Viewpoint

Understanding pathogens in the era of next generation sequencing

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What is a pathogen? Medical textbooks usually define a pathogen as any microorganism that causes disease. However, this widespread definition is problematic on a number of counts [1,2]. Moreover, a generally accepted definition is not forthcoming among medical microbiologists, immunologists, and physicians. Here it is argued that there is another, and more pressing question to be asked, namely: what *makes* some organisms pathogenic and others not? Asking these questions instead allows for distinguishing pathogens from non-pathogens in a more flexible way, while at the same time emphasizing the roles of ecological and evolutionary processes in determining pathogenicity in infectious diseases.

Historically, the difference between pathogenic and non-pathogenic variants was of great significance to medical bacteriologists who produced sophisticated classifications such as the *Atlas of Bacteria Pathogenic in Man* [3]. The concept of "pathogenic germ" was also embedded in Robert Koch's postulates and is partly reflected in today's concepts of "virulence genes" and "pathogenicity islands". From the birth of classical bacteriology until the present, several pathogen-like organisms were identified and classified based on morphological, cellular, or genetic criteria. As most pathogenic organisms known to nineteenth-century bacteriologists exhibited polysaccharide capsules that prevented phagocytosis, it was sensible to consider that pathogenic microbes markedly differed from those that did not cause disease [1]. More broadly, within the Koch-Pasteur tradition, "microbes" were long regarded as being potentially dangerous and healthy tissues were presumed to be germ-free [4].

Demarcating pathogens from other harmless organisms was a major theme in twentieth-century medical and biological research. Following the work of Joshua Lederberg on bacterial recombination in the 1950s, for instance, "plasmids" were characterized as self-replicating units capable of horizontally transmitting "virulence genes" and "resistance factors" with other bacteria – a process known to contribute to the evolution of antibiotic resistance [5]. Elucidating the role of plasmids during infectious processes fostered at the same time a molecular understanding of virulence and pathogenicity. Molecular developments throughout the 1970s confirmed that acquiring one or two plasmids was often sufficient to transform a harmless microorganism into one capable to cause severe disease or to enhance a bacterium's resistance to drugs, and sharpened the distinction between pathogens and non-pathogens one step further.

The formulation of a molecular version of Koch's postulates in the late 1980s [6] emphasized the centrality of virulence genes in pathogenesis while it acknowledged the existence of a fundamental distinction between pathogenic and non-pathogenic organisms [7]. At the dawn of the "genomic era" in the early 1990s, after the discovery that pathogenicity islands – large DNA regions in the flexible part of bacterial genomes – are capable of producing rapid evolutionary innovations, and that the integration of a single pathogenicity island into a bacterial genome can "in a single step, transform a normally benign organism into a pathogen" [8], the boundary between pathogens and harmless organisms appeared more firmly established than ever. Such "islands", indeed, were initially thought to be present only in pathogenic strains or species, and were often characterized as

"unique genetic elements which contribute to bacterial virulence" [9].

Yet deciding where (and how) to draw the boundary between commensalism and pathogenicity remains problematic for several reasons. The difficulty stems partly from the fact that the concept of pathogen is not absolute but relative [10]. In effect, although medical textbooks usually define pathogens as any organism that causes disease, the concept of a pathogen cannot be defined without also considering the host and the nature of their ecological interactions [11]. Metazoan organisms are complex ecosystems constantly interacting with microbiota (*i.e.*, commensals) forming vast, multi-species complexes such as the human's gut flora. Understanding the regulative function of the microbiata in maintaining human and animal health is now a crucial task [12]. Most of the time, also, a microorganism can act as a commensal or as a pathogen depending on where it establishes a niche in the infected body to replicate itself [2]. Also significant in relativizing the distinction between pathogen and non-pathogen is that a bacterial strain described as being very virulent *in vitro* could turn out to be mild *in vivo* (and vice-versa), and that virulence can be lost and restored (for instance by serial passages of the strain in a host). Finally, it is hypothesized that a microorganism can, through evolutionary time, switch between commensal and pathogenic states [7].

Despite considerable conceptual and technological advances, including the development of next generation sequencing apparatuses, several "omics" sciences contributed in the past 20 years or so to "blur" the distinction between pathogens and non-pathogens [7]. Indeed, thanks to high-throughput technologies and the construction of powerful databases which allow for performing large-scale comparisons between different genomes, pathogenomics gained momentum in the mid-1990s and revealed the geographical and ecological scope of microbial (pathogenic) diversity. An important outcome of the rise of pathogenomics is that pathogenicity islands are not specific to pathogenic lineages or species [13]. On the contrary, the underlying mechanisms in pathogenicity islands are much more common than was first suggested and exist in phylogenetically distinct species where they have different biological functions. For instance, *Yersinia pestis*, the agent of plague, contains a "high pathogenicity island" coding for virulence genes (an iron uptake system) also found in about 30% of non-pathogenic members of the species that were isolated from the human digestive tract [14]. Following the discovery of several similar islands in non-pathogenic strains, the concept of the pathogenicity island had to be redefined, and it progressively acquired the status of a "more general genetic entity" whose function is largely ecologically mediated [15,16].

Host-parasite interactions are better described as forming a continuum ranging from commensalism and mutualism to parasitism and pathogenicity [17], not as discrete biological categories. However, pathogens and non-pathogens can be distinguished – even if only temporarily – with respect to their (micro) evolutionary history: as microbiologist Stanley Falkow put it, "The pathogen, through evolution, has gained the inherent capacity to breach host cell barriers, while commensal species and opportunists ordinarily cannot do so" [18]. Given the wealth of data obtained by metagenomics, pathogenomics, and phylogenetics (*e.g.*, the ubiquity of lateral gene transfer), and thanks to the output of projects such as the Human Microbiome Project (http://nihroadmap.nih.gov/hmp), the main challenge now is to understand what are the factors (human, technological, environmental, *etc*.) conducive to evolutionary and ecological processes that lead to those functional differences between pathogens and non-pathogens, and to assess their impact on emerging disease events worldwide. In sum, answering the question "what is a pathogen?" requires us to think beyond the biology of both hosts and pathogens taken in isolation and to ask instead, "what *makes* organisms pathogenic, and why?"

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