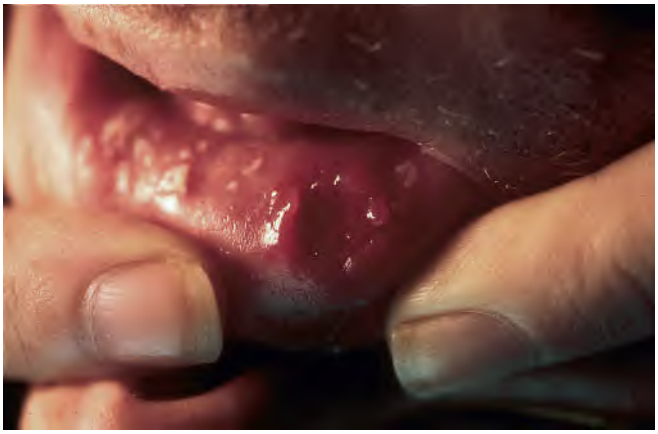

FIG. 122.7 Aphthous ulcer. *(Courtesy Dr. Stephen Raffanti.)*

lymphocytic infiltrates and dermal mononuclear cell infiltrates.[154] In addition to aseptic meningitis, neurologic symptoms occur in a minority of patients and may include encephalitis, peripheral neuropathy, and an acute ascending polyneuropathy (Guillain-Barré syndrome).[155] Physical examination frequently reveals cervical, occipital, or axillary lymphadenopathy; rash; and, less commonly, hepatosplenomegaly. Oral aphthous ulcerations (Fig. 122.7) have been reported in several cases; these may involve the esophagus. Oral and esophageal candidiasis during the seroconversion illness has been reported. Symptoms generally resolve in 10 to 15 days. A wide range of acute opportunistic infections have been reported in patients with acute retroviral syndrome including *P. jirovecii* pneumonia, cryptococcal meningitis, and *Candida* esophagitis. Their occurrence is probably due to the depression of the $CD4^+$ cell count that generally accompanies acute HIV infection.

Laboratory evaluation of patients with acute retroviral syndrome shows a reduced total lymphocyte count, elevated sedimentation rate, negative heterophil antibody test, and elevated aminotransferase and alkaline phosphatase levels.[146] When lymphocyte phenotyping is performed, a characteristic pattern is observed.[156] Initially the total lymphocyte count including both $CD4^+$ and $CD8^+$ T lymphocytes decreases with a normal $CD4^+/CD8^+$ ratio. Within several weeks the $CD4^+$ and $CD8^+$ cell populations both begin to increase. The rise in $CD8^+$ cell numbers is relatively greater than the rise in $CD4^+$ cells, and the $CD4^+/CD8^+$ ratio is inverted. In the weeks that follow the $CD8^+$ cell population increases markedly because of HIV-specific $CD8^+$ T lymphocytes. The $CD4^+/CD8^+$ ratio usually remains inverted as the acute illness resolves, primarily because of excess numbers of $CD8^+$ cells. In patients with neurologic symptoms the cerebrospinal fluid may show a lymphocytic pleocytosis with normal levels of protein and glucose.[157]

Tests for detecting acute HIV infection include plasma HIV RNA, which becomes positive about 5 days after infection, and HIV p24 antigen, which may be detected after 10 days; antibody reactivity on enzyme immunoassay testing is not found until 14 to 21 days.[158] Fiebig and colleagues[158] characterized the stages of acute HIV infection by the timing of the appearance of viral markers and immune responses in plasma donors who were seroconverting. In Fiebig stage 1 (3–8 days), HIV-1 RNA is detectable, but p24 antigen and anti-HIV antibodies cannot be detected. Stage 2 (4–8 days) is marked by the appearance of p24 antigen in blood and cerebrospinal fluid. Stage 3 is marked by detection of anti-HIV antibodies by sensitive enzyme immunoassay, stage 4 is marked by the first appearance of bands on a Western blot, and stage 5 is marked by conversion of the Western blot to positive on multiple bands.[157,158] The cumulative time of Western blot seroconversion ranges from 15 to 28 days. Use of a fourth-generation, ultrasensitive enzyme immunoassay shortens the time to detection of antibodies.[159] Therefore plasma HIV RNA is the most sensitive marker for acute HIV infection and is the preferred test when evaluating patients suspected to have acute retroviral syndrome.[160] Typical RNA levels range from 10^5 to more than 10^6 copies/mL of plasma, and the titers decline as the $CD8^+$ cytotoxic T-cell and antibody responses subsequently increase. Low-level ($<10^4$) false-positive HIV RNA tests may occur, but high-level viremia is diagnostic of acute infection in the absence of anti-HIV antibodies.

The differential diagnosis of acute retroviral syndrome includes a number of other illnesses—infectious mononucleosis and other viral infections such as influenza, viral hepatitis, measles, rubella, primary herpes simplex virus (HSV) infection, and CMV and secondary syphilis. Evaluation of patients presenting with an illness consistent with acute retroviral infection should include a careful history to elicit risks for HIV infection; laboratory tests to rule out mononucleosis, CMV, and syphilis; HIV antibody and plasma RNA tests; and complete blood cell counts and differential. There is potential benefit in treating acute HIV with ART because there is evidence that this may lower the viral setpoint, lead to enhanced $CD4^+$ and $CD8^+$ HIV-specific responses, decrease the severity of acute disease,[161,162] and decrease levels of immune activation and inflammation.[163] Early treatment does not appear to prevent establishment of reservoirs of latently infected resting $CD4^+$ cells, although it may decrease the size of the reservoirs in adults.[164] In infants, very early treatment may result in remission of HIV viremia following cessation of treatment for several years, as illustrated by the "Mississippi Child."[165] However, the remission of viremia is not permanent.[166] Current guidelines recommend ART for all HIV-infected individuals; thus initiating treatment in early HIV infection (the day of HIV diagnosis if resources and support are available) is appropriate (see Chapter 128).[167–169]

Persistent Generalized Lymphadenopathy

Infection with HIV is associated with a high prevalence of persistent generalized lymphadenopathy (PGL), often beginning with acute retroviral syndrome. In the early 1980s, PGL was recognized as a prodromal state to the development of AIDS in MSM who were otherwise healthy.[15,17] The pathogenesis of PGL is related to the rapid infection of $CD4^+$ cells in lymph nodes by HIV after initial infection. Although enlarged peripheral lymph nodes may be the most evident sign of recent HIV infection, intestinal lymph nodes and Peyer patches in the gut are an even more important target of early HIV infection with infection of large proportions of $CD4^+$ T cells and dendritic cells resulting in obliteration of normal nodal architecture and loss of function. Disruption of the intestinal mucosa can then lead to bacterial translocation and systemic immune activation, a process that may persist throughout the course of infection.[170,171]

The syndrome of PGL is defined as the presence of two or more extrainguinal sites of lymphadenopathy for a minimum of 3 to 6 months for which no other explanation can be found. Biopsy specimens of lymph nodes from such patients usually reveal a follicular hyperplasia without specific pathogens. PGL develops in 50% to 70% of HIV-infected individuals. The most frequently involved node groups are the posterior and anterior cervical, submandibular, occipital, and axillary chains; epitrochlear and femoral nodes may also be enlarged. Physical examination usually reveals symmetrical, mobile, rubbery lymph nodes ranging from 0.5 to 2 cm. Localized (i.e., asymmetrical) adenopathy and rapid nodal enlargement are not characteristic and suggest another infectious or malignant process. Mediastinal and hilar adenopathy is not characteristic of the syndrome; however, abdominal computed tomography often reveals enlarged mesenteric and retroperitoneal adenopathy in patients with HIV infection. The natural history of HIV infection in patients with PGL does not differ significantly from that of HIV infection in patients without PGL.[86,172] Involution of enlarged lymph nodes, with degeneration of follicular germinal centers and loss of hyperplasia, often accompanies progression of HIV infection to advanced disease.

In patients treated with ART, previously involuted lymph nodes may again enlarge as HIV-specific and other T cells are replenished. In addition, focal lymphadenitis with constitutional symptoms may occur in patients with previously silent infections 1 to 2 months after starting ART. These reversal or unmasking reactions, or immune reconstitution syndromes, are reminiscent of reversal reactions seen in multibacillary forms of leprosy, heralding a return of pathogen-specific T-cell responses.

The differential diagnosis of PGL includes HIV infection and a wide variety of other processes associated with generalized lymphadenopathy such as sarcoid, secondary syphilis, and Hodgkin disease. In patients with

HIV infection, lymphadenopathy may also be caused by mycobacterial infections, Kaposi sarcoma, and lymphoma.[173] An uncommon cause of lymphadenopathy in patients with HIV infection is multicentric Castleman disease.[174,175] Castleman disease is an angioproliferative, hyperplastic process of lymph nodes and other lymphoid tissues showing characteristic histologic findings, with either hyaline vascular or plasma cell variants. In patients with HIV in particular, multicentric Castleman disease is the most common presentation, with involvement of lymph nodes, liver, spleen, and other organs. Although the pathogenesis of Castleman disease is not fully understood, infection with Kaposi sarcoma–associated herpesvirus (human herpesvirus type 8) is believed to underlie a large proportion of cases. Nearly all patients who have HIV infection and Castleman disease are also infected with human herpesvirus type 8.[176,177] In contrast to PGL, multicentric Castleman disease is associated with constitutional symptoms and multiple organ involvement in most patients with HIV infection. The diagnosis is established on histopathology. Treatment with the anti-CD20 monoclonal antibody rituximab, alone or in combination with chemotherapy such as liposomal doxorubicin, has led to an overall survival of >90% at 5 years.[178,179]

Constitutional Disease and Wasting

HIV infection is often completely asymptomatic; however, some patients present with nonspecific constitutional symptoms in the months or years after primary infection but before opportunistic disease is diagnosed. Patients commonly report being easily fatigued and the need to reduce their normal activities. Debilitating fatigue is uncommon in the early years of infection. Low-grade fevers (temperature <38°C [100.4°F]), occasional night sweats, and intermittent diarrhea are also reported. Severe wasting, with loss of more than 10% of body weight, is generally a finding of advanced HIV disease. The exact incidence of constitutional symptoms, fatigue, and weight loss is not known, and the cause is varied and often multifactorial. The differential diagnosis of these findings includes intercurrent minor illnesses, endocrinologic abnormalities, anemia, and psychological or psychiatric disorders.

In African patients with HIV infection, a wasting illness termed *slim disease* has been described.[180] These patients have debilitating fatigue, fevers, sweats, protracted diarrhea, and severe weight loss. Opportunistic or conventional pathogens are not found, but the patients waste away and die of severe malnutrition and terminal secondary infections. This illness has been encountered in developed countries as well but far less commonly than in Africa—a pattern that suggests underdiagnosis of opportunistic diseases in Africa. Several studies of African patients with enteropathic slim disease found that most had enteric pathogens or microsporidia when a thorough evaluation was performed.[181,182] In Abidjan, Côte d'Ivoire, 37% of patients who died with a diagnosis of slim disease were found at autopsy to have disseminated tuberculosis,[183] and the presence of tuberculosis at autopsy was strongly associated with the degree of wasting.[184] The definition of wasting syndrome in the United States is the presence of unexplained constitutional disease for longer than 1 month with a temperature higher than 38.3°C (100.9°F), diarrhea, and loss of more than 10% of baseline body weight. A thorough evaluation to identify specific pathogens that would explain the symptoms and be amenable to treatment is essential before wasting syndrome is diagnosed, and usually a specific cause can be implicated.

In patients with more advanced HIV disease with high viral loads and severe depletion of $CD4^+$ cells, constitutional disease (fatigue, weight loss, malaise, fever) usually heralds the onset of opportunistic infections or malignancies. In one study of HIV-infected outpatients with fever, a specific cause could be identified for 83%.[185] Common causes of fever in these patients included *P. jirovecii* pneumonia, *M. avium* complex bacteremia, catheter-related bacteremia, bacterial pneumonia, sinusitis, lymphoma, and drug reactions. Fever lasting longer than 2 weeks was more often associated with AIDS-defining illnesses.

Anxiety disorders and depression are common in patients with HIV infection,[186–188] and studies have suggested an increased prevalence of affective disorders among HIV-infected individuals. Injection drug users, in particular, have a high prevalence of affective disorders that may result in somatic complaints. Moreover, the physical effects of opiates and withdrawal from stimulants such as cocaine and amphetamines cause fatigue and other constitutional symptoms.

Metabolic and Endocrine Abnormalities

A number of metabolic and endocrinologic disturbances have been identified in patients with HIV infection.[189–191] The pathogenesis of metabolic and endocrine dysfunction in people with HIV is thought to be multifactorial and may result from activation of proinflammatory cytokines and procoagulant mediators by HIV infection, T-cell activation resulting directly and indirectly from HIV, bacterial translocation due to intestinal mucosal disruption, and activation of other infectious agents such as CMV by HIV-induced immunodeficiency.[192] The spectrum of complications appearing in ART-naïve patients differs from that seen in treated patients with viral suppression, and in the latter group, aging and lifestyle may contribute to some manifestations.

Hypogonadism, particularly depression of testosterone or dihydrotestosterone levels, has been reported in men and women with HIV infection and weight loss or wasting.[193,194] Elevated levels of myostatin-immunoreactive protein, a muscle catabolic agent, have been found in men with HIV and wasting.[195] In most clinical studies, however, wasting has been found in association with decreased caloric intake, elevated catabolism caused by opportunistic infections, or chronic diarrhea.[196–199] In advanced HIV, severe wasting, whatever the cause, is strongly associated with mortality risk.[200] Weight loss has remained an important predictor of mortality even in the era of ART.[201]

The prevalence of osteoporosis is more than three times greater in HIV-positive individuals than HIV-negative control subjects.[202] HIV-positive individuals are also at increased risk of bone fracture.[203]

In contrast to the wasting illness seen in patients with advanced untreated HIV infection, patients receiving ART may have lipid abnormalities including hypertriglyceridemia and high low-density lipoprotein cholesterol and low high-density lipoprotein cholesterol levels. They may also have lipodystrophy, obesity, and insulin resistance.[76,204] These abnormalities are seen more commonly with protease inhibitor–based therapy, but they also occur with nonnucleoside reverse-transcriptase inhibitor therapy. Significant weight gain and diabetes mellitus have been reported in patients switching from efavirenz to integrase strand transfer inhibitors.[205,206] The lipid and glucose abnormalities may contribute to an increased risk for cardiovascular disease including myocardial infarction.[207] Recent abacavir use has been associated with an increased risk of myocardial infarction.[208] In the Multicenter AIDS Cohort Study, HIV-infected men on ART with suppressed viral loads were overall less likely to have good metabolic health,—a composite of normal blood pressure, serum triglycerides, serum cholesterol, fasting blood glucose, and waist circumference—than HIV-seronegative men. However, these differences were largely seen in nonobese men, although the prevalence of obesity was slightly lower in HIV-infected men.[209]

Oral Disease

Abnormalities of the oral cavity occur throughout the course of HIV infection. Primary HIV infection has been associated with severe aphthous stomatitis and with oropharyngeal and esophageal candidiasis. As the infection progresses and immunologic impairment proceeds, numerous oral complications arise. In the late stages of disease, oral manifestations are highly prevalent and frequently severe.[210–212] A number of studies have demonstrated that the occurrence of oral lesions such as candidiasis and hairy leukoplakia is associated with an increased risk for progression to AIDS.[213–215]

Oral Candidiasis

Candida infections of the hard and soft palates, buccal mucosa, tongue, pharynx, and hypopharynx are observed frequently. *Candida albicans* is the species most commonly identified, but *Candida tropicalis, Candida glabrata,* and *Candida krusei* infections also occur. Contrary to systemic *Candida* infections, which appear to result from defects in phagocyte function and number, mucosal *Candida* infections result from impaired cellular immunity. The incidence of candidiasis increases with progressive cellular immunodeficiency, particularly as $CD4^+$ counts fall below 200 to 300 cells/mm^3.[216]

The most common form of candidiasis is thrush (pseudomembranous candidiasis). Characteristic cottage cheese plaques that can be removed with a tongue blade are seen on the soft palate, tonsils, and buccal mucosa (Fig. 122.8). Less often, thrush involves the lateral and posterior

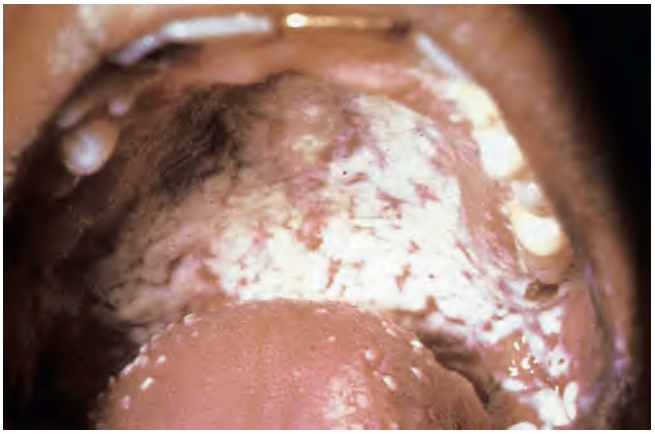

FIG. 122.8 Oral candidiasis (thrush). *(Courtesy Dr. Stephen Raffanti.)*

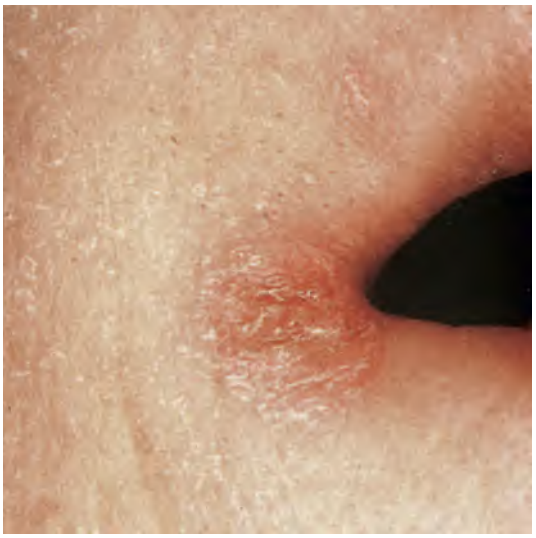

FIG. 122.9 Angular cheilitis. *(Courtesy Dr. Stephen Raffanti.)*

aspects of the tongue, the hard palate, and the hypopharynx. *Candida* infection can produce flat erythematous plaques distributed in the same way as the pseudomembranous form of the disease but without the characteristic white exudate. This atrophic form of candidiasis is underdiagnosed because many clinicians are unfamiliar with its appearance. Atrophic candidiasis of the tongue also occurs. Less frequently, *Candida* can cause a nonscrapeable white plaque similar to that seen in hairy leukoplakia (see next section). In contrast to the corrugated lesions and hairlike projections seen in oral hairy leukoplakia, candidal lesions are smooth. This hypertrophic form of disease may involve the lateral border of the tongue, palate, and buccal mucosa. *Candida* infection of the lateral lip (angular cheilitis) is another common complication. Angular cheilitis can cause pain, fissures, erythema, and difficulty opening the mouth (Fig. 122.9). Physical examination, wet mount preparation, and response to antifungal therapy establish the diagnosis.

The diagnosis of candidiasis is frequently made on the basis of physical examination alone. A potassium hydroxide preparation of scraped material from a plaque is diagnostic and can be performed easily in most clinical settings. Cultures for *Candida* are rarely necessary unless antimicrobial resistance is suspected in patients with thrush refractory to azole therapy. A biopsy specimen of oral lesions can be used to distinguish various forms of leukoplakia. A therapeutic trial of antifungal agents can also help establish a diagnosis.

Oral Hairy Leukoplakia

Oral hairy leukoplakia is a raised white lesion of the oral mucosa that is usually seen on the lateral margin of the tongue.[217] The frequency of occurrence of oral hairy leukoplakia increases as the $CD4^+$ count decreases.[216] Oral hairy leukoplakia appears to be caused by the replication of Epstein-Barr virus in the epithelium of keratinized cells on the surface of the tongue and buccal mucosa.[218] Other herpesviruses have also been isolated from cultures of biopsy specimens of lesions; however, their role in the pathogenesis of oral hairy leukoplakia is unclear. The diagnosis is established by visual inspection, failure to scrape off the lesion with a tongue blade, failure of the lesion to respond to antifungal therapy, and biopsy material or scrapings in which Epstein-Barr virus can be identified. Oral hairy leukoplakia is usually asymptomatic, although large lesions may impair taste, hinder eating, and cause discomfort. There is usually clinical improvement with ART.

Gingivitis and Periodontitis

Severe gingivitis (linear gingival erythema) and periodontitis (necrotizing ulcerative periodontitis) have been observed in patients with HIV disease.[219] The onset of symptoms is often insidious but may be abrupt. Pain is often severe; patients may note foul breath, bleeding gums, and loosening of teeth. Physical examination may reveal a bright red marginal line on the gingiva, necrosis and ulceration of interdental papillae, gingival erosion, exfoliation of enamel, and loose teeth. The cause of gingivitis and periodontitis is unclear. Cigarette smoking may be an important cofactor in the pathogenesis of periodontitis. Mixed cultures of aerobic and anaerobic flora have been obtained from gingival biopsy samples. More severe, ulcerating gingivitis can be caused by infections with gram-negative bacilli, particularly *Klebsiella pneumoniae* and *Enterobacter cloacae*. Infections tend to be chronic, but débridement, irrigation, and topical antiseptic agents or metronidazole therapy may control some cases.

Oral Ulcers

A number of ulcerative lesions may occur in the oral cavity of patients with HIV infection. HSV types 1 and 2 may cause primary or recurrent oral ulcers. These lesions generally appear as small smooth ulcers on an erythematous base on the lips, buccal mucosa, hard palate, or gums. The ulcers may be single or multiple and are often painful. Episodes may last for several weeks; acyclovir may be beneficial. CMV may rarely cause solitary large ulcers in patients with disseminated CMV infection. Aphthous stomatitis is manifested by single or multiple painful ulcers, often with exudate or necrosis, which may appear on the buccal and labial mucosa and lateral margin of the tongue (see Fig. 122.7). These ulcers do not occur more commonly than in HIV-seronegative individuals, but episodes are more severe and prolonged.[220] The ulcers may be treated with topical corticosteroids or thalidomide if persistent.[221–223] The cause of oral ulcers is best determined by biopsy and viral culture, although minor lesions may be observed without specific therapy in many cases. Several drugs including zalcitabine, zidovudine, and dapsone have been reported to cause oral and gastrointestinal ulcers.

Other Oral Lesions

The purple-red lesions of Kaposi sarcoma may occur at any site in the mouth, but the palate is most common. Lesions may become large and nodular. Non-Hodgkin lymphoma may arise in the mouth as a swelling or ulcers; biopsy is required for diagnosis. Oral warts caused by human papillomavirus infection may be seen, but they are not malignant precursors. Ketoconazole and zidovudine can cause brown oral pigmentation. Salivary glands such as the parotid gland may be enlarged by infiltration with $CD8^+$ lymphocytes or benign lymphoepithelial cysts. These cysts often respond to ART.[224]

Musculoskeletal Complications

Polymyositis complicates HIV infection in a small number of patients and can occur at any stage of HIV infection.[225] Clinical features include myalgias, weakness of the proximal muscles, muscle tenderness, wasting, and fatigue.[226] Creatine kinase and other muscle enzyme concentrations are usually elevated, although they do not correlate with disease severity; electrophysiologic studies are consistent with a myopathy.[227,228] Nucleoside reverse-transcriptase inhibitors have been associated with a polymyositis-like clinical picture in a small proportion of patients who receive these agents. Initially described with zidovudine, the mechanism of this

myopathy is inhibition of mitochondrial DNA polymerase, which is distinguished on electron microscopy by ragged myofibrils.[229] Other nucleoside reverse-transcriptase inhibitors also can cause mitochondrial toxicity resulting in neuropathies, liver impairment, and lactic acidosis.[230] The more recently developed agents in this class such as tenofovir, emtricitabine, and lamivudine are less likely to cause mitochondrial damage.

Pyomyositis has been reported in patients with advanced HIV. Skin flora, particularly *Staphylococcus aureus,* are usually recovered from wound cultures, and preexisting skin diseases such as prurigo nodularis are a risk factor.[231,232]

Although rheumatologic findings in patients with HIV disease are not unusual, the extent to which HIV infection is associated with these disorders is not always clear. Defining a specific arthropathy caused by HIV is difficult because many patients with HIV infection are already at increased risk for inflammatory joint disease. For example, injection drug users may develop septic arthritis caused by pyogenic bacteria, particularly *S. aureus.* MSM may have an increased risk for gonococcal arthritis or postinfectious reactive arthritis (formerly Reiter syndrome) associated with genital or gastrointestinal tract infections. Immune complex deposition related to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection may also be associated with arthritis in patients with HIV infection. Although some animal retroviruses are clearly associated with arthropathies, the situation with HIV remains unclear.

Osteopenia and avascular necrosis of bone occur at a higher frequency in HIV-infected individuals than in HIV-seronegative people in the general population,[233–235] and the incidence of avascular necrosis appears to be increasing.[236] Risk factors for avascular necrosis include corticosteroid therapy, lipid-lowering agents, and testosterone.[235] Although avascular necrosis has been described in individuals receiving ART,[237] such therapy does not appear to affect the risk for avascular necrosis.[238] Bone mineral density decreases modestly shortly after ART initiation but does not appear to decrease with time on ART, and in some studies it has increased.[233]

Cutaneous Manifestations

Dermatologic consequences of HIV infection include primary cutaneous opportunistic infections and malignancies, which may also disseminate to the viscera, and systemic opportunistic diseases with skin involvement.[239,240]

Viral Infections of the Skin and Mucous Membranes

A wide range of viruses involve the skin in immunosuppressed patients with HIV infection. The exanthem of acute HIV infection is an erythematous morbilliform eruption of the trunk and upper arms that occurs 2 to 4 weeks after infection and is usually associated with fever, headache, arthralgias, night sweats, pharyngitis, or thrush.[136,241,242] The rash resolves within 5 to 7 days. HSV (see Chapter 135) frequently causes morbidity in patients with advanced HIV disease.[243] Serology shows previous infection with HSV-2 in more than 90% of MSM with HIV infection; it is less prevalent in other groups. Although HSV-2 recurs frequently even in nonimmunosuppressed hosts, it recurs more frequently and for prolonged periods in patients with HIV infection. HSV-2, a common pathogen of the sacral root dermatomes, often causes outbreaks in the buttocks, perineum, scrotum (or vulva), and shaft and glans of the penis. Characteristic lesions of HSV appear first as painful erythematous papules; later, they vesiculate and ulcerate, and pustules may form. Chronic ulcers may become granulated, verrucous, or bloody (Fig. 122.10). Herpes simplex proctitis is associated with severe rectal pain, fever, tenesmus, and obstipation. External lesions may be absent, and the diagnosis is established by anoscopic or sigmoidoscopic examination and cultures. Giant perirectal ulcers and lesions at other sites that yield thymidine kinase–resistant strains of HSV-2 have occurred in patients who were previously treated with acyclovir. HSV infections are diagnosed by the typical appearance and distribution of the lesions, viral culture, or polymerase chain reaction assay. Tzanck preparations may show giant cells, which suggest HSV infection but generally have low sensitivity. Some physicians base diagnosis on how patients respond to an empirical trial of acyclovir. Orolabial HSV infections in HIV-infected patients may be caused by HSV-1 or HSV-2. Although primary infections may occur after patients acquire HIV, recurrences are the more common manifestation of HSV infection. Often a prodrome of tingling and pain precedes the appearance of painful vesicles and ulcers. Lesions may be found on the lips, buccal mucosa, gingiva, soft palate, uvula, and tongue. HSV disease may recur chronically in patients with advanced immunosuppression.

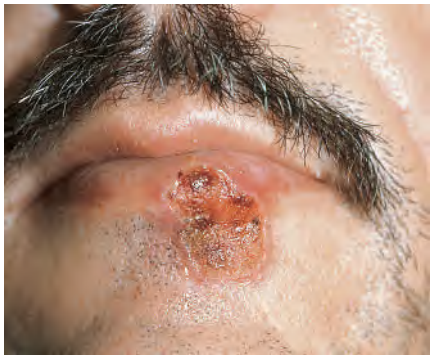

FIG. 122.10 Herpes simplex cheilitis. *(Courtesy Dr. Stephen Raffanti.)*

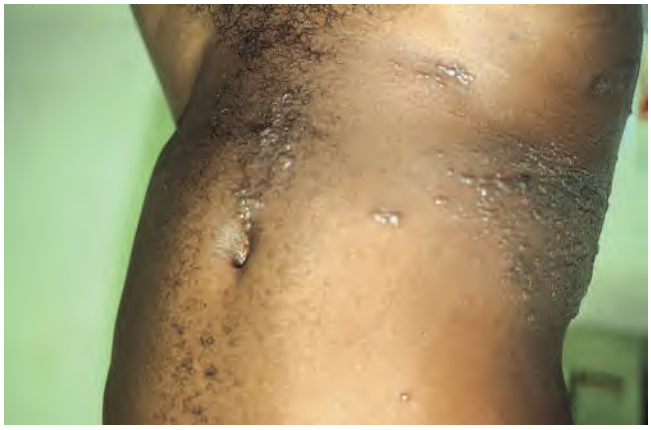

FIG. 122.11 Herpes zoster in dermatomal distribution. *(Courtesy Dr. Stephen Raffanti.)*

Varicella-zoster virus (shingles) is often reactivated in patients with HIV infection (see Chapter 136),[244] typically when the $CD4^+$ count is 200 to 500 cells/mm^3. There have been reports of herpes zoster after initiation of ART,[245] suggesting a role of the host immune response in this clinical manifestation. Herpes zoster may occur early in the course of HIV infection, but the incidence in late HIV disease is 5% to 10% annually.[238,246–249] Dermatomal outbreaks are most common, and a substantial proportion of patients may have several dermatomes involved (Fig. 122.11). Recurrent episodes at the same or different sites, chronic (nonremitting) zoster, and dissemination are often seen.[250] Shingles is often characterized by radicular pain and itching several days before erythematous papules appear, and vesiculation occurs within several days. Lesions are often extremely pruritic, and excoriation with secondary bacterial infection commonly occurs. Over a period of 4 to 7 days, lesions form bullae and crusts and begin to heal, although some patients have chronic herpes zoster. Cranial and thoracic dermatomes, followed by lumbar and sacral roots, are most often involved. Outbreaks along the ophthalmic branch of the trigeminal nerve may result in corneal involvement and lead to scarring and opacification that impair vision. A substantial proportion of patients may experience postherpetic scarring and pain. In patients with HIV infection who acquire primary varicella (chickenpox), the acute infection may progress to a chronic form in weeks to months.

Despite the frequency of disseminated CMV disease in late-stage AIDS, cutaneous manifestations are unusual. However, vesicles, bullae,

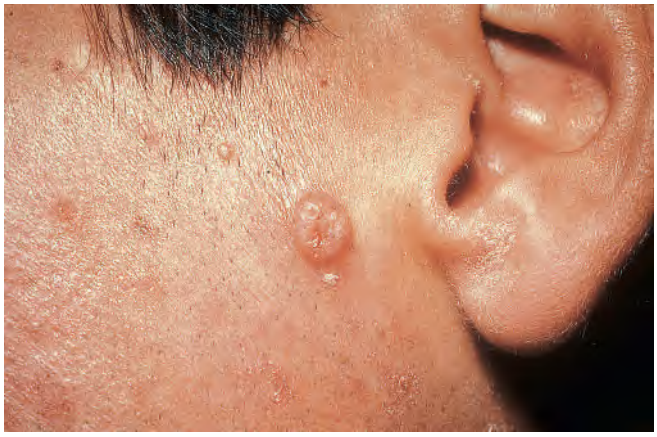

FIG. 122.12 Molluscum contagiosum. *(Courtesy Dr. Stephen Raffanti.)*

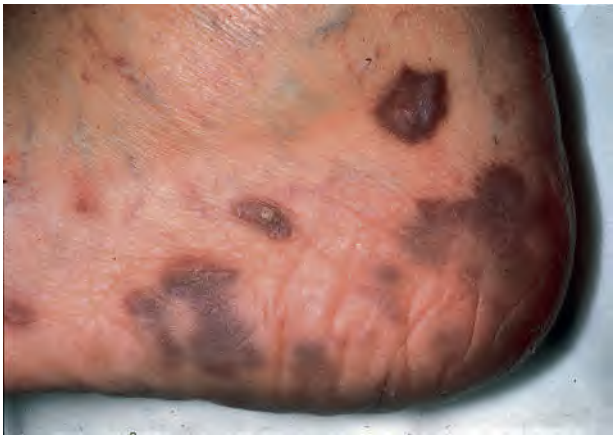

FIG. 122.13 Kaposi sarcoma. *(Courtesy Dr. Stephen Raffanti.)*

and hyperpigmented indurated plaques all have been described. Infections with human papillomavirus, the causative agent of condylomata acuminata, are more prevalent in HIV-infected people than in the general population. In addition to genital lesions (see Chapter 143), warts are often seen in periungual locations, on the feet (plantar warts), and in bearded areas of the face.

Molluscum contagiosum, a cutaneous poxvirus infection, is seen more often in HIV-infected people than in other populations (see Chapter 133). Most patients have $CD4^+$ counts less than 200 cells/mm^3. The agent is transmitted by sexual or other close contact; reactivation of remote infection may cause outbreaks in immunosuppressed hosts. Molluscum lesions are small firm papules with a pearly white umbilicated surface distributed on the face, trunk, or genital areas. The lesions are usually painless and can be differentiated from herpetic lesions by the absence of erythema, smaller size, and resolution of lesions without ulcerating or crusting (Fig. 122.12). Biopsy may be necessary to exclude more serious causes of cutaneous lesions such as cryptococcosis, pyogenic granuloma, and basal cell carcinoma. Liquid nitrogen is used effectively to treat this condition. Lesions may also resolve after initiation of ART, with a resultant increase in $CD4^+$ count.[251]

Bacillary Angiomatosis

Bacillary angiomatosis is associated with cutaneous and visceral involvement that produces lesions characterized by vascular proliferation, hemorrhage, and necrosis.[252–256] The disease was first described in 1983 in a patient with AIDS with subcutaneous nodules with vascular proliferation and evidence of bacterial involvement by electron microscopy.[255] Subsequently the cause of bacillary angiomatosis was attributed to the organisms *Bartonella henselae* and *Bartonella quintana* (see Chapter 234). *B. henselae* infection has been associated with cat and flea exposure and *B. quintana* infection with low socioeconomic status, homelessness, and exposure to lice.[257] *Bartonella* organisms have been cultured from skin lesions, blood, liver, bone, and other sites.[252–254,256]

Patients with bacillary angiomatosis usually present with one or several cutaneous lesions, although disseminated disease is common. The typical skin lesions are purple-red nodules or plaques that can ulcerate and crust. Lesions may be mistaken for cutaneous Kaposi sarcoma, skin tags, or basal cell carcinoma. Visceral disease may include hepatitis (bacillary peliosis), splenic or osseous lesions, bacillemia, pneumonitis or, less often, involvement of other organs.[258] Bacillary peliosis is a characteristic illness in which patients present with fever, right upper quadrant pain, hepatomegaly, and elevation of liver enzyme levels, particularly alkaline phosphatase.[259,260] Imaging studies of the liver may reveal echogenic defects; histologically, lesions have a cystic appearance, with vascular proliferation, hemorrhage, and necrosis.

The diagnosis of bacillary angiomatosis is best made by biopsy of involved sites. Hematoxylin and eosin stains of biopsy specimens from skin lesions show proliferation of small blood vessels in the dermis or cutis, enlarged endothelial cells with abundant cytoplasm, and necrotic and granulomatous changes. Warthin-Starry stains show perivascular accumulations of bacilli; these findings may be confirmed by electron microscopy, although this is not usually necessary. The diagnosis can also be established by culture of the organism in several special media or by detection of *Bartonella* DNA by polymerase chain reaction assay.[261] Serologic assays for anti-*Bartonella* antibodies are available through the Special Pathogens Branch of the CDC. The natural history of the infection in patients with HIV is for relapses to occur in the absence of prolonged therapy with erythromycin or doxycycline. Fluoroquinolones, other macrolides, and trimethoprim-sulfamethoxazole also have activity against *Bartonella*.

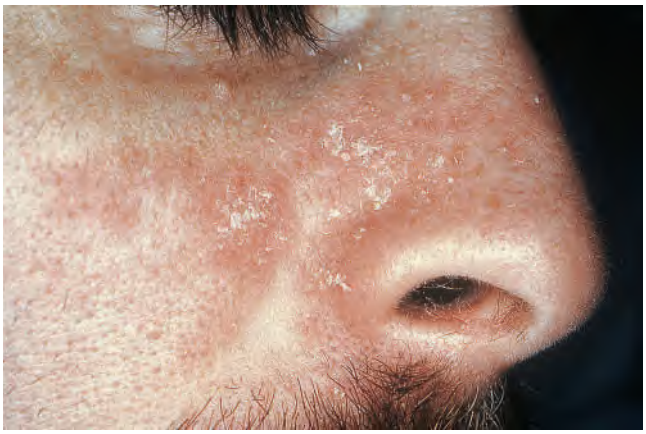

FIG. 122.14 Seborrheic dermatitis. *(Courtesy Dr. Stephen Raffanti.)*

Kaposi Sarcoma

Kaposi sarcoma is a vascular neoplastic disorder that in the United States is seen predominantly in HIV-infected MSM. Human herpesvirus type 8, which is transmitted sexually, has been implicated in the pathogenesis of Kaposi sarcoma (see Chapter 140).[226–265] Although Kaposi sarcoma also affects visceral organs, the characteristic findings are cutaneous red-purple nodules or plaques (Fig. 122.13). Sites commonly involved include the legs, feet, mucous membranes, hard palate, nose, trunk, and scalp.[266] Lesions of Kaposi sarcoma are often difficult to distinguish from lesions of bacillary angiomatosis; biopsy is required for diagnosis.

Other Cutaneous Manifestations

Various other skin disorders have been described in patients with HIV infection. Seborrheic dermatitis, an inflammatory condition of sebaceous glands that may be associated with dermatophytic superinfection, is an early complication (Fig. 122.14). Erythema and scaling of midline areas of the forehead, face, and groin are typical findings. Psoriasis occurs in 5% of HIV-infected individuals, with scaly reddish plaques, onycholysis, nail pitting, and subungual hyperkeratosis. Associated psoriatic arthritis

occurs more frequently than in HIV-seronegative individuals with psoriasis. Tinea infections of the scalp, trunk, inguinal and perineal areas, extremities, and feet are also common. Onychomycoses, or fungal infections of the fingernails and toenails, are common, although usually asymptomatic, causing only cosmetic changes. Bacterial folliculitis may be localized or disseminated in patients with HIV infection, and relapses frequently occur. *S. aureus* is the most common causative pathogen. Community-acquired methicillin-resistant *S. aureus* skin infections occur with increasing frequency in HIV-infected patients; risk factors include high-risk sex, drug-using behavior, and environmental exposure, but not immune status.[267] Eosinophilic folliculitis (Fig. 122.15) is an inflammatory condition associated with raised pruritic nodules with a pustular head on an erythematous base; it is similar to bacterial folliculitis. Biopsy specimens of these lesions reveal intense infiltration of eosinophils and absence of polymorphonuclear cells and organisms. Xerosis and ichthyosis are also common in patients with advanced HIV disease and may be refractory to therapy with emollients and antiinflammatory agents; antihistamines may provide symptomatic relief. Prurigo nodularis appears as nodules and papules caused by chronic rubbing and scratching. This is precipitated by one of the many causes of pruritus in HIV-infected patients, such as xerosis, eosinophilic folliculitis, and atopic dermatitis.

Disseminated cryptococcosis and histoplasmosis may cause mucocutaneous papules, nodules, pustules, or ulcers (Figs. 122.16 and 122.17). Biopsy and culture establish the diagnosis. Molluscum contagiosum can also cause small papules; the causative agent is molluscum contagiosum virus, a poxvirus. In Thailand and southern China, *T. marneffei* is a common opportunistic fungal infectious agent in patients with AIDS.[268] Patients can present with umbilicated papules, subcutaneous nodules, or morbilliform eruptions. Diagnosis is established by identifying the ellipsoid organism with central septation and characteristic red pigment production when grown in culture. Nontuberculous mycobacteria such as *M. avium* complex and *M. haemophilum* may cause cutaneous papules, pustules, abscesses, lymphadenitis, or ulcerations. Culture is required for diagnosis.

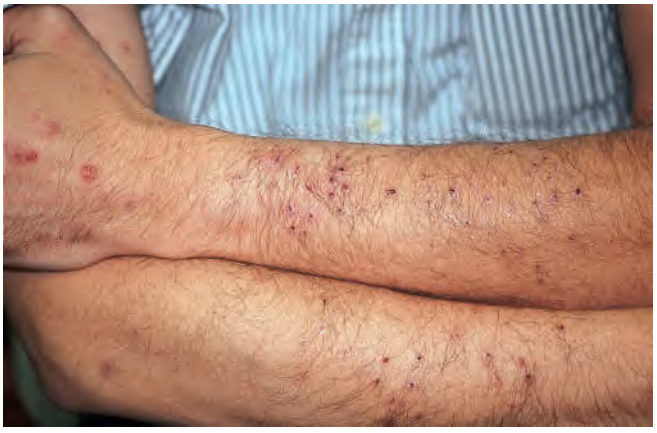

FIG. 122.15 Eosinophilic dermatitis. *(Courtesy Dr. Stephen Raffanti.)*

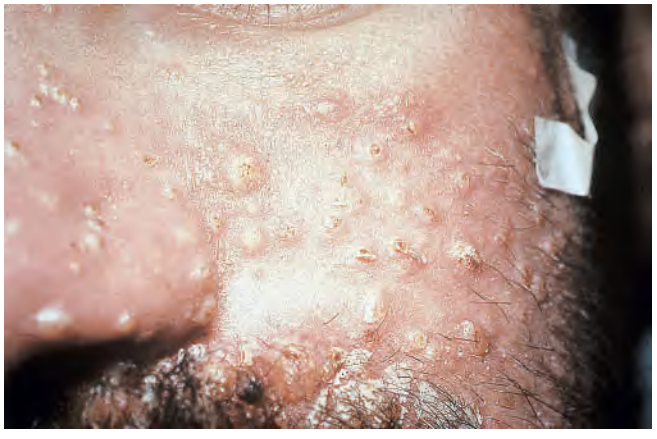

FIG. 122.16 Cryptococcosis. *(Courtesy Dr. Stephen Raffanti.)*

Scabies

Sarcoptes scabiei var. *humanus* is the mite responsible for scabies, a common ectoparasitic infestation, in HIV-infected individuals. Scaly pruritic papules or hyperkeratotic plaques may occur on the palms, soles, trunk, or extremities. Characteristic burrows between the fingers and on the wrists are not always seen. Norwegian (crusted) scabies (Fig. 122.18) is a severe and highly contagious manifestation of this disease, seen particularly with advanced immunosuppression.[269] Permethrin 5% cream and ivermectin are effective therapies.[270]

Renal Disease

A number of renal abnormalities have been described in patients with HIV infection since early in the epidemic, and the spectrum of conditions recognized has expanded over time, as has our understanding of the pathogenesis of these disorders.[247] Similar to metabolic disease, kidney dysfunction may result from direct effects of HIV itself, immune-mediated responses to HIV infection, opportunistic infections, and chronic inflammation in individuals successfully treated with ART. In addition, drug toxicity from antiretroviral agents and drugs used to treat opportunistic diseases and other complications is a major cause of renal problems in patients with HIV infection. Metabolic disorders such as

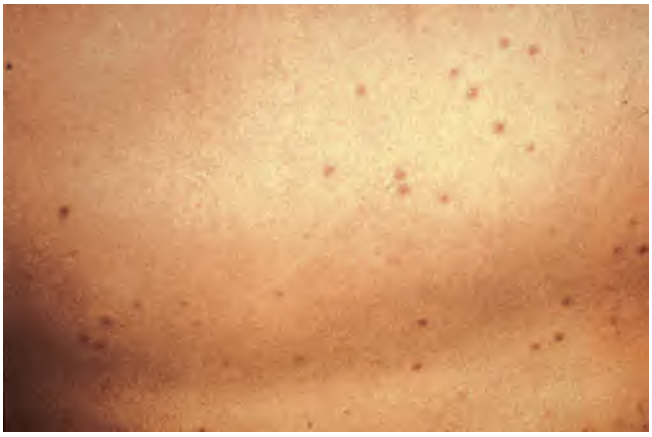

FIG. 122.17 Histoplasmosis. *(Courtesy Dr. Stephen Raffanti.)*

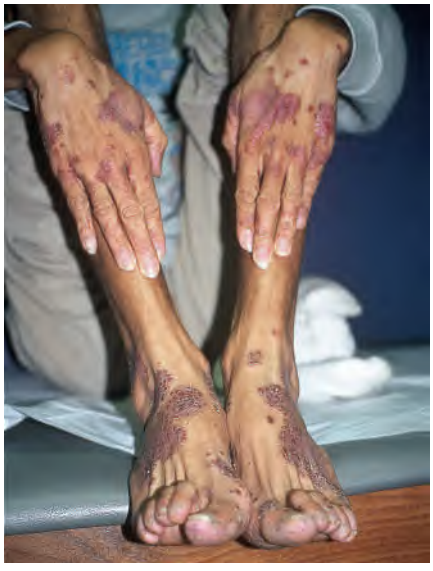

FIG. 122.18 Norwegian scabies (in a patient with wasting syndrome). *(Courtesy Dr. Stephen Raffanti.)*

diabetes and cardiovascular disease increase the risk of developing kidney damage as well, and risk factors for cardiovascular disease and chronic kidney disease are multiplicative.[271] Other conditions that mediate renal pathologic processes including injection drug use and HBV and HBC infection may also affect the kidneys.

Pentamidine, foscarnet, and the aminoglycosides can cause acute tubular necrosis, and indinavir, sulfadiazine, and intravenous acyclovir can cause intratubular obstruction by crystal formation. The widely used nucleoside reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF) accumulates in renal tubular cells via active transport by organic anions and can cause tubular dysfunction including proteinuria, diabetes insipidus, acute tubular necrosis, and, unusually, Fanconi syndrome. Patients with a normal glomerular filtration rate at the onset of treatment with TDF experience modest declines in glomerular filtration rate over time but rarely progress to chronic kidney disease. Individuals with underlying kidney impairment or low body weight may have a marked loss of renal function after initiating TDF, and the drug is not recommended for patients with chronic kidney disease. A newer formulation of a tenofovir prodrug, tenofovir alafenamide (TAF), does not undergo organic anion transport and does not accumulate in renal tubular cells, although it does achieve higher intracellular concentrations in peripheral blood mononuclear cells. TAF had less nephrotoxicity in clinical trials and resulted in improvement in renal dysfunction in patients switched from TDF-containing regimens. TAF is now recommended to replace TDF in US and other treatment guidelines.[162] Thrombotic microangiopathy and thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome have been reported in HIV-infected individuals and are associated with high HIV-1 viral loads and advanced immunosuppression. Hypertension may be a prominent feature, and the condition may respond to ART.[272] Immune complex–mediated glomerular diseases such as those associated with immunoglobulin A (IgA) nephropathy[273] and HCV infection[274] have also been reported.

The predisposition of patients of African descent to develop renal disease has been linked to polymorphisms in the *APOL1* apolipoprotein gene, which is hypothesized to have provided evolutionary protection against trypanosomal infections in Africa.[275,276] Although a variety of renal pathologic processes occur in patients of all races, and chronic renal insufficiency due to a variety of causes is not uncommon, black patients develop end-stage renal disease (ESRD) considerably more often than white patients, a consequence of the *APOL1* polymorphisms.[277] One study noted that more than 90% of patients with biopsy-proven HIV-associated nephropathy (HIVAN) had *APOL1* risk alleles. The pathogenesis of *APOL1*-associated nephropathies is not well understood but may involve changes in ion flux, mitochondrial dysfunction, and increases in inflammation.[278]

Manifestations of HIVAN include proteinuria, mildly elevated serum creatinine levels, and normal-sized echogenic kidneys on renal ultrasonography. Biopsy specimens show focal and segmental glomerulosclerosis on histopathology with glomerular injury, mesangial proliferation, tubular degeneration with microcyst formation, and capillary collapse.[279] Patients usually have detectable HIV RNA in the plasma.[279,280]

Renal dysfunction in patients with HIV disease is usually diagnosed incidentally when patients present with opportunistic infections and have a $CD4^+$ count less than 200 cells/mm^3.[281] Asymptomatic proteinuria, up to 5 g/day, is often the initial finding, and the serum creatinine level is often normal or only mildly elevated. The albumin concentration is almost always low, as is true for most patients with AIDS with opportunistic infections, and the blood pressure is usually normal. Renal biopsy most often shows focal and segmental glomerulosclerosis (collapsing glomerulosclerosis) with severe tubulointerstitial disease and proliferative microcyst formation. Immunofluorescence studies often reveal deposits of IgM and C3, and electron microscopy shows tubuloreticular inclusion bodies,[281] underscoring the association of this genetic association, but the presence of HIVAN in the small number of individuals without risk alleles indicates that other factors are also involved.[282,283]

The clinical course of HIVAN progresses quickly, usually because many other opportunistic processes occur simultaneously.[284] The optimal management for HIVAN includes rapid initiation of ART,[285,286] short courses (8–12 weeks) of corticosteroids,[287] and use of angiotensin-converting enzyme inhibitors for patients who can tolerate them.[288] Immunosuppressive therapy with cyclosporine has been tried but is probably not superior to corticosteroid therapy.[289]

A recent large observational cohort study demonstrated that the risk of ESRD remains high among HIV-infected individuals but is declining with improvements in viral suppression.[290] ESRD remains more frequent in black patients with HIV infection. Kidney transplantation to HIV-positive recipients appears to be feasible from HIV-negative as well as HIV-positive donors as long as viral suppression is maintained.[291,292]

Ocular Complications

Ocular diseases are extremely common manifestations of HIV disease with a wide variety of causes ranging from a benign HIV retinopathy to sight-threatening viral opportunistic infections.[293–295] As HIV disease progresses, the risk for ocular complications increases appreciably.

Before widespread initiation of ART, HIV retinopathy occurred in approximately one-third of all patients with HIV, with the prevalence higher in patients with low $CD4^+$ counts. The most frequent finding is a cotton-wool spot, a small pale lesion that is thought to represent transient focal retinal ischemia. HIV retinopathy may also produce microaneurysms and retinal hemorrhages. The condition is generally benign, although some patients have developed visual defects attributed to this condition.

The most common and serious ocular complication of HIV disease is retinitis, most often caused by CMV (see Chapter 137). CMV is ubiquitous in patients with HIV infection and causes serious morbidity in patients with AIDS. CMV is transmitted by the same routes as HIV, and almost all patients with sexually acquired HIV infection are also infected with CMV. Similar to other herpesviruses, CMV may infect cells latently and be reactivated when host defenses are impaired. Asymptomatic CMV viruria and viremia may be found in one-third to one-half of patients with advanced HIV disease. The risk for CMV retinitis is determined largely by the $CD4^+$ count and by the CMV DNA level in the peripheral blood. For patients with $CD4^+$ counts less than 200 cells/mm^3, the annual risk is 4% to 12%. Further risk stratification is aided by CMV DNA measurements, with aviremic patients having less than 1% risk per year, even at low $CD4^+$ counts.[296]

CMV has a unique predilection for the retina, with 90% of end-organ disease in patients with HIV infection being retinitis.[297] Other involved sites include the colon, esophagus, stomach, adrenals, pancreas, brain, and lungs. The onset of CMV retinitis may be insidious or rapid. Patients complain of painless, progressive visual loss, blurring, and floaters. CMV retinitis usually arises unilaterally, although it may subsequently progress to the contralateral retina. Funduscopic examination of the involved eye typically reveals coalescing white exudates in a vascular pattern, with surrounding hemorrhage and edema. Lesions are often peripheral initially, involve the fovea later, and result in visual loss. Retinal detachment may occur as a late complication.

The advent of ART has resulted in astonishing declines in the incidence of CMV retinitis. Several population-based studies have reported 60% to 90% reductions in the incidence of this disease. Improvement in cell-mediated immunity with ART results in suppression of CMV DNA in plasma, with a subsequent decrease in the risk for disease. It appears that patients with treated CMV retinitis who receive ART may safely discontinue anti-CMV therapy if their $CD4^+$ count rises to more than 100 cells/mm^3 for at least 3 to 6 months.[298,299] For patients with $CD4^+$ counts less than 50 cells/mm^3, education regarding retinitis symptoms and regular ophthalmologic examinations are recommended. Patients complaining of ocular symptoms should undergo a thorough ophthalmologic examination. Retinal findings may include cotton-wool spots or lesions of infectious retinitis. Less common retinal infections include toxoplasmosis, pneumocystosis, varicella-zoster virus, and ocular syphilis, which is usually a diffuse intraocular process. Cotton-wool spots are prevalent in patients with AIDS but do not appear to predict the development of other retinal disease. The cotton-wool spots are distributed in a vascular pattern similar to that of CMV but do not have the irregular pattern of full retinal exudates and hemorrhages characteristic of CMV retinitis. An ophthalmologist or other highly trained observer should examine any patient with signs or symptoms of retinitis promptly because delay in therapy can result in irreversible visual loss. Cultures of the blood and urine yield CMV in 60% and

80% of cases, respectively, although the diagnosis rarely rests entirely on these results.

Patients who have had CMV retinitis frequently experience acute retinal detachment. Erosion of the retinal border at the site of a necrotic lesion allows the retina to be lifted off underlying tissues. Patients complain of sudden loss of vision ("like a curtain falling" in front of the affected eye). Surgical reattachment is often partially successful in restoring vision, although progressive visual loss may ensue. Treatment options include oral valganciclovir, intravenous ganciclovir, intravenous foscarnet, and intravenous cidofovir in combination with ART (see Chapter 137).[298]

Varicella-zoster retinitis is a severe necrotizing retinitis (progressive outer retinal necrosis) that may occur in patients with advanced HIV disease and low $CD4^+$ cell counts, although some cases occur at earlier stages of HIV disease.[300] Patients most often note rapid visual loss. Funduscopic findings include peripheral necrosis, occlusive vasculopathy, optic neuritis, and vitreal and scleral inflammation, and the syndrome is termed *acute retinal necrosis.* Varicella-zoster retinitis usually occurs in the absence of zoster at other sites, but a history of varicella-zoster virus disease is common, and the virus may be isolated from tissue samples in some patients.[301] Blindness ensues despite therapy in most patients, although responses to intravenous ganciclovir and foscarnet plus intravitreal injections of ganciclovir or foscarnet or both have been reported.[298] Optimization of ART is also recommended.

Ocular toxoplasmosis occurs in patients with advanced immunodeficiency, and many, but not all, have cerebral toxoplasmosis. *Toxoplasma* retinitis is characterized by discrete, rounded, pale exudates. Lesions are usually discrete foci of retinal inflammation without hemorrhage or vasculopathy. Vitreal inflammation is common. The diagnosis is made by observation by an experienced ophthalmologist.

P. jirovecii may cause a choroiditis that mimics CMV retinitis.[302] The lesions are typically posterior, are yellow-orange, and do not cause vitreal inflammation. Choroidal pneumocystosis occurs most often in patients with previous *P. jirovecii* pneumonia, particularly in patients taking aerosolized pentamidine for prophylaxis.

Cardiac Manifestations

Early clinical observations suggested that HIV infection spared the heart, as memorialized in the activist/playwright Larry Kramer's award-winning play *The Normal Heart.* Subsequent experience showed that cardiac involvement in HIV and AIDS is not unusual.[303,304] Cardiac abnormalities in patients with HIV infection may include opportunistic infections or diseases of the myocardium (e.g., *T. gondii, Trypanosoma cruzi*) or pericardium (e.g., mycobacteria, Kaposi sarcoma), left ventricular dysfunction and dilated cardiomyopathy, cardiac autonomic abnormalities, and vascular heart disease such as pulmonary hypertension. Infectious endocarditis may also occur in patients with HIV infection, especially injection drug users, but there is little evidence to suggest that the risk is increased after accounting for behaviors. Marantic endocarditis is a manifestation of late-stage HIV disease and is sometimes noted at autopsy.

In patients with long-standing but treated HIV infection, accelerated atherosclerosis with myocardial infarction is increasingly common.[207] Population-based studies have demonstrated that rates of myocardial infarction and cardiac death are increased in HIV-infected veterans compared with control subjects, and HIV-infected men in the Multicenter AIDS Cohort Study have an increased prevalence of coronary calcification and total (but not calcified) coronary artery plaque than HIV-negative men.[305,306] The risk for coronary disease is associated with age, duration of HIV infection, nadir $CD4^+$ lymphocyte count, and duration of ART, suggesting in part that chronic immune activation plays a mechanistic role in the development of cardiac disease. This is consistent with the results of the Strategies for Management of Antiretroviral Therapy (SMART) study,[307] in which patients randomly assigned to interrupted ART had increased rates of cardiac death, which was associated with elevations in proinflammatory cytokines and prothrombotic markers.

Myocardial disease in HIV-infected individuals is surprisingly common, particularly in late-stage disease. Infectious myocarditis has been reported with a number of opportunistic infections, notably *T. gondii.* In an autopsy-based study in France, for example, 12% of patients with AIDS who died were found to have cardiac toxoplasmosis.[308] Most patients with cardiac toxoplasmosis also had cerebral involvement, although several patients had isolated toxoplasmic myocarditis. Myocarditis may be seen in acute *T. gondii* infection, and coincident toxoplasmic pneumonitis may present a clinical picture of diffuse pulmonary infiltrates and cardiac insufficiency. Other opportunistic agents causing myocardial involvement include mycobacteria and fungi.

A more common and underdiagnosed disorder in HIV-infected individuals is cardiomyopathy with left ventricular dysfunction, which may result in congestive heart failure.[309] Several large cohort studies in the pre-ART era found that 8% to 12% of patients with HIV infection had echocardiographic evidence of left ventricular dysfunction, and the incidence of dilated cardiomyopathy with severe congestive heart failure (New York Heart Association class III or IV) was 15% to 18% per year.[310,311] Clinical evaluation of patients may reveal only complaints of fatigue and exertional dyspnea, and physical examination may show tachycardia without rales or overt signs of congestive heart failure. Echocardiography shows global hypokinesis and enlargement of all four chambers, with a modestly to severely reduced left ventricular ejection fraction and increased end-diastolic volume index. Chest radiography is frequently unhelpful. The cause of HIV-related cardiomyopathies is multifactorial, with conflicting data on the causative role of cardiotropic viruses, vitamin deficiencies, and cardiotoxic drugs. However, there is compelling evidence that HIV itself is involved in the pathogenesis of cardiomyopathy in a large proportion of patients.[310,311] HIV has been identified in myocardial tissue by in situ hybridization in several studies and found to be positive in one-third to two-thirds of patients with myocarditis. In addition, an inflammatory cellular infiltrate composed of major histocompatibility complex class I–expressing $CD8^+$ cells is found in a large proportion of patients, suggesting an autoimmune mechanism in this process. Other viruses also implicated in dilated cardiomyopathy in patients with HIV infection include Epstein-Barr virus, coxsackieviruses, and CMV. Supportive treatment with digoxin, diuretics, and afterload reduction is usually of symptomatic benefit, and some patients with inflammatory myocarditis respond to corticosteroid therapy. Nonetheless, the prognosis for patients with this finding is poor,[312] although combination ART might change this.

Pericardial effusions have been reported in varying proportions of patients in a number of studies, many of which have had selection bias as a limitation. In patients with more advanced illness in hospital-based settings, pericardial effusions can be common. For example, a Portuguese study found that 41% of 181 HIV-infected patients had pericardial effusions.[313] Most effusions in this setting are asymptomatic or arise with signs and symptoms of opportunistic disease at other sites. The causes of pericardial disease in patients with HIV infection are diverse, but opportunistic infections and malignancies are the agents most commonly implicated. Mycobacterial infections, especially tuberculosis, are frequently associated with pericardial involvement in areas in which coinfection with HIV and *M. tuberculosis* is common, and nontuberculous mycobacteria may also invade the pericardial space. Other infections seen in the pericardium include bacterial infections, fungal infections (e.g., *Cryptococcus*), and viral cardiopulmonary infections. Kaposi sarcoma and non-Hodgkin lymphoma may also cause pericardial effusion. Cardiac tamponade or cardiac dysfunction resulting from effusions is unusual in most series, and in most cases pericardiocentesis is not necessary as a therapeutic maneuver. One study, however, reported that 40% of patients with an HIV-related pericardial effusion had signs of tamponade.[314]

Hematologic Manifestations

HIV infection can affect all three hematologic cell lines—leukocytes, red blood cells, and platelets. Although neutropenia and anemia are seen primarily in advanced disease, thrombocytopenia can occur in early-stage disease (e.g., HIV-related thrombocytopenia) or late-stage disease (thrombotic thrombocytopenic purpura). Neutropenia may be caused by HIV infection itself or may be an adverse effect of therapy. In one study, neutropenia was an independent risk factor for bacterial infection after controlling for $CD4^+$ count.[315] In another study of 71 patients with an absolute neutrophil count less than 1000 cells/mm^3 for a median of 13 days, only 6 (8%) developed culture-proven infection.[316]