Disease consequences of pathogen adaptation

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Experimental evolution studies demonstrate that pathogens evolve rapidly, have a large capacity for increased virulence and cause disease in many different ways. A large proportion of genetic diversity for host susceptibility to infectious, autoimmune and 'genetic' diseases, and to cancer, is probably caused by pathogens and/or host counteradaptations. Recent advances in diverse fields support this claim and suggest many underused approaches for identifying and experimentally dissecting the complicated host–pathogen interactions that often lead to disease.

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Abbreviations

ATL	adult T-cell leukemia
CTL	cytotoxic T lymphocyte
HSV	herpes simplex virus
HTLV	human T-cell leukemia virus

Introduction

Pathogens evolve rapidly and they are usually up to no good, at least from the host's perspective. Although this statement is not controversial, the breadth and importance of pathogen adaptation are often under-appreciated. Pathogen adaptations are often demonstrably negative for the host but, more often, the negative consequences to hosts are cryptic, poorly understood or assigned a non-pathogen etiology altogether. This review summarizes recent advances in pathogen-mediated disease and attempts to classify the myriad of disease consequences of pathogen adaptation. We also develop two case examples involving autoimmunity and cancer that help highlight this important, but difficult, area.

Experimental pathogen evolution

The best evidence for rapid pathogen adaptation to hosts comes from the accumulating body of experimental work involving serial-passage studies. Ebert [1] reviewed 43 serial-passage studies involving diverse infectious agents and documented the following four major patterns: first, virulence increased in 23 out of 24 studies (96%); second, pathogen fitness increased in 9 out of 9 studies (100%); third, pathogen growth rate increased in 15 out of 15 studies (100%); and fourth, pathogen virulence to a former host decreased in 32 of 34 studies (94%). These results are profound because they demonstrate that rapid pathogen evolution must be occurring in natural populations and that it is almost always negative for current host species. However, virulence attenuates in former host species, presumably due to costs and tradeoffs associated with pathogen adaptations. This pathogen-driven selection will favor counteradaptations by the host and a never-ending molecular 'arms race' will ensue [2•].

Pathogen adaptation occurs so rapidly during passage that it can be used to experimentally identify molecular and functional changes occurring at the host–pathogen interface. Surprisingly, it has seldom been used this way because most passage studies were conducted to either develop an attenuated pathogen for use in vaccines or to adapt a pathogen to an animal model. A few recent studies have been used as an experimental tool to understand pathogen adaptation. Influenza passaged 12 or 20 times with three-day cycles increased in virulence by four or five orders of magnitude, respectively, as measured by LD_{50} [3•]. Genomes of post-passage virus were sequenced and 14 amino-acid substitutions were identified. These substitutions were found in 8 out of 10 influenza genes and influenced a variety of functions, including nuclear localization signals, sites of protein and RNA interaction, and pH optima of fusion. The first two functions represented previously unknown modulators of virulence and highlight one of the powers of this approach — selection experiments can be used to identify the molecular sites of important host–pathogen interactions.

A similar approach was used to identify substitutions that confer neuroadaptation of yellow-fever virus [4]. Several recent studies have also used serial passage *in vitro* to identify genetic changes that account for the resulting attenuation of virulence *in vivo* for Japanese encephalitis virus [5], *Staphylococcus aureus* [6], yellow-fever virus [7] and *Toxoplasma gondii* [8]. *In vivo* readaptation of an *in-vitro*-passaged strain of feline immunodeficiency virus (FIV) led to characterization of viral sites responsible for resistance to antibody neutralization [9]. If sexual reproduction is possible in the pathogen, it can be used to speed-up evolution, as has been shown in *T. gondii* [10]. In short, serial-passage methodologies are powerful but underused approaches both for dissecting host–pathogen interactions and for directed evolution to produce pathogens optimized for gene therapy or vaccine strategies [11•].

Pathogen adaptation to host genotypes

Many of the diseases discussed in this review require that pathogens adapt to different genotypes within the same host species. Although this is a common assumption, serial-passage studies provide little supporting data because most involve passage through a new host species. Some of the best evidence for host-genotype-specific adaptation comes

from recent studies where pathogens from two host populations are reciprocally transplanted. Such studies in trematode parasites of snails [12•] and a bacterial pathogen of *Daphnia* [13] show that these parasites are better adapted to their own local host genotypes. Another clear example of host-genotype-specific adaptation is the evolution of viral variants that escape MHC-dependent immune recognition, as demonstrated in HIV, simian immunodeficiency virus (SIV) (reviewed in [14]), human T-cell leukemia virus (HTLV) [15•] and hepatitis C virus [16] (see also Update).

Despite these examples, it remains to be demonstrated whether the strong and rapid adaptation that emerges in serial-passage studies between two species will also occur between two genotypes within a host species. This is a particularly important problem for vertebrate hosts, because one could argue that most pathogen evolution may be directed toward escaping the adaptive immune response, where the effector/recognition molecules are largely generated by somatic mechanisms. A recent genome scan for positively selected amino acid sites in hepatitis C virus uncovered 13 such sites, 8 of which were B-cell epitopes [17•], indicating that most of the detectable positive selection operating on this virus resulted from somatically generated host antibodies. Approximately half of the genetic variability for susceptibility to infectious disease is MHC related [18], suggesting that MHC-, T-cell- and B-cell-mediated immunity is a focus of host–pathogen evolution. Still, half of the genetic variability for susceptibility to pathogens involves non-MHC germline diversity.

Evolution of antibiotic resistance and compensatory mutations

The evolution of antibiotic resistance is a powerful experimental model for studying pathogen adaptation to a host phenotype. In general, antibiotic resistance evolves rapidly and usually reduces microbe fitness. The big surprise has been that when the antibiotic is removed, compensatory mutations usually restore microbial fitness without the loss of antibiotic resistance [19•]. The general explanation seems to be that there are simply more compensatory than reversion mutations. These findings suggest that evolutionary solutions may be less limiting than previously thought, making pathogen adaptation to both antibiotics and host defenses an even bigger problem. Analogous compensatory mutations have recently been reported in influenza [20], herpes simplex virus (HSV) [21], hepatitis B virus [22] and possibly in HIV, where escape mutations persist long after selection favoring them is eliminated [23] (see also Update). Such host-genotype-specific adaptations should favor host genetic polymorphisms [2•,13]. As discussed below, there seem to be an abundance of such host polymorphisms.

Why is there so much genetic variation for susceptibility to infectious disease?

One is struck by the staggering genetic variability associated with susceptibility to infectious disease [24]. Because of the importance of infectious disease, one might predict just the opposite; susceptible genotypes should be eliminated. If susceptibility alleles persist, it is probably due to one of two possible mechanisms. First, alleles that are effective against one pathogen might be ineffective against another pathogen or for some other function (antagonistic pleiotropy), resulting in a stable polymorphism. Sickle-cell hemoglobin is a classic example. The second possible mechanism is that pathogens are rapidly evading host defense genes, causing host counteradaptation. Such antagonistic coevolution can maintain host polymorphism due to negative-frequency-dependent selection. Common host alleles are the primary targets of pathogen evolution, which gives a disadvantage to these alleles and an advantage to rare host alleles. Polymorphisms are maintained because of this frequency-dependent selection [2•,13]. This process seems to account for the protein polymorphisms of the CCR5 gene that confers susceptibility to SIV in African green monkeys [25•], as well as susceptibility to HIV in humans.

Both of these mechanisms are examples of host counter-adaptations resulting in disease and both have been used to explain polymorphism of MHC genes, where demonstrably 'bad' genes that confer susceptibility to one or more diseases have been maintained for large periods of evolutionary time. But what about non-MHC genes? Estimates indicate that about half of the host genetic variability for resistance to infectious disease is attributable to non-MHC genes [18]. Many of these genetic polymorphisms will probably be maintained by one of these two mechanisms and consequently will contribute to lowered host fitness (decreased disease resistance) under some circumstances.

'Immune' genes evolve rapidly

If molecular arms races exist between pathogens and host defense genes, then these immune system genes should show signs of rapid protein evolution. They are among the fastest-evolving genes [26,27] as assayed by comparing rates of non-synonymous substitution across entire genes. This assay underestimates the rate of evolution for two reasons. First, positive diversifying selection only acts on a small proportion of sites. Second, identifying actual host defense genes is difficult. For example, a recent gene-expression study found that many genes not thought to be 'immune' genes were upregulated during infection [28•]. These genes, however, can still be targets of pathogen adaptation and so will be incorrectly categorized as 'non-immune' genes. Scanning genes for amino acid sites showing positive selection can be useful for identifying genes important to host–pathogen coevolution [17•,29,30].

'Genetic diseases' that are not rare (>1%) are likely to have pathogen involvement

Genes that cause disease should be eliminated by natural selection, yet there are many important, non-rare disease genes [31]. What is maintaining these bad genes? Recently Cochran and coworkers [32•] have argued that such 'genetic disease' genes are likely to have a cryptic pathogen etiology, thus offering a solution to the paradox of the persistence of

seemingly 'bad' genes. Cochran and coworkers developed the concept of 'fitness load' that takes into account both the severity and prevalence of each disease. For example, a disease that was found in 1% of the population and reduced the fitness to 80% of wild type would have a fitness load of $0.01 \times 0.8 = 0.008$. The goal was to discriminate between diseases that can be explained by recurring mutations, versus those that cannot. All diseases with a fitness load greater than 0.01 and known etiology have pathogen involvement. On the basis of this analysis and other arguments, the authors conclude that the many diseases with high fitness load (>0.001) and obscure etiology are likely to have pathogen involvement. Such diseases include atherosclerosis, endometriosis, polycystic ovary disease, breast cancer, cerebral palsy, endomyocardial fibrosis, juvenile diabetes (insulin-dependent diabetes mellitus [IDDM]), pre-eclampsia, eclampsia, rheumatoid arthritis and schizophrenia. Because fitness is difficult to measure and generally underestimated [33], it is likely that many diseases with fitness loads >0.001 will also be caused by pathogens, rather than mutations.

Pathogens and autoimmunity: molecular mimicry

If pathogens mimic host T-cell antigens, they might evade some immunity because self-tolerance mechanisms eliminate or anergize relevant T cells, but such mimicry could lead to increased host autoimmunity [34]. Four critical lines of evidence support the molecular-mimicry hypothesis for initiation and/or exacerbation of autoimmunity: first, epidemiological studies associating various pathogens with particular autoimmune diseases [35•]; second, sequence and structural homology between many pathogen peptides and self proteins (reviewed in [36]); third, isolation of T cells from autoimmune patients that are cross-reactive with both self and pathogen-derived peptides; for example T cells that are cross-reactive with B4 Coxackie virus and GAD have been isolated from patients suffering with IDDM [35•,36]; and fourth, experimental mouse models of autoimmune disease (reviewed in [37–39]).

Mouse models demonstrate that molecular mimicry of T-cell epitopes by pathogens can supply the specific antigenic signal to break tolerance in cross-reactive T cells: first, it has been established that some autoreactive T cells to a specific self antigen expressed in the thymus can escape central tolerance and be activated in the periphery (reviewed in [37]); and second, transgenic mice that express viral antigen as a self protein and therefore possess autoreactive T cells in the periphery do not develop overt autoimmune disease until infected with the virus [39]. Recently it has become clear that autoimmunity due to molecular mimicry may be exacerbated because T-cell specificity is far more degenerate than was generally believed; this mechanism appears to contribute to the probability of cross-reactivity [40,41].

Some of the most compelling evidence linking autoimmune disease with molecular mimicry comes from the mouse model of herpes stromal keratitis (HSK). HSK develops in mice infected with HSV-1 because autoreactive T cells target corneal tissue. Infection of susceptible mice with HSV-1 lacking the putative mimic epitope fails to induce disease [42•]. Autoimmune disease also failed to develop when the cross-reactive epitope was present but was altered, suggesting that molecular mimicry was essential and mere tissue damage or inflammation was not sufficient for induction of autoimmune disease in animals with low numbers of autoreactive T cells. By contrast, when infected with the altered epitope, disease did develop in TCR-transgenic mice (with high numbers of autoreactive T cells) that only recognize the 'wild' mimic epitope. Taken together, these data suggest that molecular mimicry may be crucial for development of HSK in individuals where numbers of autoreactive T cells are limited [43•]. Similar results have been found in a transgenic model of diabetes induced by infection with lymphocytic choriomeningitis virus (LCMV) [44].

Although molecular mimicry as a direct cause for development of autoimmune disease has received much attention and been reviewed extensively [36,40,45,46], no model system provides definitive evidence. More experimental work is needed where the mimic molecule is altered or removed in both pathogen and host. Such experiments would help determine if pathogens gain an advantage during molecular mimicry, an idea that has largely been ignored in experimental work since the original formulation of the molecular-mimicry hypothesis. One humbling conclusion emerges; the immune system is complicated and each infectious agent presents many novel differences. Experimentally disentangling the myriad of interactions leading to autoimmunity will be a major challenge.

Pathogens and cancer

Not too long ago, tumorigenesis was never thought to have an infectious etiology, but recent evidence suggests that pathogens cause 15% of all human tumors [47•]. Examples of associations include: first, hepatitis C and hepatocarcinoma (reviewed by [47•]); second, papillomavirus and cervical cancers (reviewed by [48]); third, Epstein–Barr virus and both Burkitt's lymphoma and nasopharyngeal carcinoma (reviewed by [4,49]); and fourth, HTLV and adult T-cell leukemia (ATL) (reviewed by [47•]). It is interesting to note that almost all of these infectious agents also cause an acute, often more-benign disease and only rarely result in cancer. For example, papilloma viruses usually cause warts and Epstein–Barr virus usually causes infectious mononucleosis, but occasionally they are associated with the cancers described above. Although both DNA and RNA viruses have been implicated in carcinogenesis, the mechanisms resulting in transformation are different. DNA tumor viruses generally target cellular suppressor genes and their products, whereas RNA tumor viruses, almost exclusively retroviruses, either encode viral oncogenes or insert themselves near a critical proto-oncogene. The unifying theme is that infection with tumor viruses alters

Table 1

Classification of diseases caused by pathogen adaptation.

Category	Subcategory	References
Increased virulence (pathogen fitness)	Escape of immune recognition (e.g. HIV)	[14]
	Immune modulation	[51]
	Emerging disease (e.g. HIV adaptation to humans)	[52]
	Antibiotic resistance	[19•]
	Tissue-specific adaptation (e.g. HTLV neuroinvasiveness)	[50]
	Virulence increase due to imperfect vaccines	[53]
Autoimmunity	Molecular mimicry	[35•,36,46]
	Bystander damage/innate immunity	[54–57]
Cancer	RNA viruses (e.g. oncogenes or insertion near proto-oncogenes)	[47•]
	DNA viruses that target tumor-supppressor oncogenes	[47•,48,49]
	Escape of immune surveillance (e.g. HTLV)	[15•,50]
Genetic diseases resulting from host counteradaptation	Many subcategories (e.g. sickle cell anemia)	[24,58]

Examples are drawn from the recent literature and reviews when available.

cell cycle controls (in the case of DNA viruses this is critical for their replication) and infection occasionally results in transformation.

A particularly clear example of pathogen adaptation resulting in cancer involves HTLV-1, which is associated with either myelopathy/tropical spastic parapesis (HAM/TSP) or ATL [15•]. HLA-A*02 patients usually have low proviral titers and are protected from HAM/TSP but despite this a number of HLA-A*02 HTLV carriers do develop ATL. The immunodominant cytotoxic T lymphocyte (CTL) epitope is derived from viral transcription transactivator protein (Tax) and the viral populations isolated from these patients have mutations in this protein. The majority of these mutations prevent expression of Tax, which renders HTLV invisible to the host's CTL response, but also significantly impairs HTLV's ability to replicate. This tradeoff explains why Tax-mutant viruses never come to predominate viral populations in these patients. Thus, escape from CTL response comes at a high cost to the virus, yet these mutations are still selected for, as indicated by the high dN:dS ratio in Tax [50], and appear to contribute to ATL.

Classification of diseases resulting from pathogen adaptation

Table 1 organizes major categories of disease resulting from pathogen adaptation. Recent examples or reviews are included. Many categories are not mutually exclusive and many examples treated in this paper may fit into more than one category. Since host counteradaptations involve most aspects of host physiology, we have not provided subcategories for this category because the list would be too extensive.

Conclusions

Advances in many fields indicate that pathogen involvement in disease processes is more pervasive than generally appreciated. Pathogen adaptation to host defenses and host counteradaptation are important for understanding and/or preventing disease processes including infectious, autoimmune, genetic and emerging disease, and cancer. Experimental pathogen-evolution studies can be used to identify the pathogen and host genes involved in antagonistic interactions. They can also be used for practical applications such as production of 'designer' pathogens for gene therapy and vaccination vectors.

Update

Moore *et al.* [59] searched for correlations between HIV polymorphisms and HLA genotype in 473 HIV patients. They found 64 significant correlations, which indicates that HIV is adapting to specific host (HLA) genotypes. These HLA-associated HIV polymorphisms were a strong predictor of viral titers, suggesting that the polymorphisms were pathogen adaptations resulting in increased virulence. Only 21 of the 64 mutations were associated with known class I epitopes and these are thought to function by escaping HLA-dependent immune recognition. The authors suggest that the other 43 associations could be associated with unknown epitopes. An alternative possibility is that some of these polymorphisms could be mutations that compensate for defects caused by an initial HLA escape mutant. This correlative study provides evidence for antagonistic coevolution that maintains polymorphisms in both pathogen and host through frequency-dependent selection.

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