Point, Counterpoint: The Evolution of Pathogenic Viruses and their Human Hosts

Michael Worobey, Adam Bjork, and Joel O. Wertheim

Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721; email: worobey@email.arizona.edu, bjork@email.arizona.edu, wertheim@email.arizona.edu

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Abstract

Viral pathogens play a prominent role in human health owing to their ability to rapidly evolve creative new ways to exploit their hosts. As elegant and deceptive as many viral adaptations are, humans and their ancestors have repeatedly answered their call with equally impressive adaptations. Here we argue that the coevolutionary arms race between humans and their viral pathogens is one of the most important forces in human molecular evolution, past and present. With a focus on HIV-1 and other RNA viruses, we highlight recent developments in our understanding of the human innate and adaptive immune systems and how the selective pressures exerted by viruses have shaped the human genome. We also discuss how the antiviral function of cellular machinery like RNAi and APOBEC3G blur the lines between innate and adaptive immunity. The remarkable power of natural selection is revealed in each host-pathogen arms race examined.

INTRODUCTION

The battles played out between humans and their pathogenic viruses are some of the most dramatic of all the conflicts studied by evolutionary biologists and ecologists. Viral pathogens play a dominant role in human health in terms of both morbidity and mortality and, as such, they represent one of the most potent selective forces acting on human populations (Vallender & Lahn 2004, World Health Org. 2003). Their habit of hiding within host cells, their large population sizes, rapid reproductive rates, and, above all, high rates of mutation and recombination (Domingo & Holland 1997, Worobey & Holmes 2001) make them especially formidable adversaries. Viruses are masterpieces of nature: compact, streamlined, highly flexible bundles of protein-coated genetic material with the audacious ability to hijack the sophisticated biochemical machinery within their hosts' cells.

It is becoming increasingly clear that humans (and their predecessors and relatives) have risen to the evolutionary challenge posed by viral pathogens with some elegant defenses and remarkably rapid immunological, genetic, and genomic changes of their own, ones that reveal a long history of host-virus coevolution that holds valuable lessons for biologists and clinicians. How can giant, long-lived targets like human beings defend against the asymmetric threat posed by viral pathogens? The answer lies, in large part, in another evolutionary masterpiece: the vertebrate immune system. This gives some of the most complex, slowly evolving organisms the ability, at both the individual and population level, to keep up with pathogens that represent the other evolutionary extreme. Given that 23 of 94 vertebrate-specific gene families and (conservatively) 1 out of every 30 human genes code for defense and immunity proteins (Int. Hum. Genome Consort. 2001), it is clear that much of the last 400 million years of our own evolutionary story has involved the battle with pathogens.

Here, to bring into sharper relief what we believe might be the most crucial part of that story, we attempt to answer the following questions: How have viruses shaped the human immune system, our genes, and our genomes? Have they done so differently than other pathogens, and, if so, in what ways? What evolutionary pressures have humans and other vertebrates imposed onto viruses, and what are the consequences of this feedback for them and us? To answer these questions we dissect a variety of host-pathogen arms races and conflicts in light of recent advances in the understanding of the human genome and virus-relevant innate and adaptive immunity, with an emphasis on HIV-1. We also consider newly discovered antivirus defenses that do not fit neatly within the conventional definitions of innate or adaptive immunity. These include RNA interference, as well as intrinsic immunity, whereby constitutively expressed host gene products can poison viral genomes or otherwise disrupt viral life cycles, even in the absence of virus-triggered signaling (Bieniasz 2004). Throughout, we highlight how lessons learned at the intersection of evolution, ecology, genetics, virology, and immunology can contribute both to a better understanding of each of these fields and to improving therapeutic and preventative control measures against viral pathogens.

INNATE IMMUNITY: THE FIRST LINE OF DEFENSE

The front-line component of the immune system, the innate immune response, has extremely deep roots. Every multicellular organism has a complex innate immune system that allows it to discriminate between self and nonself on the basis of a limited set of more or less generic cues, molecular patterns normally present in the invader but not the host (Beutler 2004). Receptors recognizing different classes of pathogens, if activated, rapidly unleash anti-invader responses, such as the interferon response that temporarily makes the body much less hospitable to viruses (Akira et al. 2006). It is the interferon response that is behind the sore muscles, fever, and other flu-like symptoms associated with so many viral infections.

The defining characteristic of the innate immune system is its ability to mount rapid and effective responses to a wide variety of pathogens without the requirement of previous exposure. However, recent findings demonstrate that the innate immune system can operate with a previously underappreciated level of specificity. The Toll-like receptors (TLRs) provide a good example.

The Toll receptor pathway was first identified in *Drosophila* for its role in establishing dorsal-ventral polarity in the developing embryo (Hashimoto et al. 1988), but it was later hypothesized (Belvin & Anderson 1996) and confirmed (Lemaitre et al. 1996) to play an important role in innate immunity as well. A large, multigene family of homologous proteins, known as Toll-like receptors, has since been identified in vertebrates, including mammals (reviewed in Takeda et al. 2003).

TLRs are transmembrane proteins that distinguish self from nonself by detecting pathogen-associated molecular patterns (PAMPs). Recognition of these ligands on invading pathogens, by TLRs located on the surface of immune cells, triggers the induction of genes responsible for the inflammatory (innate) immune response and also contributes to stimulating the adaptive immune response (reviewed in Akira & Hemmi 2003). Most mammalian genomes code for between 10 and 15 TLRs; 10 have been identified in humans (TLR1–TLR10).

Some TLRs can recognize a variety of viral and/or bacterial pathogens. On the viral side, for example, TLR2 can identify PAMPs found on measles virus, cytomegalovirus, and hepatitis C virus (HCV) (Bieback et al. 2002, Compton et al. 2003, Dolganiuc et al. 2004). Others are more restricted: Respiratory syncytial virus and herpes simplex virus are identified by TLR4 and TLR9, respectively (Kurt-Jones et al. 2000, Lund et al. 2003). TLR3 is something of an all-purpose virus alarm, as it identifies double-stranded RNA (dsRNA) (Alexopoulou et al. 2001), found in most viruses at some point in their life cycle; it is the trigger for the interferon response.

A key point here is that, unlike the main players in the adaptive immune system—for example, major histocompatibility complex (MHC) genes—although adept at what they do, TLRs are not very evolutionarily flexible. Viral antigen recognition mediated by MHC genes is characterized by an impressive amplification in the gene family, unrivalled levels of polymorphism at each locus (**Figure 1**), and striking levels of positive selection (MHC Seq. Consort. 1999). TLRs, however, represent the opposite: a set of good, generic signal receptors, held in place by strong purifying selection. The function and nucleic acid sequences of TLRs are highly conserved

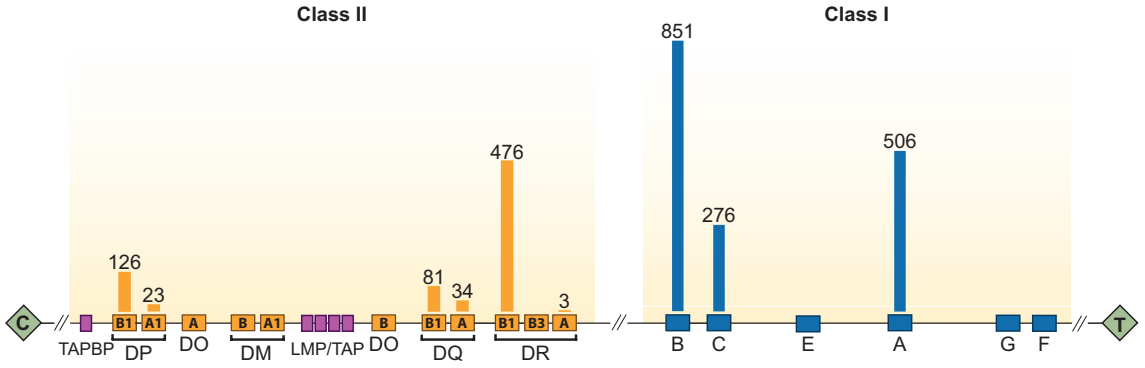

Figure 1

Human major histocompatibility complex (MHC) class I and II loci. This is a schematic diagram (not to scale) of some of the key loci in the MHC class I and II regions. Included are the nine classical human leukocyte antigen (HLA) loci, each of which is labeled with the number of alleles found so far in the human population. Several other HLA loci exist, some of which are shown here. Many non-*HLA* genes with immune function are also found in the MHC. *TAPBP*, *LMP*, and *TAP*, which are involved in antigen processing by the proteasome and transport of peptides to MHC molecules, are shown here as examples. C, centromere; T, telomere.

across vertebrates, and the TLR arsenal of extant vertebrates is remarkably similar to that of the ancestral vertebrate, even down to the level of gene copy number, which has typically remained at just one for each TLR (Roach et al. 2005). The PAMPs targeted by TLRs are presumably not at liberty to evolve to avoid recognition: hence the evolutionary stability (and the reliability) of TLRs (Smith et al. 2003).

INTRINSIC IMMUNITY: MUTATIONAL MELTDOWN AND OTHER WAYS TO KILL A VIRUS WITHOUT EVER KNOWING IT IS THERE

Our cells, it turns out, constitutively produce potent antiviral drugs. One of the most dramatic examples of the evolutionary arms race between pathogens and their hosts—one that no one knew about until just a few years ago—is the case of retroviruses (and retroelements) versus the *APOBEC* gene family of Old World primates. One player in this arms race, the HIV protein Vif (viral infectivity factor), has long been known to be important; viruses with defective or absent *vif* genes (Δ*vif*) are unable to replicate in most human cell lines (Gabuzda et al. 1992). However, the reason why HIV needs Vif in order to infect human cells remained largely obscure for over a decade. During this same time period, HIV researchers also began coming across viruses that had a bizarre mutational pattern. Their genomes had been hypermutated—fatally riddled with G-to-A (guanine-to-adenine) mutations that resulted in defective progeny (Borman et al. 1995). The solution to this small mystery would reveal a surprisingly broad new line of defense against viruses.

The breakthrough came when Sheehy et al. (2002) compared gene expression in human cell lines and identified a protein, APOBEC3G, present only in cell lines in

which *vif*-defective viruses were unable to replicate. Zhang et al. (2003) and Mangeat et al. (2003) independently drew the connection that it was APOBEC3G's function as a cytidine deaminase—a DNA-editing enzyme—that could explain both the necessity of a functional Vif protein and the hypermutation of HIV genomes. During the viral life cycle, APOBEC3G is incorporated into the viral capsid. Typically, it is subsequently degraded by the virus's Vif protein, which is effectively the antidote to APOBEC3G. However, in the absence of a functional Vif, APOBEC3G is not degraded; upon infection of a new host cell, it hypermutates the viral genome, via deamination, while the virus is reverse-transcribing its RNA genome to DNA.

It is now known that APOBEC3G is actually one of many APOBEC proteins that comprise a recently expanded part of a large gene family that includes AID (activation-induced deaminase) (Conticello et al. 2005). AID is a protein responsible for the somatic hypermutation that takes place in the immunoglobulin (Ig) genes of cells that produce antibodies, allowing them to selectively fine-tune their antigen recognition (Muramatsu et al. 2000). The common ancestry, and mechanism, of APOBEC3G and AID is a shining example of how natural selection is a great tinkerer—in this case co-opting a mechanism crucial to adaptive immunity and shaping it into a totally distinct weapon.

The antiretroviral properties of APOBEC3G have been observed in other viruses too, including HTLV-I (human T cell lymphotrophic virus), SIV (simian immunodeficiency virus), and hepatitis B virus (HBV), as well as in retroelements such as retrotransposons present in the human genome (Bogerd et al. 2006a, 2006b; Muckenfuss et al. 2006; Turelli et al. 2004). Retroelements compose approximately 42% of the human genome (Int. Hum. Genome Consort. 2001), and it has been suggested that their activity may sometimes provide an adaptive advantage to individual hosts by helping to create new genes, including some with roles in adaptive immunity (Agrawal et al. 1998, van de Lagemaat et al. 2003). However, retroelement activity has demonstrably negative fitness effects as well, such as disrupting the expression of crucial genes and causing malignancies and autoimmune disorders (reviewed in Bannert & Kurth 2004). In addition, gene products encoded by some retroelements may be toxic for the host organism (Boissinot et al. 2006). These elements may have overall deleterious host fitness effects and could have selected for the expansion and diversification of the *APOBEC/AID* gene family (Sawyer et al. 2004).

Several other members of the *ABOBEC* gene family have demonstrable antiretroviral activity; the radiation of this gene family has been accompanied by remarkably strong selective pressure, with some of the highest dN/dS ratios ever measured (OhAinle et al. 2006, Sawyer et al. 2004, Turelli & Trono 2005, Zheng et al. 2004). Additional unrelated genes such as *TRIM5α* have also been highly effective at restricting retrovirus propagation and, accordingly, also exhibit evidence of extremely intense positive selection in both humans and other primate species (Sawyer et al. 2005). TRIM5α appears to disrupt the uncoating of retroviruses, which leads to their death (Stremlau et al. 2004). This mechanism of action implies that endogenous and exogenous retroviruses, rather than retroelements, likely provided the selection for TRIM5α antiretroviral activity. Much of the most

exciting research on intrinsic immunity proteins is focused on their potential use as drug treatments for HIV and other retroviral infections (Mangeat & Trono 2005).

RNA INTERFERENCE AND GENE SILENCING: AN ANCIENT VIRAL DEFENSE MECHANISM?

While the antiretroviral effects of the *APOBEC* genes likely resulted from the modification of previously existing proteins functioning in the adaptive immune system, an ancient, putatively antiviral mechanism called RNAi (RNA-mediated interference) has been co-opted and modified to perform unrelated cellular functions: transcriptional and posttranscriptional gene regulation. Surprisingly, it was these derived functions of RNAi that led to its discovery. One of the first documented instances of RNAi activity occurred not when researchers attempted to suppress viral infection but when they introduced a second copy of a gene for purple pigment into a petunia plant with the intention of producing more pigment. The result of this experiment was not the production of more purple pigment, but less (Jorgensen 1995).

Gene silencing phenomena, whereby a specific gene could be suppressed by the introduction of the homologous sequence, was subsequently observed in a variety other organisms, but it was Fire et al.'s (1998) pioneering work on *Caenorhabditis elegans* that first identified the role of dsRNA in RNAi and gene silencing. In addition, they noted that owing to the small amount of dsRNA required to initiate gene suppression, a catalytic protein cascade must be involved in this RNA-mediated gene suppression.

A crucial part of RNAi activity is its specificity, which is achieved by using the original dsRNA template as a guide for identifying all other copies of the gene of interest. This template is created by the enzyme Dicer (Bernstein et al. 2001), which cleaves the dsRNA into short (approximately 22-bp) RNA sequences known as small interfering RNAs (siRNAs). Once the dsRNA is cleaved, the siRNA is incorporated into the RNA-induced silencing complex. This protein complex then binds to and cleaves other copies of the target RNA, which essentially suppresses all protein products that would have been produced from the original RNA sequence (reviewed in Stram & Kuzntzova 2006).

Despite its recent discovery, RNAi is now understood to be an extremely important regulator of gene expression; however, the most parsimonious proposal for the ancestral function of this RNAi machinery is defense against RNA viruses and transposable elements, given the conservation of this function across the eukaryotic spectrum (Cerutti & Casas-Mollano 2006). The antiviral effects of RNAi, owing to its ability to identify and degrade homologous nucleotide sequences, are thus a good example of tremendous specificity in organisms traditionally thought to have only nonspecific innate immune responses.

Like the interferon response, RNAi is triggered specifically by dsRNA, albeit by shorter nucleotide strands. Unlike the interferon system, which can result in a more general response that upregulates a variety of antiviral functions such as proteasome function and MHC class I production, the RNAi machinery specifically targets the

gene of interest, allowing the rest of the cell to continue to function normally. Moreover, RNAi is a more general antiviral mechanism than interferon, as the latter is expressed only in a limited range of cell types.

Note that although RNAi is still used as an antiviral defense in plants and invertebrates, it is possible that in mammals its antiviral function has been largely supplanted by the interferon and adaptive immune responses (Cerutti & Casas-Mollano 2006). Regardless of whether it survives only as a derived, gene-regulatory mechanism in mammals, it can still induce potent antiviral responses. One of the most promising avenues of biomedical research today involves the artificial induction of RNAi in humans by introducing synthetic siRNAs designed to target specific genes, including essential viral genes, which can lead to the suppression of viral replication within the host cell. Encouraging results have been seen in a large group of viruses ranging from single-stranded RNA viruses (influenza virus and SARS) to retroviruses (HIV-1), hepadnaviruses (HBV), and even DNA viruses (herpesviruses). These studies are reviewed in Stram & Kuzntzova (2006), who note that RNAi may have an impact on human health in the coming century comparable to that of antibiotics in the previous century. Like antibiotic use, therapeutic RNAi represents the successful harnessing, for medical purposes, of evolutionary adaptations devised by natural selection during ancient host-pathogen arms races.

ADAPTIVE IMMUNITY: EDUCATION BY PATHOGEN

What is Adaptive Immunity?

The vertebrate adaptive immune response is highly specific and extremely dynamic, and it can recognize and respond to virtually any pathogen, including viruses within infected cells. It mimics many of the features that make viruses dangerous, including the ability to rapidly generate extensive genetic diversity through recombination and mutation and select for successful variants.

Unlike in viruses, this selection in B and T cells takes place across somatic generations; successful variants possess a cell-surface receptor that physically binds with high affinity to some foreign molecule (i.e., an antigen), allowing the immune system to see an invading pathogen, to remember it, and to respond rapidly in the event of subsequent encounters. B cell receptors are known as immunoglobulins. They search for, and bind to, macromolecular antigens on foreign invaders. Daughter B cells then secrete increasingly specific Ig (i.e., antibodies) that circulate throughout the intercellular space. T cell receptors, however, bind specifically to smaller peptide regions presented by special molecules encoded by class I and class II genes of the MHC (**Figure 2**).

During development, human fetuses generate an extremely large population of B cells and T cells, each one bearing a slightly different cell-surface receptor. Cells that recognize antigen are culled at this point because only self antigens are present (Burnet 1959). An almost unlimited combinatorial explosion of receptor diversity is generated through somatic recombination, whereby sets of gene segments drawn from a diverse but finite pool are fused into unique, irreversible mosaics (Janeway et al. 2005).

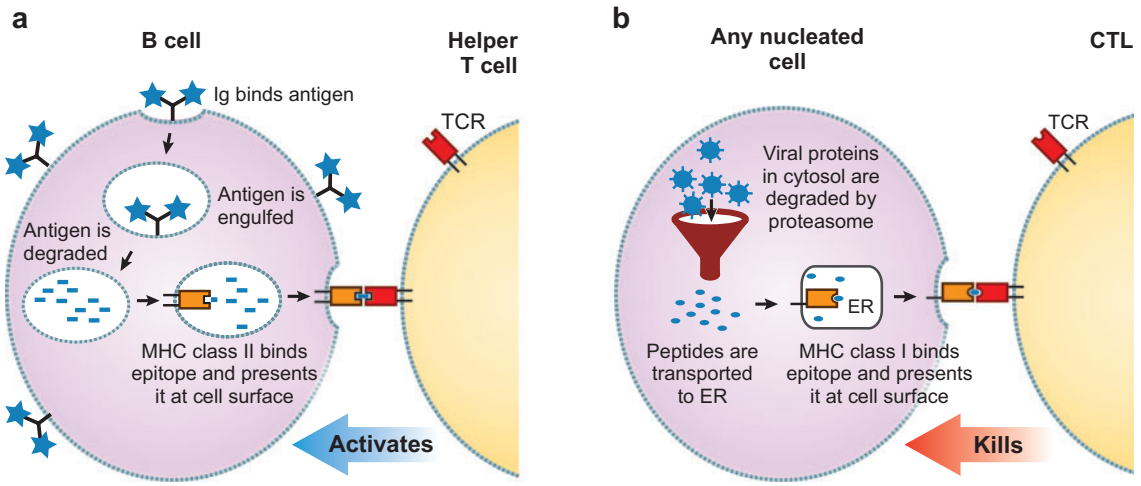

Figure 2

Antigen presentation by major histocompatibility complex (MHC) molecules. (*a*) B cells bind viral antigen with their immunoglobulin receptors and engulf it. The antigen is then degraded within vesicles into peptides 13 amino acids or greater in length. Peptides that bind MHC class II molecules are then presented at the cell surface where the epitope/MHC complex can be recognized by helper T cells with matching T cell receptors. If a match (binding) occurs, the helper T cell activates the B cell (signals it to proliferate). (*b*) Cells infected with virus degrade viral proteins found in the cytosol, via the proteasome. The resulting 8–10 amino acid peptides are then transported to the endoplasmic reticulum (ER). Peptides that bind MHC class I molecules are presented at the cell surface. Cytotoxic T lymphocytes (CTLs), whose receptors bind the epitope/MHC complex, induce apoptosis in the virus-infected cell.

B cells further the refinement of Ig specificity via somatic hypermutation, driven by AID (see above discussion of ABOBEC3G). It is the remaining pool of hundreds of millions of circulating lymphocytes that is called upon when the host is challenged by pathogens. One or more lymphocytes, by chance, might bear a receptor that is a good fit for some antigenic region; the tighter the fit, the stronger the signal for the lymphocyte to proliferate. The upshot, after several rounds of lymphocyte division, is an adapted population of lymphocytes. The successful variant in the original pool is amplified into a massive army of clones with the ability to home in on the pathogen with great accuracy and precision, then kill or neutralize it. These cells remain ready for quick action should the pathogen ever return.

Major Histocompatibilty Complex Molecules versus Viral Epitopes

Any attempt to understand how selection imposed by viruses has shaped the human genome rests on an appreciation of the MHC (**Figure 1**). The MHC is an extremely gene-dense region of chromosome 6, housing approximately 130 expressed genes, almost half of which have immune system function (Meyer & Thomson 2001, MHC Seq. Consort. 1999). These include the diverse and rapidly evolving MHC class I and

II genes that broker the antigen recognition performed by T cells and B cells, as well as many other genes with immune function. Without MHC molecules—better known as human leukocyte antigen (HLA) in humans—there can be no effective neutralizing antibody (NAb) response from B cells, of the sort that helps us eliminate influenza virus (**Figure 2*a***), and no cytotoxic T lymphocyte (CTL) response to destroy cells infected with virus, of the sort that helps us fight HIV-1 (**Figure 2*b***).

MHC class I molecules have a direct and special connection to viruses: They play the central role in the task of alerting CTLs to cells that have been breached by virus and are an unambiguous antivirus adaptation (**Figure 2**). Expressed by all nucleated cells, class I molecules bind to and transport intracellular, cytosolic viral peptides. These are derived from larger proteins that have been minced into short fragments in the proteasome, a sort of meat grinder that cleaves any proteins found in the cytosol into bite-size pieces for MHC molecules. They present these peptides on the infected cell's surface (physically bound in pockets or grooves) for inspection by roving T cells; because of the specificity discussed above, a given T cell will recognize only specific epitope/MHC complexes (Doherty & Zinkernagel 1975, Zinkernagel & Doherty 1974). If a CTL binds to the peptide/MHC complex, it induces apoptosis in the cell that presented the antigen. MHC delivers a constant efflux of cytosolic peptides to the cell surface, allowing the immune system to effectively see what is going on deep within almost every cell of the body and to respond accordingly.

MHC class II molecules, however, are involved in transporting and displaying antigens derived from vesicles within the cell (**Figure 2**). These can be of two main sorts. First, B cell–mediated responses, including the NAb response, require a go-ahead signal from $CD4^+$ helper T cells. This is where MHC class II genes come into play. A successful antibody response to a viral pathogen begins with a helper T cell recognizing a viral T cell epitope presented by the B cell: The B cell must capture viral antigens with its Ig receptor, engulf them, degrade them into small peptides, then spit out these minced up fragments onto its cell surface with the aid of MHC class II molecules (**Figure 2**).

Second, there are vesicles in which pathogens such as *Mycobacterium tuberculosis* seek intracellular refuge from immune attack. Class II molecules deliver antigen from such invaders to the cell surface where it can be recognized by $CD4^+$ T cells, which command the infected cell to flood the vesicle with bactericidal poison (not shown). This sort of antigen presentation, although not directly connected to viruses, is still related: One of the reasons so many AIDS patients die from tuberculosis is that the virus destroys the T cells that would normally recognize the *M. tuberculosis*–derived epitopes bound to MHC class II molecules on the surface of an infected cell.

There is a large degree of specificity between the binding of MHC and viral peptides, and this is a crucial point for understanding the evolution of both viruses and humans. A given MHC/HLA variant will only bind one or a few short peptide fragments, and changing just one amino acid can often obliterate binding (reviewed in Frank 2002). This sets the stage for CTL epitope escape, on the part of viruses (discussed below). But it also evidently puts tremendous selective pressure on humans to evolve and maintain a diverse array of MHC molecules. Heterozygosity at *HLA* loci can lead to a powerfully diverse immune response (Carrington et al. 1999,

Doherty & Zinkernagel 1975). Accordingly, *HLA* loci are by far the most polymorphic of any in our genome (MHC Seq. Consort. 1999). *HLA-B*, an MHC class I gene, leads the way with 851 alleles, followed by *HLA-A*, another class I locus, with 506, and *HLA-DRB1*, a class II locus, with 476 (**Figure 1**) (Robinson et al. 2003; **http://www.ebi.ac.uk/imgt/hla/**).

Most *HLA* polymorphism is also evidently generated de novo in each host species (Shiina et al. 2006), contrary to earlier assertions that it represents ancient variation predating the origin of humans. Shiina et al. traced single-nucleotide variation generated before and after human-chimpanzee speciation and showed conclusively that trans-species diversity could account for only a small fraction of MHC class I diversity in either humans or chimpanzees. This is important not only because it illustrates the strong incentive for constant generation and maintenance of a diverse HLA repertoire in response to a constantly evolving microbial antigenic repertoire, but also because it finally puts the MHC in line with other evolutionary genetics data that conclude that a narrow bottleneck occurred at the origin of our species (Hammer 1995).

With respect to nonself antigens, MHC class I molecules are virtually totally devoted to viruses, the only pathogens that normally replicate within the cytosol (Janeway et al. 2005). It appears, once again, that it is this group of pathogens that has driven our genes to the extremes of diversification. Viruses might even be one of the ultimate causes behind some of our more pleasurable, proximate activities: MHC gene products are aromatic, and we might unwittingly choose potential mates in part by sniffing out good, complementary MHC alleles—ones that might tilt the odds in favor of producing offspring with more robust immune systems (reviewed in Milinski 2006).

Natural experiments—cases where patients are born with defective MHC class II antigen presentation—suggest that viruses might be the main selective agents at class II loci as well: In one cohort of 10 children with MHC class II deficiency, 8 died during the study; 6 deaths were attributed directly to viral infection, and an additional death was likely due to complications arising from a viral infection (Saleem et al. 2007). This is an unsettling reminder of what lies just behind the veil of our immune system.

The MHC is associated with more genetic diseases than any other region of the genome (MHC Seq. Consort. 1999). This includes most autoimmune diseases, such as rheumatoid arthritis and diabetes. It seems that a good deal of human genetic disease has arisen via hitchhiking of deleterious mutations (MHC Seq. Consort. 1999), and it is probably not a coincidence that hitchhiking diversity reaches its peaks in the vicinity of the antigen-presenting *HLA* loci (Shiina et al. 2006). In other words, the long-standing and continuing battle to generate HLA diversity, in response to the Red Queen vicissitudes presented by pathogens, has been marked by a lot of costly collateral genetic damage—yet another indication of the utmost importance of our defenses against pathogens. The magnitude of debris dragged in the wake of selection on MHC loci says a lot about the strength of the selection and, in turn, the agents behind it.

HUMAN-VIRUS BATTLEGROUNDS WITHIN, AND BETWEEN, HOSTS

The Human Leukocyte Antigen Environment, Cytotoxic T Lymphocyte Escape, and Persistent Viruses

MHC class I–mediated antigen recognition, which places viral epitopes in the crosshairs of CTLs, puts strong selective pressure on viruses to alter those epitopes. Typically, the number of viral peptides detectable by a host's CTL population is, at any given time, quite small. Often only two or three immunodominant epitopes are detectable (Yewdell & Bennink 1999). This is why genetic variation (resulting from high viral mutation and replication rates, and large population sizes) is so crucial to viral persistence; a mutant virus with just the right amino acid substitution becomes invisible by moving outside the highly focused spotlight of the prevailing CTL response. Such CTL epitope escape mutations allow a few lucky variants to reproduce without the risk of their host cells being killed off through CTL-mediated damage control. This can allow the viral population to persist within the host for long periods—years or decades in some cases—with successive rounds of selective sweeps as viral epitopes are seen by CTLs, then altered by mutations that lead to CTL escape, then seen again by a new set of CTLs, and so on.

In HIV-1, within-host population dynamics leads to distinctive ladder-like phylogenetic trees. When samples are collected across many time points, it can be seen that the fate of most viral lineages within the host is to go extinct and that viral genetic diversity is repeatedly pruned down to one or a few variants that, for a short time, escape from immune responses. This is a nice illustration of the connectedness of viral ecology (predator-prey population dynamics) and evolution (genetic changes) and how aspects of both can be inferred from phylogenetic patterns. Grenfell et al. (2004) have termed this unified view of ecological and evolutionary dynamics phylodynamics. Interestingly, the phylodynamic patterns of HIV-1 at the population level are very different, with little evidence for selective sweeps that would be indicative of differences in host-to-host transmission fitness: The population-level phylogeny is very bushy, not ladder-like. Influenza virus phylogenies at the population level, however, look like within-host HIV-1 phylogenies. This pattern might reflect antigenic drift, whereby viruses that have mutated to escape previously infected hosts' antibody responses enjoy a selective advantage because they can reinfect previously exposed hosts (Bush et al. 1999). Recent findings, however, support a more nuanced perspective on influenza phylodynamics, one that includes a bigger role for stochastic effects than previously appreciated. Local introductions and extinctions of different viral variants often appear to be due to chance rather than fitness differences (Nelson et al. 2006), a finding that suggests that the ladder-like phylogenies of influenza virus might not be completely due to the host's population-level immune selection.

A similar ladder-like phylogeny has been inferred for Ebola virus isolates sampled over the past 30 years across central Africa. Walsh et al. (2005) sought to determine if the Ebola virus was endemic to the outbreak regions or if each outbreak represented a new introduction of the virus to a geographical region. The inferred phylogeny

indicated that the virus responsible for each new Ebola outbreak during this time period was a descendant of a preceding outbreak elsewhere and therefore was a recent introduction. Furthermore, these outbreaks followed a pattern of migration that was reflected in the viral phylogeny. This example shows that spatial and temporal dynamics (wave-like spread)—in the absence of strong immune selection—can clearly generate ladder-like phylogenies reminiscent of those shaped by immunity-driven selective sweeps. It also demonstrates that phylodynamics can be used to predict the future spread of emerging viruses.

The MHC class I alleles inherited from one's parents determine the immune system's ability to present pathogen antigens to T cells. An individual's *HLA* genotype can have far-reaching effects on the outcome of a variety of viral infections and is predictive, for example, of whether HIV is likely to kill you quickly or slowly, should you become infected. At a general level, Carrington et al. (1999) found that heterozygosity for *HLA-A*, *-B*, and *-C* translated into significantly delayed onset to AIDS among patients infected with HIV-1. More specifically, they also identified two deleterious alleles—one *HLA-B* and one *HLA-C*—associated with rapid progression to AIDS symptoms. Interestingly, they also noticed that the relative hazard of progression to AIDS or death was two- to threefold higher for homozygosity at the *HLA-B* locus versus *HLA-A*, a pattern that mirrors the higher polymorphism observed in *HLA-B* in the population at large.

The class I locus *HLA-B* turns out to be particularly important in defining the HLA environment that HIV-1 must adapt to in order to maintain a chronic infection (Bihl et al. 2006, Kiepiela et al. 2004). Kiepiela et al.'s study was a particularly elegant demonstration that *HLA-B* plays the dominant role in influencing HIV disease outcome. Among hundreds of HIV-positive patients in a South African cohort, a significantly greater number of CTL responses were HLA-B-restricted compared with HLA-A, and expression of particular *HLA-B* (but not *HLA-A*) variants was associated with extreme disease outcomes (fast or slow progression to AIDS). This is directly relevant to vaccine design because it suggests that artificial stimulation of CTLs directed against epitopes presented by HLA-A might be much less protective than ones designed for HLA-B, and that such CTL vaccines could be expected to perform very differently in patients with distinct complements of HLA alleles.

This observation also suggests an answer to the riddle of why *HLA-B* is more polymorphic in the human population than *HLA-A*, and why *HLA-B* has evolved more rapidly at the molecular level as well (Kiepiela et al. 2004). With HIV, and perhaps many other viruses, *HLA-B* is where the main action is in the battle between the pathogen and the host's CTLs. Moreover, it has recently become clear that the immune system's most effective CTL responses against HIV-1 target Gag polyprotein epitopes (Kiepiela et al. 2007, Zuniga et al. 2006). The *gag* gene, which codes for important structural components of the virus, is one of the most conserved in the entire HIV-1 genome. It is probably not a coincidence that the best place for the CTL response to lock onto viral epitopes is in a region where the virus is obviously under strong evolutionary constraints. Presumably, many potential CTLs escape mutations in *gag* come with too high a cost to be selected. At any rate, the battle within an HIV-1-infected host seems to be fought primarily between the Gag

protein of the virus and the one or two *HLA-B* alleles available to carry Gag epitopes to CTLs.

Evasion of adaptive immune responses is of course a crucial feature of other viruses that can establish persistent infections. Specific HLA alleles with either negative or positive associations with disease have also been found for HBV and HCV (Gaudieri et al. 2006, Heeney et al. 2006, Thio et al. 2003). With respect to HCV, which most often establishes a persistent infection, the HLA class II alleles *DQB1*0301* and *DRB1*11* are associated with self-limiting infection, predominantly in Caucasian populations (Yee 2004). Individuals with these alleles can likely eliminate the virus via an effective antibody response, triggered by a helper T cell population that binds particularly well to HCV peptides presented by these alleles. Polymorphisms at nonimmunity-related genes, such as *CCR5*, which codes for a cell-surface receptor that mediates the entry of HIV-1 into cells, can also have dramatic consequences for disease progression, including making some hosts effectively impervious to HIV-1 (see sidebar).

Kiepiela et al. (2004) found that HIV-1-infected infants in their Zulu/Xhosa study population had a much higher frequency of deleterious *HLA-B* alleles (B*18 and B*5802) and a much lower frequency of protective alleles (B*57 and B*5801) than the population at large. This suggests that HIV-1 can be expected to cause rapid changes in allele frequencies at HLA loci, especially *HLA-B*. It follows logically from the mechanics of MHC-based antigen presentation, and the diverse array of viral pathogens like HIV-1 and HCV-pulling antigen-recognition genes in many different

CCR5-Δ32: A VIRAL-RESISTANCE ALLELE

CCR5-Δ32 is a truncated *CCR5* allele found at high frequencies in Europeans. It leads to a defective cell-surface coreceptor, preventing HIV infection in homozygotes and decreasing the risk of infection in heterozygotes (Liu et al. 1996, Samson et al. 1996). *CCR5-Δ32* carriers also have a decreased likelihood of contracting hepatitis B virus (Thio et al. 2007) and improved outcomes during hepatitis C virus infection (Goulding et al. 2005).

The HIV/AIDS pandemic is too recent to account for current *CCR5-Δ32* frequencies. Bubonic plague and smallpox have been proposed as selective agents (Stephens et al. 1998, Galvani & Slatkin 2003), although recently it has been argued that neutral genetic drift may better explain the current frequency of *CCR5-Δ32* (Sabeti et al. 2005).

Biomedical researchers are optimistic that development of *CCR5*-antagonists (e.g., using RNAi) could lead to important therapeutic advancements for the fight against HIV (Qin et al. 2003), although there could be trade-offs. *CCR5-Δ32* carriers are now known to be at an increased risk for symptomatic West Nile virus infection (Glass et al. 2006) and are more susceptible to noninfectious diseases such as rheumatoid arthritis (Garred et al. 1998) and inflammatory bowel disease (Martin et al. 2001).

directions, that the evolutionary outcome was both an amplification in the number of *HLA* genes, and an accumulation of a diverse pool of allelic variants at these loci.

Viral Interference with Antigen Presentation, and the Host Response: Natural Killer Cells

Viruses do not limit themselves merely to fleeing the CTL response by quick evolutionary change. They also go on the offensive. They have evolved a suite of adaptations that target the heart of the antigen-recognition system: the presentation of viral epitopes by MHC molecules. In the arms race between viruses and humans (**Table 1**), it perhaps in this arena that host-virus coevolution has reached the most extraordinary level of escalation.

In HIV-1, to take one well-studied example, the accessory protein Vpu actively degrades the class I molecules, abrogating CTL responses (Kerkau et al. 1997). Another protein, Nef, downregulates MHC expression by the infected cell (Piguet & Trono 1999). Patients with a rare, *nef*-deleted virus tend to have extremely slow progression to AIDS (Deacon et al. 1995, Kirchhoff et al. 1995). In other words, downregulation of MHC seems to make the difference between a killer virus and a near-commensal virus. Other viruses have evolved similar techniques for interfering with virtually every step necessary for antigen presentation by both MHC class I and class II molecules (reviewed in Brodsky et al. 1999 and Ploegh 1998). MHC class I interference by viruses is not unexpected, but the fact that so many viruses go to the trouble of interfering with MHC II antigen presentation (Brodsky et al. 1999) is further evidence that much of the evolution in this MHC class, too, is virus induced.

The arms race does not end there, however. Our immune system includes a special set of cells, known as natural killer (NK) cells, which deal with MHC interference. These cells do not attempt to recognize viral antigens themselves. Rather, they express cell-surface receptors, called killer Ig-like receptors (KIR), that bind to MHC class I. Unless an interrogated cell can satisfy a curious NK cell that is free from interference with antigen presentation, by binding its KIR receptors with MHC, it is commanded to undergo apoptosis, just to be on the safe side. Incidentally, because NK-cell receptors need to keep up with MHC gene evolution, they evolve extremely rapidly (Khakoo et al. 2000) and have undergone amplification and exist as a large cluster of related loci on chromosome 19 (Nolan et al. 2006). The innate immune system, therefore, is not restricted to static solutions like TLRs, but can also display rapid molecular and genomic change when called upon by viral selective pressure.

Not to be outdone, viruses have evolved mechanisms for avoiding the NK response. The HIV-1 Nef protein, for instance, while downregulating the expression of *HLA-B* and *HLA-A*, the class I loci most likely to be associated with a vigorous CTL response, leaves *HLA-C*, which is much less likely to stimulate a strong CTL response, to be expressed at high levels. In doing so, it hobbles the host's CTL response but leaves enough impotent MHC molecules on the cell's surface to inhibit NK-cell activity (Collins & Baltimore 1999). It is this sort of adaptation, whereby a virus with a genome only 10 kb in length so subtly thwarts all of the intrinsic, innate, and adaptive immunity weapons deployed against it, that justifies the use of the term masterpiece.

Table 1 Key host-virus interactions and their consequences

Host-virus conflict/ interaction	Consequences for virus (within and between hosts)	Consequences for host (individual and population)
Toll-like receptors v. PAMPs (innate immunity)	Potentially unavoidable recognition	Rapid antiviral response
	↓ replication	Clearance or modulation of infection
	↓ transmission	Purifying selection on TLRs
	selection to disrupt signaling downstream of TLR binding	↓ susceptibility to cross-species transmission
RNAi v. viral dsRNA	Potentially unavoidable recognition	Specific antiviral response
	↓ replication	Clearance or modulation of infection
	↓ transmission	Tool for controlling gene expression
	Selection to usurp host RNA silencing to virus's advantage	↓ susceptibility to cross-species transmission
APOBEC3G v. Vif (intrinsic immunity)	Hypermutation of viral genome	Automatic antiviral response without need for recognition
	↓ replication	Prevention, clearance, or modulation of infection
	↓ transmission	Positive selection on *APOBEC3G*
	Selection on *vif* to block APOBEC3G	↓ susceptibility to cross-species transmission
Antibodies v. viral epitopes (B cell–mediated adaptive immunity)	↓ replication	Highly specific antiviral response
	↓ transmission	Prevention, clearance, or modulation of infection
	↓ reinfection	Selection for ↑ diversity of MHC class II molecules (gene amplification, positive/balancing selection)
	Positive selection for epitope escape mutations within hosts (persistence) and between hosts (reinfection)	
	Selection for glycan shield and other antibody-avoidance tactics	
CTLs v. viral epitopes (T cell–mediated adaptive immunity)	↓ replication	Highly specific antiviral response
	↓ transmission	Prevention, clearance, or modulation of infection
	↓ reinfection	Selection for ↑ diversity of MHC class I molecules
	Positive selection for epitope escape mutations within and between hosts	Selection to respond to viral interference with antigen recognition (NK cells)
	Selection for interference with MHC-based antigen recognition	
Natural killer cells v. viral interference with antigen recognition (innate immunity)	↓ replication	Clearance or modulation of infection by eliminating cryptic infected cells
	↓ transmission	Selection for ↑ diversity of *KIR* genes
	Selection to deceptively upregulate expression of ineffective MHC genes	

Within-Host Adaptations Can Be Costly: Cytotoxic T Lymphocyte Escape Mutant Transmission, HIV-1 Attenuation, and the Glycan Shield

In principle it is possible that selective pressures acting on viruses within hosts could conflict with those acting at, or after, transmission from one host to the next. In the case of HIV-1's resistance to NAbs, such conflicting pressures might help explain why the virus persists within humans and might expose a chink in the armor of HIV-1 through which vaccination strategies may be directed.

Thus far, attempts to develop an effective HIV-1 vaccine have been spectacularly unsuccessful. Essentially, researchers are hoping to use a vaccine to induce an immune response more effective than the body's own natural response. Although the human immune system can raise NAb against the HIV Env glycoprotein (the only viral protein exposed to the external environment), the virus has proven extremely successful at avoiding it (Albert et al. 1990). It is not just the rapid rate of mutation that helps HIV escape NAb; many of these mutations lead to the development and/or rearrangement of N-glycosylated sites, special amino acids where host sugar molecules can attach to the protein. Under NAb-driven selection, N-glycosylated sites accumulate, leading to a glycan shield that obscures the viral Env protein behind a layer of host-derived sugars (Wei et al. 2003).

This glycan shield seems like an impenetrable barrier behind which HIV should be free to replicate and transmit without worry of an NAb response. A ray of hope comes from a study of discordantly infected heterosexual couples (where one member of a presently monogamous couple is infected with HIV). Derdeyn et al. (2004) noticed that the HIV variants transmitted between these partners were significantly more likely to be ones with diminutive sugar shields. They hypothesized that while the glycan shield may be advantageous for HIV within a chronically infected host, it is a hindrance during transmission to a naïve host with no HIV-1 antibodies and likely must be lost and then re-evolved within each newly infected host. A vaccine directed at raising NAb may be more effective during this transmission event, when the glycan shield is absent, and might therefore be able to block HIV transmission.

As promising as this may be, a second study looking at homosexual male discordant couples did not find the same pattern of loss of the glycan shield upon transmission (Frost et al. 2005). Note that these patients were infected with a strain of HIV-1 that is distantly related to the strain studied in the heterosexual couples. It is not clear if this, or the different mode of transmission, explains the different glycan shield dynamics observed in the two studies. Clearly, more research is needed on the selective forces that govern the glycan shield formation/loss and how this relates to HIV transmissibility and future vaccine design.

While antibody-neutralization-sensitive variants might be selectively transmitted between hosts, there is no evidence for such selective transmission of HIV-1 CTL escape mutants (Frater et al. 2006). However, even if they are transmitted at background frequency, escape mutants clearly do sometimes move from one host to the next. What is the consequence of this for the host and virus? That depends upon the HLA environment within that host. Escape mutants must sometimes arrive in new

hosts with similar HLA to the host in which they evolved, putting the new host at an immediate disadvantage. But because of the standing HLA diversity in the human population, most often escape mutants simply go extinct because the new host has a different MHC environment (Goulder et al. 2001, Kiepiela et al. 2004, Phillips et al. 1991). There is little evidence that CTL responses within hosts are rapidly leading to a situation where most strains of HIV-1 are CTL escape mutants.

Ariën et al. (2005) found evidence that HIV might be attenuating over time: When competed against each other in head-to-head assays, strains from the 1980s exhibited significantly higher replicative fitness than strains circulating 20 years later. In other words, the later strains appeared to have lost some of their ability to infect new host cells, at least when competing directly against early strains in these tightly controlled experiments. Ariën et al. (2005, 2007) speculate that this drop in replicative fitness might be the result of the series of population bottlenecks experienced by HIV-1 each time it enters a new host, and might result in the evolution of milder strains of virus that take longer to progress to AIDS. The idea is that, despite the observation that replicative fitness actually increases throughout chronic infection within a host (Troyer et al. 2005), the genetic bottleneck that occurs when the virus is transmitted from one host to the next could obliterate and even reverse any fitness gains achieved within the first host. Moreover, if the genetic environment of the newly infected host is different, in particular at *HLA* loci, that will further penalize the newly transmitted virus: Its hard-won CTL escape mutations might no longer be beneficial because mutations that provide a cloak of invisibility in one host might have the opposite effect in the next host. With each such transmission between genetically mismatched hosts, the virus must evolve to escape the T cell responses of the new host; such evolution appears to come at the cost of replicative fitness (Martinez-Picado et al. 2006). It remains to be seen whether a decline in replicative fitness is a general feature of the unfolding HIV-1 pandemic and, if so, whether it has a real connection to intrinsic virulence, a property about which it is extremely difficult to make reliable predictions.

What Factors Prevent or Permit Viral Emergence?

Successful between-host transmission—involving hosts from different species—is one of the landmarks that every new emerging infectious disease agent must pass, and it pays to consider how it might, or might not, occur. The existence of intrinsic immunity genes that restrict viral host range may help explain why humans have acquired so few retroviruses from the many other infected primate species (Worobey 2007). Experimental evidence suggests that human APOBEC3G and its orthologs in Old World monkeys can restrict the ability of viruses to jump into new species. For example, human APOBEC3G blocks SIV from the African green monkey from infecting humans; the monkey homolog of APOBEC3G, in turn, blocks HIV. Surprisingly, the ability of these proteins to restrict viruses from other species is determined by a single amino acid substitution, which presumably prevents mismatched Vif protein from degrading APOBEC3G (Bogerd et al. 2004, Mangeat et al. 2004, Schrofelbauer et al. 2004). But for that single amino acid, there might be no human AIDS pandemic, at least as we know it.

Single amino acids can also make the difference on the viral side. Anishchenko et al. (2006) found that Venezuelan equine encephalitis virus, a mosquito-borne RNA virus that sometimes emerges out of its rodent reservoir and establishes outbreaks in horses and humans, requires just a single change in its envelope glycoprotein to do so. In this case, the main barrier to cross-species transmission might thus be ecological (rare opportunities for viruses to encounter abnormal host species) rather than evolutionary. More often, though, moving from one fitness peak to another (in a new host) might require multiple mutations, and such jumps are presumably difficult even for rapidly evolving viruses (Holmes 2006).

What is remarkable, and reassuring, is how effective our defenses must be: For every new pathogen that has become established in human populations, how many more must have died out after having infected just one or a handful of individuals? And for every hopeful cross-species transmission event where at least one human individual became productively infected, how many other exposures must have failed to even cause an initial infection, with host-restriction elements or innate immunity responses eliminating the pathogen before it gained a foothold in the host?

CONCLUSION

Clearly, a large proportion of the human genome is given over to genes that help shield us from pathogens. At some of the loci known to be directly involved in viral defense, we see allelic diversity that is unrivalled by other types of genes. We also see some of the most rapid molecular evolution and strong balancing and directional positive selection. Moreover, the genome itself may have experienced its most dynamic changes in response to viruses. Segmental duplications across the genome generally tend to be enriched for immunity genes (Gonzalez et al. 2005), and virus-relevant gene families such as MHC class I and II, *KIR*, and *APOBEC* are well represented in the top ranks of amplified loci. In the landscape of the human genome, the MHC is the richest biodiversity hot spot, and viruses are likely the main reason for this.

MHC/HLA genomic and allelic diversity represents one of the human population's most valuable genetic endowments, and the diversity of *HLA* alleles in the human population has no doubt buffered us from extinction. Nonrandom distributions of *HLA* alleles in different populations most likely echo local battles with pathogens that have come and gone. The overall diversity observed is all the more remarkable in light of the fact that periodic selective sweeps have probably been a feature of this history. The resulting MHC and other immunity-related genetic diversity presents both challenges and opportunities for therapies and immunization. We cannot, for example, expect vaccines designed to elicit beneficial T cell responses to work uniformly across patients because different individuals bring to the table different complements of antigen-recognition alleles. In the short run, this complicates matters; in the long run, we can look forward not only to drugs tailored to individual genomes, but perhaps also vaccines.

We believe the observations brought together in this review argue strongly in favor of viruses being the dominant agents shaping vertebrate and human defense and immunity, both present and past. Other ideas, such as that adaptive immunity might

have evolved in vertebrates because of a need to recognize and manage beneficial gut microbial communities (McFall-Ngai 2007), seem at odds both with the virus-driven genetic and genomic patterns inherent in the MHC and other immunity genes. The unparalleled levels of polymorphism and deleterious genetic diversity associated with the virus-specialized class I *HLA* loci, plus the fact that individuals with defective antigen-presentation genes die rapidly and almost exclusively of overwhelming viral infection, suggest that viruses have been, and remain, more important selective agents than bacterial and eukaryotic pathogens.

Viruses have shaped our battle-worn genotypes and phenotypes and may be an impetus behind many characteristics that seem far removed from pathogens, from complex gene regulation, to diabetes and arthritis, to mate choice and sexual reproduction. They are among the most important forces in human evolution, and future insights into human-virus interactions will likely play a key role both in controlling their emergence and spread and in understanding our own genetic heritage.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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