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What is a pathogen? Toward a process view of host-parasite interactions

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Until quite recently and since the late 19th century, medical microbiology has been based on the assumption that some micro-organisms are pathogens and others are not. This binary view is now strongly criticized and is even becoming untenable. We first provide a historical overview of the changing nature of host-parasite interactions, in which we argue that large-scale sequencing not only shows that identifying the roots of pathogenesis is much more complicated than previously thought, but also forces us to reconsider what a pathogen is. To address the challenge of defining a pathogen in post-genomic science, we present and discuss recent results that embrace the microbial genetic diversity (both within- and between-host) and underline the relevance of microbial ecology and evolution. By analyzing and extending earlier work on the concept of pathogen, we propose pathogenicity (or virulence) should be viewed as a dynamical feature of an interaction between a host and microbes.

A century ago, Gertrude Stein told us that a rose is a rose is a rose, but today, modern genomics is telling us that a pathogen is not a pathogen — Eric C. Keen[1]

Introduction

Medical historians describe how diseases were seen either as 'things' or 'processes', and how this led to what are now known as the 'ontological' and the 'physiological' models of disease.[2] According to the ontological model, a disease is a foreign entity (either animate or inert), or an object 'lodged in the body'.[3] Ultimately, curing disease and restoring health amounts to expelling the intruder. In contrast, the physiological model frames a disease as a disturbance or as a deviation from the norm, and includes a temporal aspect. In this dynamic conceptualisation, health corresponds to the harmony or equilibrium established between the elementary qualities of the body that can be disrupted.[4] By 1850 in Europe, the ontological view, long associated with early theories of contagion, was largely out of fashion. However, it did gain a new foothold with the rise of medical microbiology in the last quarter of the 19th century, and enjoyed a lasting influence in the 20th century.[5]

More than mere historical curiosities, these models are reflected, at least partly, in current scientific concepts. The notion of a 'pathogen', for example, was long understood along the lines of the ontological model. A pathogen was seen as an essentially static or unchanging entity, which was absolutely distinct from other types of microbes in that it was believed to possess an inherent capacity to cause disease in hosts. The German bacteriologist Robert Koch, for instance, promoted a separation between 'harmful' microorganisms and other 'kinds' of microbes.[6] In the first decades of the 20th century, American microbiologist Hans Zinsser divided microorganisms into 'pure saprophytes' (unable to grow in living tissues), 'pure parasites' (able to rapidly enter and reproduce in a healthy host), and 'half parasites' (low and context-sensitive invasive power).[7] A number of medical bacteriologists in the 1950s, microbiologist Stanley Falkow remembers, also 'focused on differentiating the "good guys" from the "bad guys," and a pathogen was simply defined as any organism that caused disease'.[8] Even nowadays 'most authorities divide microbes into those that are pathogenic and non-pathogenic', according to immunologist and bacteriologist Arturo Casadevall.[9] But what if a pathogen is not always a pathogen?

By analyzing several significant conceptual shifts in our ways of thinking about microorganisms and their hosts, we show how scientists are now embracing a version of the physiological, process-oriented model of host-parasite interactions. Here, our use of the term parasite is ecological (see the Glossary) and includes both micro-parasites (e.g., viruses and bacteria) and macro-parasites (e.g., worms), and we define the concept of host-parasite relation as an interactive biological system, whose outcome is indeterminate and depends largely on the ecological context it inhabits.

Our main thesis is that a better understanding of virulence (i.e., the decrease in host fitness due to the infection) could be achieved if we asked under what (ecological) circumstances a microorganism acquires the capacity to bring about disease in a host, rather than looking for some specific attributes that might demarcate pathogens from commensals. In fact, the boundaries between commensalism, parasitism and mutualism are fluid, and these interactions may best be viewed as a continuum rather than as fixed categories in nature (**Fig. 1**). Indeed, 'symbiotic associations' can easily go from one to the other following small ecological changes.[10]

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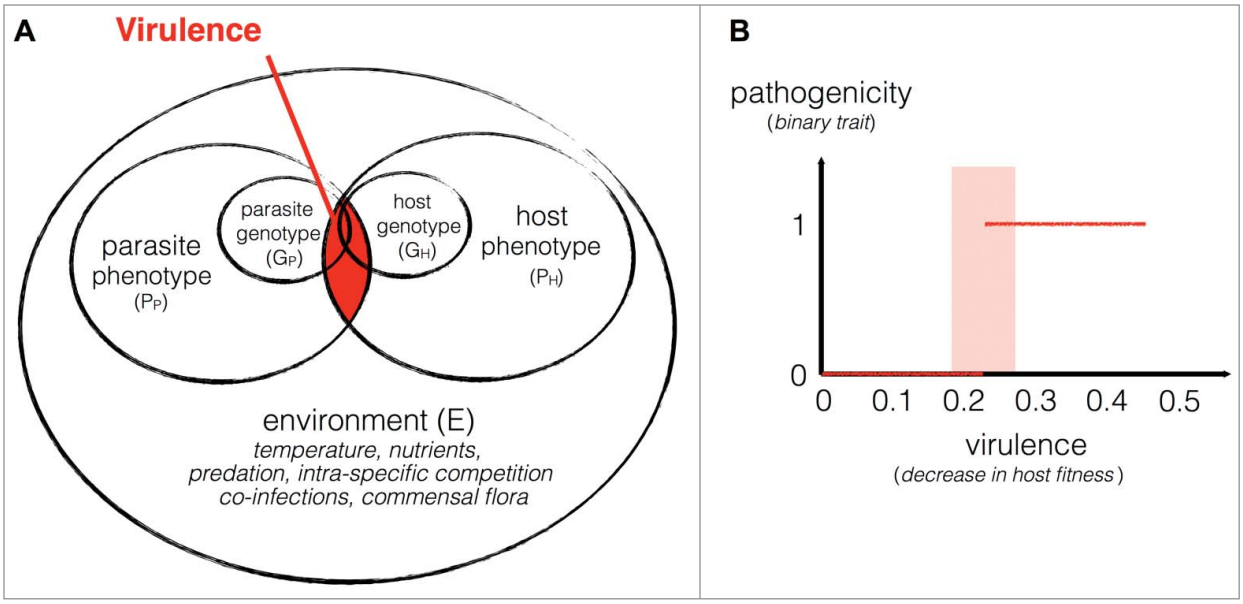

Fig. 1. Spatial schema representing how the virulence of an infection arises (**A**) and how a biological association moves from virulence to pathogenicity (**B**). Note that in (**A**) the genotype only partly determines the phenotype and that the environment includes many factors (e.g., multiple infections). In (**B**), one needs to set a threshold value in order to decide when a parasite is virulent enough to be considered a pathogen. The rectangle illustrates the uncertainty in defining such a value.

After reviewing part of the history of the study of host-pathogen interactions in the field of infectious diseases, we show that identifying the concept of 'pathogen' with that of disease-causing agent is an abstraction from a more complex biological reality that is now becoming untenable, particularly in light of ecological, immunological, and evolutionary considerations. Our claim stems from the conjunction of several lines of evidence, both historical and contemporary, which show that (i) the host and the wider ecological context usually determine whether an organism is pathogenic; (ii) the immune response can sometimes do more harm than the invading agents, or at least actively takes part in the harm caused; (iii) virulence is the outcome of a specific host-parasite interaction, not a fixed property of either the germ or the host; (iv) healthy carriers illustrate that the current method for experimentally distinguishing between pathogens and safe microorganisms, namely Koch's postulates, is often inapplicable; and lastly, (v) pathogens lack structures unique to them that would set them apart from commensals unambiguously. Many of these points are already present in the literature; however, we argue that in bringing them together we provide a stronger rationale for treating pathogens' identities as transient and open to evolutionary and ecological changes. Finally, we illustrate how an interactionist approach can help in clarifying the origin of pathogenicity and refining the principles employed by practitioners to classify organisms as pathogens or commensals.

From Foes to Friends: Thinking About Host-Parasite Interactions Historically

Microorganisms have long had an ambivalent status in the life sciences. When Charles Sédillot introduced the term 'microbe' in 1878 to designate microorganisms, those were 'negatively valued' by man in his concrete life and had yet to be 'positively valued' as objects of research in their own right.[11] As the concept of a 'pathogenic germ' progressively emerged in microbiology during the second half of the 19th century, healthy organisms were redefined as living entities free from germs[12], where 'germ' meant bacteria and viruses, as well as fungi and microscopic protozoans. Early medical bacteriologists conceptualized the relation of microorganisms to human health as antagonistic, and, following Robert Koch's call to arms, devoted substantial efforts to tracking germs in their 'remotest nook and cranny'.[13] In short, the 'germ theory of disease' led to a view that could have been summed up as 'man plus germ equals disease'.[4]

This sustained fear of germs was backed up by the rapid identification of a number of causative agents like tuberculosis, plague, typhus, smallpox, and malaria, among others, during the 'golden age' of bacteriology. At the turn of the 20th century biochemist Paul Ehrlich, a former student of Koch, developed the concept of a 'magic bullet', which reinforced the image of medical scientists as 'microbe hunters', to use the words of Paul de Kruif.[14] Solving the problem of infectious diseases was apparently straightforward: identify and eliminate the intruders with the help of magic bullets. When antibiotics became widely available after the Second World War, they appeared to confirm to Erhlich's vision. The development of antibiotics was seen by many as 'the dawn of a new era in the control of infectious disease'.[15] This age of optimism witnessed the development of various means to successfully treat syphilis, staphylococcus infections and tuberculosis. In turn, the production of vaccines led to a dramatic decline of common and deadly diseases such as diphtheria, polio, pertussis, tetanus, and mumps. The large-scale use of DDT to control insect-borne diseases not

only fostered hopes of conquering tropical diseases like malaria but also led to the development of global eradication programmes by the World Health Organization (WHO), which announced the eradication of smallpox in 1979.

In light of these achievements, and despite some warnings about potentially unwanted effects, medical officers in the 50s, 60s, and 70s were generally optimistic about the coming end of infectious diseases.[16] This optimism was also based on the view that microorganisms were largely static entities, unlikely to evolve, and on the belief that natural selection would lead to a decline in virulence between hosts and parasites.[17] The long-term trend, it was held, was toward the evolution of avirulence: excessively virulent microbes would, in killing their hosts, destroy themselves, as their own transmission would be prevented. Though dismissed, this view is gaining a renewed currency as microbiological ecology reveals that the vast majority of microorganisms are innocuous and even beneficial to humans, plants, and animals.[18]

Starting in the late 1970s and culminating with the global AIDS pandemic, the emergence of 'new' diseases challenged this account of the forthcoming conquest of infectious diseases.[19] A recent report has outlined over 300 'emerging events' between 1940 and 2004, caused largely by socio-economic, environmental, and ecological factors.[20] Furthermore, a large number of these emerging events are due to the evolution of drug resistance.[21] Facing the constant challenge of infectious diseases, a number of scientists are now rediscovering the 'natural history' tradition of host-parasite interactions. Running somewhat parallel with the 'microbe hunting' and 'magic bullet' paradigms, this other, less visible tradition characterizes host-parasite relations in broader ecological and evolutionary terms.[22] Early proponents include Theobald Smith, René Dubos, Frank Macfarlane Burnet, Frank Fenner, and more recently Joshua Lederberg. For most of these researchers, dissatisfied with reductionist claims as embodied by classical bacteriology, health and disease rest not only on the presence or absence of specific bacteria or other germs in the body but also on the balanced relationship established between hosts and parasites.[23] The co-evolutionary history of humans and microbes supports the view that we have learned to cohabit peacefully with many, if not most, potentially pathogenic germs and that ecological or environmental modifications can disturb this equilibrium and induce disease.[24]

Observing that public health policies – often oriented toward eradication programs – have tended to be 'permeated with Manichean images between good and evil, or life and death', Lederberg advocated the 'need to pay much closer attention to ecological relationships among various microorganisms'.[25] In an influential essay published a year later[26], he invited scientists to reject the 'metaphor of war' in favor of an ecological one – one that would be better suited to capturing the complex and dynamic relations human have evolved – and are evolving – with their microorganisms. To gain the upper hand over infectious diseases, some suggested, we would be better off if we could 'embrace notions of balance that have been eclipsed by Pasteur's germ theory and the quest for magic bullets'.[27] A landmark report issued in the wake of Lederberg's call, *Ending the War Metaphor: The Changing Agenda for Unravelling the Host-Microbe Relationship*, acknowledged the diversity and evolutionary potential of microbial life forms.[28] Replacing the war metaphor with an ecological one shifts the focus of inquiry away from an ontological model of microbial disease toward the intricate and diverse nature of hosts, microbes, and their processual relationships, as assumed by the physiological model described above.

Moving from warfare to ecological concepts also affects the definition of the concept of a pathogen. As the authors of *Ending the War Metaphor* noted, 'it has become exceedingly difficult to identify what makes a microbe a pathogen'.[28] Advances in genomics and microbial ecology have led to a clearer picture of the origin of virulence factors, others have argued, but they have also 'helped to blur the distinction between pathogens and non-pathogens'.[29] Another recent US report entitled *Sequence-Based Classifications of Select Agents: A Brighter Line*, pointed out that 'biology is not binary' and that there are 'no clear-cut boundaries that separate a pathogen from a non-pathogen'.[30] Even more recently, it has been suggested that 'modern genomics is telling us that a pathogen is not a pathogen'[1] and that 'attempts to classify microbes as pathogens, non-pathogens, opportunists, commensals, and so forth are misguided because they attribute a property to the microbe that is instead a function of the host, the microbe, and their interactions'.[31]

In the end, large-scale sequencing not only shows that identifying the roots of pathogenesis is much more complicated than previously thought; it also forces us to reconsider what a pathogen is and how we should think about it. The static definition of pathogens has been challenged several times in the past and authors have proposed alternative ways to analyze virulence,[31-33] e.g., the damage response framework that we discuss in a later section. Building on this previous work and on recent elements from genomics studies, we criticize further the 'fixist' view.

Pathogen in Post-Genomic Science

Opportunities make pathogens

Several pathogen-like organisms regularly colonize places in the human body and persist without causing clinical symptoms.[34] Conversely, some potentially pathogenic organisms (*S. pneumoniae*, *N. meningitidis*) transiently colonize the nasopharyngeal milieu even though they are not part of the normal flora.[35] The fungus *Candida albicans* commonly found in humans, for instance, can be both a commensal saprophyte and a pathogen at different times.[36] Even genetically identical members of a species of microorganism (i.e., clones), such as uropathogenic *Escherichia coli*, can be highly pathogenic in one environment but not in another.[37] Furthermore, several species of microorganism cause disease only in immunocompromised organisms, not in immunocompetent ones.[38] Previously non-pathogenic organisms, in contrast, can penetrate a host's immunological barriers during medical surgery, or thanks to immune-suppressive therapies. However, predicting the consequences of a treatment is not straightforward. For instance, if immunosuppression is achieved in mice by using a chemotherapeutic agent, the host is more sensitive to infection by the fungus *Aspergillus*

fumigatus than if the immunosuppression is achieved using corticosteroids.[39]

Until recently, the determinants of health and disease due to microorganisms composing the 'normal flora' of individuals were largely ignored.[40] Host-microbial interactions in the microbiota, however, are subject to change as some residential microorganisms have the ability to protect against some invaders while at the same time causing disease.[41] The relation between a host and its microbial communities is now being investigated using metagenomics tools and in light of the concept of 'microbiome' – the totality of microbes living in constant interaction with their hosts. Changes in the genetic composition of the microbiome, it has been shown, can seriously affect disease risk.[42-43]

Microbiologists have long distinguished between 'primary' and 'opportunistic' pathogens.[32] Whereas opportunistic (or facultative) pathogens require an injured host to cause infection, primary pathogens do not. For example, microorganisms living in reservoirs (e.g., soil, water, or other animals) are often called 'facultative pathogens' as they cause disease only when they encounter a susceptible host.[44] Primary pathogens are thus seen as 'irreversible specialized parasites', in contrast to 'Jekyll-and-Hyde pathogens' that can switch from commensalism to pathogenicity via the acquisition or loss of genes.[1] Primary pathogens' capacity to cause disease enables them to use other organisms as a source of nutrition and as a locus for replication and transmission to new hosts.[32]

As has been pointed out, for instance by Casadevall and Pirofski, a strict demarcation between primary and opportunistic pathogen is problematic because primary pathogens that are able to cause disease in immunocompetent hosts (e.g., *M. tuberculosis*) usually cause disease at an even higher frequency in immunocomprised hosts, and so could be considered to be opportunistic just as well.[45] It is also important to stress that primary pathogens do not always express pathogenicity. For instance, most *Salmonella typhimurium* bacteria, when infecting the gut, do not produce virulence factors (Type III secretion systems). The production of these toxins is due to phenotypic noise such that a strong minority of the (isogenic) bacteria express the gene.[46] This plasticity in pathogen phenotype can sometimes be very adaptive in terms of the ability to react to changes in the environment.[47]

To summarise, non-pathogens or commensals can become pathogens following changes in the host environment. As we will see later on, the reverse is also true and 'pathogens' can end up protecting their host against more virulent parasites.

Immunopathology: when the enemy is us

Parasites are not always solely to blame for the nature and scope of host damage: the immune system is often involved as well. In a healthy host, the immune system usually detects pathogens and responds to parasitic invasions (e.g., it secretes cytokines, phagocytises bacteria, etc.). The main function of the immune system is to keep growing microbial populations at bay, a regulative process occurring at the mucosal interface that often determines whether a microorganism should be deemed a pathogen or not.[48] Toll-like receptors (TLR) are molecules capable of detecting specific patterns of prokaryotes and bacteria that, in turn, inform the immune system that those potentially dangerous invaders have crossed the immune barrier.

Innate and adaptive forms of immunity usually work together to prevent the body from falling prey to the countless microorganisms present in the environment. Many of the visible signs and symptoms usually associated with infection and disease (e.g., swelling, redness, production of pus), however, are the result of the immune system attempting to defeat the microbial intruders, not of the microbes' offensive mechanisms themselves – despite being induced by them. Mounting a strong immune response can also have significant fitness-related costs to the host, which may explain why such responses are often suboptimal.[49]

In fighting infection and clearing out parasites from the body, the immune system can sometimes turn against itself – a phenomenon known as 'immunopathology'. In some cases, the exaggerated immune response is responsible for more harm than is the pathogen load thriving in the blood stream. Immuno-pathological responses able to augment the size of the skin lesions, for instance, are increasingly considered to be a very common cause of infectious diseases. Extensive tissue damage generated by resistance to leishmaniasis (causing more than 50000 deaths annually) would be an example of this. Hence, in addition to pathogen-induced damage, there is evidence that immunopathology is significantly involved in the etiology of at least 10 tropical diseases ranked as high priority by the WHO and including tuberculosis, malaria, Chagas disease, and leprosy.[50] In some cases, experiments permit the amount of damage due to the immune system to be quantified, for instance in some rodent *Plasmodium* infections.[51] In the case of tuberculosis, the underlying mechanisms of immunopathology are particularly well understood, with an important role granted to chemokine molecules.[52]

In brief, even though pathogens can seriously disable the host using a range of virulence determinants (e.g., adhesins, invasins, toxins, etc.), pathogenesis usually results from the complex interplay between the immune system of the host and microbial communities forming the microbiota.

Measuring and conceptualizing virulence

Virulence is commonly thought of as a microbial property. However, microbiologists have long known that virulence is not a constant property of the causal germ but that it can vary both experimentally (in vitro and in vivo) and spontaneously, and that it can be enhanced, lost, and restored.[53] As a consequence, the same microorganism can exist in both pathogenic and commensal forms in natural or experimental contexts. This makes measuring levels of virulence challenging. One of the most common measures of virulence in virology is the LD_{50} test, namely the infective (lethal) dose needed to kill 50% of the hosts. The corresponding measure of virulence is the number of host deaths per unit of time. However, this measure (dead/alive) is entirely qualitative and cannot take account of quantitative changes such as non-fatal infections in prolonged host-parasite interactions.[54] Other relevant measures of virulence, including the production (and size) of lesions, a decrease in host fitness, a reduction in host mobility, and the speed at which death occurs, demand a more quantitative approach. Conversely, for some pathogens like HIV,

the LD_{50} measure may not be very informative. (In the absence of treatment, HIV always kills its host.)

In the second half of the 20th century molecular approaches to pathogenesis gave rise to the concept of 'virulence gene'. This concept was particularly successful in plant biology[55], and carrying 'virulence genes' came to be regarded as a reliable indicator of pathogenicity outside the field of phytopathology as well.[56] As noted by microbiologist David Relman, for instance, '[o]ften the only difference between a pathogenic and a non-pathogenic strain of the same species, e.g., enteropathogenic and nonenteropathogenic *Escherichia coli*, is a small set of virulence genes'.[57] Despite the success achieved by this concept, the expression of those genes and the production of a diseased phenotype strongly depends both on the physiological and ecological environments. Moreover, the function(s) of those genes hinges on the definition of virulence that is adopted,[58] but no definition is universally accepted.[59] Whereas microbial genes perform a range of biological functions capable of harming the host, several opportunistic microorganisms are able to bring about debilitating effects in a host even without possessing any so-called virulence genes or virulence factors.[60] Some have therefore proposed distinguishing between 'true virulence genes' (directly involved in host damage), 'virulence associated genes' (involved in the regulation or expression of virulence factors), and 'virulence life-style genes' (involved in colonization, immune evasion, survival and reproduction), knowing that, in the end, where to draw the line is a matter of perspective, emphasis, and definitional criteria.[58]

Overall, measuring virulence is often done in a comparative way (i.e., strain A is more virulent than strain B in host C in environment E). Importantly, different assays of virulence can yield different – and sometimes inconsistent – results depending on the inoculation route, the quantity of inoculum, and the genetic profile of the host organism chosen.[61] The relativity of virulence measures is another problem for the definition of what pathogens are.

Koch's postulates in microbiology and molecular biology

To identify and classify pathogenic microorganisms, Friedrich Löffler, one of Koch's colleagues in Berlin, developed a sequential set of criteria known as 'Koch's postulates'. Though their number vary in the literature, these can be summarized in 4 methodological steps: (1) the presumed causative microorganism must be observed in every occurrence of the disease; (2) the organism must be successfully isolated and grown in pure culture; (3) once inoculated to new susceptible hosts, it should yield the same patho-phenotype; (4) the microorganism must be recovered from the experimental animal that was inoculated. The postulates rapidly became entrenched in experimental medicine as they provided new ways for identifying and controlling infectious diseases.[62]

As bacteriologists such as Emile Duclaux and others soon pointed out, Koch's postulates face a number of limitations, including the impossibility of cultivating some bacteria or viruses in the laboratory, the artificial aspects of experimental inoculations in animal models, and the fact that the same bacterium does not always produce the same disease in different hosts.[63] Edwin Oakes Jordan, an early 20th century American public health scientist and bacteriologist, for example, argued that 'no sharp line can be drawn between pathogenic and non-pathogenic microorganism (. . .). The conception of a pathogenic microorganism is a relative, not an absolute one; that is to say, no microbe is known that is capable under all conditions of producing disease in all animals'.[64]

One underlying assumption of the postulates is that pathogenic germs should only be observed in infected, and not in otherwise healthy, individuals. Subclinical or latent infections and 'healthy carriers' harbouring pathogenic germs in sufficient quantity, however, turned out to be very common in human and animal populations, particularly during epidemic outbreaks.[13] One of the most famous of these asymptomatic carriers was 'Typhoid Mary', an American cook who between 1901 and 1907 was accused of having caused more than 25 cases of typhoid in the houses where she was employed, without herself expressing any symptom. After her arrest by police, laboratory tests confirmed 'she carried an almost pure culture of *Salmonella typhosa* in her bowels'.[65]

The postulates were regularly updated to reassess causation in relation to the rise of technological tools in the fields of virology, epidemiology, and molecular microbiology and molecular medicine.[62] In the late 1980s, Stanley Falkow reformulated the initial postulates to make them more readily applicable to the analysis of bacterial virulence in the context of modern microbiological research as follows: (1) the phenotype or property under investigation should be associated with pathogenic members of a genus or pathogenic strains of a species; (2) specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence; (3) reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity.[66]

While the 'molecular Koch postulates' provided a conceptual framework in experimental biology they also 'rest on the assumption that there is an essential distinction between pathogens and non-pathogens'.[29] As Pallen and Wren have argued, this dichotomy is problematic, particularly in post-genomic science context, and leads to a dilemma: insisting on the first postulate means that any factors common to pathogenic and non-pathogenic organisms cannot count as virulence factors (because a potential virulence factor should only be present in pathogenic species); whereas if the first postulate is left out, then many virulence factors exist in non-pathogenic individuals as well.

Failure to satisfy Koch's postulates does not entail that the putative microorganism (or factor) plays no role in pathogenesis; however, it shows that pathogenicity cannot solely be attributed to an encounter with particularly virulent organisms (or its parts) but needs to involve consideration of the host response as well.

Pathogens have no structure or function unique to them

From the last 3 decades of the 19th century until today several pathogen-like organisms have been classified on the basis of their physiological, morphological, cellular, and molecular traits.[67] For example, Frederick Griffith's bacteriological work on pneumococci in the 1920s led to the identification of virulent organisms with a transmissible physical feature: the polysaccharide capsules, which allows organisms to evade phagocytosis.[31] In the

mid-20th century, the distinction between pathogenic and non-pathogenic organisms was carried further, following the discovery that 'plasmids' – self-replicating and heritable entities – encode resistance and virulence factors.[68] As Falkow emphasized: 'Thus in *some but not all* strains the acquisition of these 2 plasmids was enough of an addition to a strain's genetic potential to tip the balance from that of a non-pathogenic commensal of the normal flora, to that of a strain now capable of producing overt disease'.[69]

At the dawn of the genomic era in the 1990s, an attempt at formulating at the molecular level the distinction between pathogens and harmless organisms was proposed. This attempt was made on the basis of the discovery that the integration of 'pathogenicity islands' – large DNA regions in the flexible part of bacterial genomes – into a bacterial genome can 'in a single step, transform a normally benign organism into a pathogen'.[70] As microbiologist Jörg Hacker and his colleagues remarked: 'comparative genome analysis provides a promising instrument to investigate the differences between non-pathogenic and pathogenic strains as well as between different pathotypes on the nucleotide level'.[71] Pathogenicity islands, indeed, were initially thought to exist only in pathogenic strains or species, and were often characterized as 'regions in the genomes of certain pathogens that are absent in the non-pathogenic strains of the same or closely related species and that contain large continuous blocks of virulence genes'.[72]

The molecular mechanisms operating in pathogenicity islands, however, are much more common than it was first suspected. Indeed, they are found in phylogenetically distinct species where they perform different biological functions, often not related to pathogenicity. For instance, *Yersinia pestis* (bubonic plague) contains a 'high pathogenicity island' coding for an iron uptake system (a virulence factor) that is also present in about 30% of non-pathogenic members of the species that were isolated from the human digestive tract.[73] Following the discovery of several similar 'islands' (termed 'symbiotic', 'fitness' or 'ecological') in non-pathogenic strains, pathogenicity islands were redefined and are now regarded as 'more general genetic entities' whose function is largely ecologically mediated.[74,75] Several 'virulence systems', for instance, are commonly found in both pathogenic and non-pathogenic bacteria. Those include: type III (e.g., *Shigellae, Salmonellae*) and type IV secretion systems (e.g., *Vibrio cholerae*), ESAT6 secretion system (e.g., *Mycobacterium tuberculosis*), and invasion genes (e.g., in *E. coli*).[29]

The possibility of demarcating pathogens from non-pathogens from a functional or structural point of view was also frequently debated in the field of immunology. Recognizing that the 'self and non-self paradigm'[76] was 'reaching the asymptote' Charles Janeway, for example, introduced the 'non-infectious self' model in an attempt to reconcile research on adaptive and innate immunity.[77] He also suggested that pathogens display specific characteristics. In particular, he argued that the innate immune system does not recognize 'non-self' but foreign 'patterns' that are typical of pathogenic microorganisms. Antigen-presenting cells (APCs) of the host have evolved, according to Janeway, to interact and detect such widespread and invariant molecular patterns present in all pathogenic microorganisms of a same class: 'The receptors of the innate system [. . .] are specific for structures found exclusively in microbial pathogens (pathogen-associated molecular patterns), which is why they function to signal the presence of infection'.[78]

Those microbial motifs, called 'pathogen-associated molecular patterns' or PAMPs (e.g., lipopolysaccharide, bacterial lipopolysaccharide, bacterial DNA, etc.), are assumed to be conserved throughout the evolutionary history of the microbial species (as they promote survival) and are recognized in hosts by 'pattern-recognition receptors' (PRRs) and other kinds of receptors. PAMPs are thus evolutionary conserved molecules that distinguish pathogens from other commensal microorganisms. The PAMP model, however, was widely criticized for suggesting that it conceives of pathogens 'as mere bags of PAMPs'.[79]

As it turned out, non-pathogenic microbes can activate PRRs,[80] and thus PAMPS are unable to demarcate pathogens from non-pathogens. In fact, PAMPs have later been renamed 'microbe-associated molecular patterns', or MAMPs, accordingly.

Within-Host Diversity and Evolution of Pathogens

The damage response framework (DRF)

Shortly after Lederberg's emphasis on disease ecology, a dynamical dimension was conferred on the concept of host-parasite relations, in particular thanks to the development of the 'damage-response framework'. This framework rests on 3 general principles: (i) virulence is the outcome of host-parasite interactions; (ii) 'damage' to the host is the main criterion that determines the pathological outcome of the interactions; and (iii) host damage is the result of both microorganism and host factors acting together.[81] The DRF explicitly defines virulence as the outcome of host-parasite interactions, not as a microbe or a host property. Here, the concept of pathogen means 'a microorganism that is capable to cause damage to a host'[81] where damage is characterized as a function of the host response and can be measured with respect to the whole organism or some of its parts (cell, organs, tissues, etc.).

Since its inception the damage-response framework has fared well in microbiology and immunology. However, DRF remains relatively silent with respect to the ecology and evolution of microbial strains. We think this is a significant shortcoming of the model as the outcome of a given host-parasite interaction is not only dependent on the immune state of the host but also on the wider environment, both within the host and outside the host. In what follows, we improve on this framework by considering how evolutionary and ecological factors come together in determining whether a biological association is pathogenic or not. Particular emphasis is placed on within- and between-host processes.

Multiple infections and within-host diversity

The implicit notion that a single germ is causally responsible for pathogenesis diverts from considering within- and between-host selection pressures, among other relevant factors shaping virulence. Competition between microbial strains within a host and between hosts means that a microorganism does not strike alone (or at least

very rarely). On the contrary, individuals often become sick because they are infected by a number of microbial strains, sometimes of different species, that can act together or in competition with one another. Synergy between microorganisms, or microbe-microbe interactions is commonly found in the case of the influenza/pneumococcus interaction, for instance, and can potentially disrupt the host' immune response and facilitate pathogenesis.[82]

Several examples illustrate that infection is a population and ecological concept, and that multiple infection is the norm rather than the exception. For instance, not only are the majority of infected adults simultaneously infected by more than 5 *Plasmodium falciparum* strains,[83] but also recent results obtained using next-generation sequencing suggest that this number is perhaps a severe underestimate.[84] One of the processes that make these **co-infections** so common (and so important to understanding virulence) is co-transmission, i.e., the simultaneous transmission of co-infection genotypes (in the case of *Plasmodium falciparum* infections).[85] Multiple infections, or co-infections, can involve different pathogen species. In Africa, *Plasmodium* and HIV cause many co-infections, and these have been shown to have epidemiological implications by speeding the spread of HIV.[86] This phenomenon could be due to the fact that HIV viral load increases during malaria febrile episodes, which could in turn increase virus transmission.[87]

Multiple infections challenge our ability to define what a pathogen is because the presence of a third party can radically alter the relationship a microorganism has with its host. For instance, many multi-drug resistant bacteria will not create disease in most hosts (partly because drug resistance mutations tend to incur fitness costs) but become a major issue in hosts co-infected with HIV.[88] More recently, it has been shown that co-infection by influenza virus and bacteria in mice leads to a decrease in the host's ability to tolerate tissue damage (several genes involved in tissue repair are down-regulated), which could explain the virulence of these co-infections.[89] The ability of pathogens to resist treatments obviously affects our readiness to classify a microbe as a pathogen. Indeed, for similar levels of virulence, a drug-resistant micro-organism will represent a much more serious threat to society.

Note also that interactions between the resistance of a strain and its virulence may occur. For instance, for some fungi of the *Candida* species, resistance can be associated with changes in the cell wall that also allow immune evasion.[90] Therefore, resistant strains could also cause more virulent infections even in untreated hosts. Being infected by a (mild) pathogen, however, can sometimes yield protection against more virulent ones. Infection by the apparently non-pathogenic flavivirus GB virus C (also referred to as hepatitis G), for instance, has been reported to prolong survival in patients infected by HIV.[91,92] In insect or bacterial diseases, however, there is much more evidence (e.g., recent results showing how the bacterium *Wolbachia* protects mosquitoes against dengue, plasmodium and chikungunya infections).[93] In fact, some have even proposed using this conflict between HIV and other viruses as a tool to develop new therapeutics.[94]

Abiotic factors such as temperature or humidity are known to affect the virulence of many infections in model organisms under laboratory conditions. But biotic factors are also indirectly linked to multiple infections, as in the case of *Plasmodium falciparum* where adults seem to be protected from developing virulent infections after 2 infections. Acquired immunity plays a key role in understanding the virulence of these infections[95] and some epidemiologists have suggested that the virulence of an infection could increase as a pathogen becomes more prevalent because the access to health care becomes more difficult.[96] More generally, multiple infections provide an illustration of pleiotropic interactions, i.e., the fact that the environment of the host, meaning in some cases the outside world, determines whether a microorganism is a pathogen or a mutualist, a situation further complicated by the fact that the composition of the commensal flora could also greatly affect the pathogenicity of a microorganism.[97]

Microbial evolution

Multiple infections and within-host diversity raise a related issue, namely microbial evolution. Traditionally, not only were most microorganisms classified as pathogens, they were also implicitly assumed not to change over evolutionary time. The germ theory of disease formulated by Pasteur and Koch assumed the constancy of bacterial species.[98] Of course, bacteria were known for their ability to physiologically adapt to new environments but until the 1940s, these changes were not considered to have a genetic basis.[99] This assumption that the microbial world is largely static was only recently challenged although it still prevailed among microbiologists in the 1970s.[17] As Lederberg pointed out 'the historiography of epidemic diseases' was 'one of the last refuges of the concept of special creationism'[100] (but see Mendelsohn who argues that changes in microbe virulence were already analyzed as an evolutionary process by Pasteur and Koch).[101] Yet part of the reason why some microbes are deadly is precisely their ability to evolve and be a 'moving target'. A direct effect of microbial evolution is that an infection that was harmless to the host can become pathogenic over the course of an infection. This has been put forward as an explanation for the virulence sometimes caused by *Neisseria meningitidis*. In the majority of infected hosts, the bacterium is a commensal. However, in some cases the same bacteria is responsible for bacterial meningitis, an extremely severe disease in which bacteria infect the cerebrospinal fluid (CSF).

Levin and Bull argued that this pathogenic outcome is the result of bacterial within-host evolution selecting for mutants that are adapted to colonising the CSF.[102] This strategy is clearly non-optimal on the long term as hosts often die before the bacteria are transmitted, but natural selection is blind and always favors the best competitor in the short term. More recently, Margolis and Levin have shown that the scenario hypothesized in the cased of *N. meningitidis* occurs for another bacterial infection (*H. influenzae*) in rats.[103] The question is then, are these bacteria pathogens? Or are only some of them, the mutants that can appear during an infection, pathogens? In most cases the pattern will not be as striking because the virulence will only change gradually. For instance, there is evidence that the replicative capacity of HIV increases (albeit slightly) throughout the course

of an infection[104] and that virulence has been increasing over time at the host population level.[105]

It is crucial to recognize that pathogens have histories and that some microorganisms have evolved, in a distant or more recent biological past, the ability to breach a host's defenses under specific circumstances. And it is no less important to acknowledge that because they are subjected to diverse selective pressures that depend on changes in the environment, those organisms are also, and for the most part, currently evolving.[106] Since the seminal work of Anderson and May and Ewald, epidemiological models have postulated that the pathogenicity of infectious diseases, usually referred to as 'virulence', can evolve as the disease spreads in a population.[107,108] This provides theoretical foundations for controlling virulence, the idea being that changing cultural practices could redirect selective pressures, for instance by decreasing opportunities for microbial transmission, and orientate the evolution of parasites toward decreased levels of virulence. Similar models have spread the idea that virulence can be adaptive from the point of view of the microbe, which explains why not all infectious diseases are avirulent.[23] The 'evolvability' of virulence is well demonstrated in serial passage experiments, where a microbe is transferred manually from one host to the next, thus removing the cost of transmission. As summarised by Ebert, it takes only 10 passages to evolve a mild strain of the bacterium *Salmonella thyphimurium* that kills less than 5% of infected mice to a strain that will decimate 90% of the infected mice.[109]

Such rapid variations in virulence form the basis of attenuated vaccines such as Sabin's polio vaccine: by adapting a pathogen to a host and making it extremely virulent on this host, one can obtain an evolved strain that is almost avirulent to the host of origin. There are some documented cases of rapid evolution in the field. Arguably, the most striking of these is the evolution of Marek disease virus (MDV) in response to vaccination. In the 1950s, this virus would cause only mild disease with paralytic symptoms in poultry and host mortality was negligible. The virulence of the virus has increased over the years and some of the strains that currently circulate cause up to 100% mortality. These increases in virulence seem to have been caused by vaccination campaigns against the virus, each new vaccine selecting for more virulent strains.[110]

This shows that the pathogenic nature of a microbe is a quantitative trait and that it can evolve rapidly, especially in response to public health policies. Pathogen evolution thus raises issues at the population level: not only is it important to treat those individuals that are infected, but also it is imperative to do so by avoiding an arms race with pathogens as each public health policy is likely to trigger an evolutionary response. This dual perspective (cure patients and control pathogen evolution) has been put forward as a fundamental goal of **virulence control practices**[111] and is becoming central a central tenet for avoiding drug resistance in the future.[112]

Conclusion

During the last decades of the 20th century, the relation of man to microbial communities was reclaimed as symbiotic and integrated, not as inimical and antagonistic. At the same time, the fragility of this ecological equilibrium was demonstrated by the steep rise of emerging infections, leading to the development of global public health perspectives and to the genesis of a 'world on alert'.[113] Research in genomics and pathogenomics fostered a new understanding of agent-host relations but also tended to blur the distinction between pathogen and non-pathogen.[114] The absence of definite criteria by which to identify pathogens has important policy implications, most notably for biosecurity issues concerning the regulation of so-called 'select agents'.[115]

As we have shown, context-sensitivity, immunopathology, discrepancy in virulence measurements, the limited applicability of Koch's postulates, in addition to the absence of definitive structural or functional differences between pathogen and commensal organisms, all support the view that a strict pathogen versus non-pathogen distinction, a legacy of the ontological model of disease, omits several relevant details involved in pathogenesis of most infectious pathologies. Such a distinction becomes increasingly difficult to maintain as soon as we venture into a richer description of biological activities and processes and start to conceptualize microbes as evolving biological entities constantly interacting with their hosts. To address the theoretical challenges posed by the pathogen concept in post-genomic science, we have presented and discussed the implications of recent results from ecology and evolution that embrace the genetic diversity and underscore the relevance of microbial evolution. We think that looking at pathogens as evolving ecological entities could lead to a more interactionist and systemic perspective on virulence and pathogenicity, and to a better understanding of the selective pressures favoring the transition from harmless commensals to infectious agents.

Answering the question 'what is a pathogen?' requires us to think beyond the biology of both hosts and pathogens taken in isolation. Host and parasite genetics do matter, but classifying a microbe as a pathogen requires more than knowing its genome. The environment of the host and its history are key factors. We argue that one possibility to clarify the debate is to focus on the virulence expressed by an infected host (i.e., the decrease in host fitness) in order to define pathogenicity. This does not solve the problem that there needs to be a threshold in switching from a quantitative trait (virulence) to a binary trait (pathogenicity) (see **Fig. 1**). Where to put the threshold is a decision that has to go beyond the scientific field (for instance, as mentioned above, the existence or not of a cheap and efficient treatment should be a significant factor in deciding what the critical virulence is). At any rate, studying virulence forces us to acknowledge that hosts are not passive vehicles at the mercy of putative infectious germs but rather are themselves agents that take an active part, in several ways, in the processes of pathogenesis. Note that this definition can also bring new insights regarding the ecology and evolution of infectious diseases, because although the importance of $\mathbf{G} \times \mathbf{G} \times \mathbf{E}$ interactions (that is, the idea that virulence is the result of the interaction between the parasite genome, the host genome and the environment) is recognized in biology, the role of host history (e.g., priori exposure to diseases) is less so in medicine.

Arguably, there is no single criterion of pathogenicity that adequately captures the mechanisms of pathogenesis. Although

some 'virulence factor' can typically be identified, in most cases disease to the host is the result of several factors acting together.[116] In the case of HIV for instance, though we now have evidence that variations in virulence may sometimes be due to virus differences, we still lack evidence for clear 'virulence factors.'[117,118] Immunology, medical bacteriology, and evolutionary biology offer distinct conceptual tools to explain what makes some relations or processes pathogenic and why. Importantly, none provides an overarching perspective from which the 'essence' of a pathogen could be grasped. Searching for the essence of a pathogen based on the detection of unique attributes, or characterizing organisms dichotomously as either pathogenic or non-pathogenic, indeed, is another example of the 'binary' mode of thinking so entrenched in Western societies, and a legacy of the ontological model of disease. The binary conception according to which microorganisms are either one or the other is an abstraction from a more complex biological reality that ignores the facts that virulence is relatively transient, that commensals and parasites form a continuum, and that microorganisms constantly evolve. The processual nature of pathogens is a compelling illustration of the recent argument to the effect that biological phenomena in health and disease may be better understood as forming a hierarchy of entangled processes, rather than a hierarchy of static things.[119]

Glossary

Commensalism/Parasitism: A commensal relationship is one where one of the 2 partners derives some benefits, while the other receives neither harm nor assistance. In a parasitic/pathogenic relation, in contrast, one of the partners incurs costs while the other derives benefits.

Co-infection: Simultaneous infection of a host by more than one parasite strain or species. Also referred to in the literature as multiple infection, mixed infection or polymicrobial infection.

Ecological context: all the factors that are involved in the infectious process but beyond the control of the pathogen genotype itself, i.e., the host genotype, its physiological status and even its environment. Note also that the notion of ecological context can be defined at the within-host level and, for a virus for instance, it could describe within-cell environments.

Fitness: For a host, fitness is the ability to have many offspring. It has 2 components: host survival and host fecundity. For a parasite, fitness is the ability to spread in the host population.

GxGxE interaction: This notation summarizes the idea that a trait (e.g., virulence) is the result of the interaction between 2 genomes (that of the host and that of the parasite) and the environment. Of course, we could increase the number of G, for instance when we account for co-infections.

Host-parasite interaction (or infection): An interactive and dynamical biological system whose outcome is indeterminate and depends largely on the ecological context.

Immunopathology: An outcome of host-parasite interactions, immunopathology describes a decrease in host fitness due to the immune response following infection.

Opportunistic parasite: Parasite that cause virulent infections at higher rate in weakened hosts but are also able to cause disease in normal individuals, albeit less commonly so.

Parasite: any organism that decreases the fitness of its host by infecting it. This ecological definition includes both micro-parasites (e.g., viruses and bacteria) and macro-parasites (e.g., worms).

Pathogen/pathogenicity: Organism that causes virulence to the host upon infection. An infection in a given ecological context is either pathogenic or not.

Resistance (host): Ability of the host to prevent the infection or to decrease parasite load. This leads to a decrease in parasite fitness (See also tolerance.)

Tolerance (host): Ability of the host to decrease the virulence of an infection without affecting parasite load. Contrary to resistance, tolerance increases the fitness of the parasite.

Virulence (host/parasite): Virulence is one of the possible outcomes of a host-parasite relation. This term can be defined in several ways. Evolutionary ecologists, for instance, define virulence as a quantitative trait that measures the decrease in host fitness due to an infection.

Virulence control practices: Also defined as 'virulence management', these aim at making public health interventions more efficient by studying parasite reproduction and transmission as well as between- and within-host competition in order to anticipate virulence evolution.

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