Evolutionary conflicts between viruses and restriction factors shape immunity

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Abstract | Host restriction factors are potent, widely expressed intracellular blocks to viral replication that are an important component of the innate immune response to viral infection. However, viruses have evolved mechanisms that antagonize restriction factors. Through evolutionary pressure for both host survival and virus replication, an evolutionary ‘arms race’ has developed that drives continuous rounds of selection for beneficial mutations in the genes encoding restriction factors and their viral antagonists. Because viruses can evolve faster than their hosts, the innate immune system of modern-day vertebrates is for the most part optimized to defend against ancient viruses, rather than newer viral threats. Thus, the evolutionary history of restriction factors might, in part, explain why humans are susceptible or resistant to the viruses present in the modern world.

Restriction factors are proteins of the innate immune system encoded in the germline genome that inhibit the replication of viruses during their life cycle in host cells. These host proteins are dedicated antiviral factors that are often induced by interferon (IFN) signaling as part of the innate immune response. They are antagonized by viral factors and are rapidly evolving. The term ‘restriction factor’ was historically adopted by laboratories studying retroviruses following the characterization of the mouse Fv1 locus, which conferred resistance to murine retroviruses1. However, this term can also be applied more broadly to host-encoded gene products that inhibit the intracellular replication of any animal virus. Recent work has shown that host susceptibility to viral infection and disease is determined, in part, by the components of the innate immune system (such as restriction factors) and the viral proteins that have evolved to evade or destroy these host defences. In this Review, we describe the general characteristics of restriction factors and show how the evolutionary conflict between viruses and restriction factors has shaped the immune systems of modern-day vertebrates. We use examples of host restriction factors that block primate lentiviruses, although we believe that many of the principles are generally applicable to other viruses and other hosts. These topics are of particular relevance today, as there have been many recent discoveries of restriction factors and determinants of viral susceptibility.

Characteristics of restriction factors
Classical innate immunity against viruses is mediated by specialized cells such as natural killer cells, dendritic cells and macrophages. By contrast, restriction factors are germline-encoded factors that mediate a ‘cell-intrinsic’ immune response. They are part of the broader innate immune repertoire of cellular molecules that detect and respond to viral infections in the absence of previous exposure. Typically, viral infections are detected by cytoplasmic or membrane-bound pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), which trigger an IFN response that induces a programme of expression of IFN-stimulated genes with broad-ranging effects on cell growth and metabolism (reviewed in REFs 2,3). Many of these IFN-stimulated genes are restriction factors that specifically inhibit viral growth within infected cells. TABLE 1 lists the general features of the restriction factors that target retroviruses and other viruses that are described in this Review. TABLE 1 is not a comprehensive list of restriction factors, but contains some of the best-studied examples.

There are several distinguishing characteristics of restriction factors that allow one to make inferences about their role in the evolution of both the host and the virus. Typically, we define a host gene as a restriction factor gene if it encodes a protein that: has antiviral activity as its major biological function; is induced by IFNs or by virus infection; is antagonized by a viral protein; and shows evolutionary ‘signatures’ of genetic conflict (positive selection).
The majority of true restriction factors share these features, as described in detail below. However, the exceptions to these definitions are also highlighted in Table 1, as they can be enlightening with regard to understanding the additional cellular roles that restriction factors might have.

Expression and activity of viral restriction factors. Many restriction factors are IFN-stimulated genes (Table 1), which is consistent with their fundamental role in antiviral responses. The IFN-mediated induction of many restriction factors is also an indication that their major activity is in combating pathogens, rather than some central metabolic or developmental role in the organism. Moreover, as many restriction factors cause destructive events, such as protein modifications or nucleotide mutations, their expression needs to be tightly controlled to avoid deleterious effects on cell growth in the absence of viral challenge. However, IFN-mediated induction is not a universal property of restriction factors, as some are expressed constitutively. In cases in which expression of the protein is constitutive, it is probable that the restriction factor also has a role in restricting endogenous events. For example, in the APOBEC3 family of cytidine deaminases, APOBEC3G is constitutively expressed by many cell types, including T cells and germ cells. For example, TRIM5α (tripartite motif-containing protein; ZAP, zinc-finger antiviral protein (also known as ZC3HAV1), PKR, RNA-activated protein kinase; SAMHD1, SAM domain- and HD domain-containing protein 1; SIV, simian immunodeficiency virus; TRIM, tripartite motif-containing protein; ZAP, zinc-finger antiviral protein (also known as ZC3HAV1). *Viruses are listed by family, which refers to a group of viruses with similar genomic structures and replication strategies.
Paralogues are generated within a species and homologous to another gene within a species. A gene that is homologous to another gene within a species is referred to as a paralogue. Paralogues are generated through gene duplication and then diverge.

Because it inhibits viral replication by means of a specific interaction with retroviral capsid proteins, tethersin (also known as BST2) can restrict enveloped viruses across several virus families, because it is nonspecifically incorporated into the cell and virus membranes and prevents efficient viral release by tethering enveloped viruses to the cell (TABLE 1).

We propose that the major biological activity of restriction factors is to inhibit viral replication. In many cases in which restriction factor function can be examined by gene knockout in mice, ablation of the restriction factor has no untoward effect on mouse development. For example, mice lacking the single mouse Apobec3 gene are viable, and the only reported phenotype is that they are more susceptible to murine retroviruses than are their wild-type counterparts. In fact, natural mutations in Apobec3 and the Mx locus that abolish function exist in inbred mouse strains. Similarly, mice with natural or engineered mutations in the tetherin, viperin (also known as Rsad2) or interferon-induced transmembrane protein 3 (Ifitm3) genes are also viable but are more sensitive to some viral infections. However, it is possible that some restriction factors have other cellular roles in addition to viral restriction. For example, TRIM5α has a more general role in antiviral signalling, in addition to its specific role in retroviral restriction. In many cases, the inability to identify a viral antagonist is more likely to be attributable to the fact that the relevant sets of viruses and host species have yet to be examined.

Viral antagonists can overcome restriction factors using several mechanisms. For example, viral antagonists can couple the restriction factor to protein degradation pathways; cause the mislocalization of the restriction factor and thus downregulate functional expression; or function as mimics of the restriction factor substrate (FIG. 1). To antagonize the restriction factor SAMHD1, the Vpx protein encoded by HIV-2 and related primate lentiviruses targets SAMHD1 for ubiquitylation followed by proteosomal degradation (FIG. 1a) by simultaneously binding to SAMHD1 and an adaptor protein in the cullin 4 ubiquitin ligase complex. The lentiviral Vif protein antagonizes APOBEC3G by a similar mechanism. By contrast, the lentiviral Vpu protein antagonizes the restriction factor tetherin by altering its normal subcellular localization (FIG. 1b). Through a direct protein–protein interaction, Vpu sequesters tetherin in the trans-Golgi network and redirects it from the cell membrane to endosomes, where it is unable to restrict viral budding from the cell membrane. A third mechanism of antagonism is illustrated by K3L, a poxvirus-encoded antagonist of the host antiviral RNA-activated protein kinase (PKR) pathway. Following recognition of double-stranded RNA, PKR inhibits protein translation by phosphorylating eukaryotic initiation factor 2 (eIF2α). K3L is structurally homologous to eIF2α and competes for binding to PKR (FIG. 1c). By acting as a mimic of eIF2α, K3L prevents the phosphorylation of eIF2α and the translational shutoff that the PKR pathway would otherwise induce (reviewed in REF. 28). Viruses might also use other strategies that have not yet been characterized to allow viral replication despite the presence of restriction factors. A key feature common to all these modes of antagonism is that, it is possible that the virus can escape restriction through mutation of the viral protein targeted by the restriction factor, as is the case for lentiviral evasion of TRIM5α-mediated restriction through viral capsid muta- 

**Box 1 | Coordination of restriction factors with other arms of the immune system**

The relationship between restriction factors and the rest of the innate immune system is a growing area of research. In many ways, restriction factors are similar to pattern-recognition receptors (PRRs) because they recognize structural patterns on pathogens. In fact, TRIM5α (tripartite motif-containing protein 5a) — which binds to a viral capsid lattice structure and accelerates capsid uncoating to cause viral restriction — has recently been shown to also function as a PRR for retroviruses. After binding to retroviral capsids, TRIM5α causes the activation of nuclear factor-κB (NF-κB) signalling and a distinct innate immune response. Moreover, even in the absence of retroviral capsids, TRIM5α has been shown to have a role in innate immune responses, as it functions as a constitutive signalling intermediate in the NF-κB cas- 

**Viral antagonists of restriction factors.** Viruses have evolved antagonists to restriction factors. These viral proteins are often encoded by ‘accessory genes’ that are not needed for viral replication except in the presence of restriction factors. Restriction factors such as tetherin that inhibit the replication of multiple virus families can be antagonized by diverse viral proteins from the different virus families (TABLE 1). In cases in which there is no known viral antagonist to a particular restriction factor, it is possible that the virus can escape restriction through mutation of the viral protein targeted by the restriction factor, as is the case for lentiviral evasion of TRIM5α-mediated restriction through viral capsid mutations. It is also theoretically possible that a recently evolved restriction factor might not yet have selected for a viral antagonist. However, in most cases, we think that the inability to identify a viral antagonist is more likely to be attributable to the fact that the relevant sets of viruses and host species have yet to be examined.

**Parologue**

A gene that is homologous to another gene within a species. Paralogues are generated through gene duplication and then diverge.

**Restriction factors.** Proteins that are not needed for viral replication except in the presence of restriction factors. The TRIM family of proteins are examples of restriction factors.

**TABLE 1**

| Restriction Factors | Examples |
|---------------------|----------|
| TRIM5α              | Retrovirus capsid receptor |
| SAMHD1              | RNA-activated protein kinase (PKR) |
| tetherin            | Tetherin (also known as BST2) |
| Ifitm3              | Interferon-induced transmembrane protein 3 (Ifitm3) |
| Rsad2               | Interferon-induced transmembrane protein 3 (Ifitm3) |
| APOBEC3G            | APOBEC3G |
| K3L                 | Poxvirus-encoded antagonist |
| Vpu                 | Lentiviral Vpu protein |
| Vif                 | Lentiviral Vif protein |

**FIG. 1**

A schematic diagram illustrating the interaction between restriction factors and viral antagonists. (A) SAMHD1 is targeted for ubiquitylation and proteosomal degradation by lentiviral Vif. (B) Tetherin is sequestered in the trans-Golgi network by lentiviral Vpu. (C) K3L mimics eIF2α and competes for binding to PKR.
Many non-coding regions of the RNA viruses main 34–37 by targeting it for degradation. The lentiviral accessory protein Vpx antagonizes the host restriction factor SAMHD1 (SAM domain- and HD domain-containing protein 1) by targeting it for degradation. The subsequent development is evolutionary system, that states: “For an evolutionary system, continuous development is needed just in order to maintain its fitness relative to the systems it is co-evolving with.”

Positive selection as a fundamental principle of virus-host interactions. Many non-coding regions of the genome evolve under neutral selection; for example, non-synonymous (amino acid-altering) and synonymous mutations are predicted to accumulate at the same rate in pseudogenes. Most host protein-coding genes evolve under negative (purifying) selection, which removes non-synonymous mutations from the population to maintain the function of the protein. By contrast, the interactions between restriction factors and viral antagonists evolve under positive selection, a selective regime that results in an excess rate of non-synonymous mutations compared with synonymous mutations (BOX 2). Positive selection is often a result of two genetic entities evolving in conflict with one another, as illustrated by the ‘Red Queen’ hypothesis, which describes an evolutionary system in which continuous adaptation is required to maintain the status quo29. Virus-host interactions are examples of ‘Red Queen’ competition, as host restriction factors exert a selective pressure on virus replication and pathogenic viruses exert fitness costs on their hosts. Mutations that allow a restriction factor to evade a viral antagonist provide a means for the host to escape the fitness costs conferred by the virus. This imposes a selective pressure on the viral antagonist to evolve specificity for the new restriction factor encoded by the host species. As a result, a prey–predator-like ‘arms race’ dynamic is established, leading to the rapid evolution of both the host and the virus [FIG. 2a]. Thus, nearly all of the restriction factors described in TABLE 1 contain genetic ‘signatures’ of positive selection.

Because viruses have existed throughout vertebrate evolution, the arms race between hosts and viruses is ancient30. In fact, many host restriction factors have evolved under positive selection for many millions of years. Under a long-term or recurrent viral selection pressure, a single amino acid in a restriction factor that directly interacts with a viral antagonist may repeatedly be mutated many times during evolution, or a restriction factor may accumulate mutations at multiple residues to escape from antagonism by many different viruses. This leads to an unusually high ratio of the non-synonymous mutation rate (dN) to the synonymous mutation rate (dS) — dN/dS — at single residues and across entire proteins [FIG. 2b]. To estimate the dN/dS ratio of a gene, ancestral gene sequences can be reconstructed using orthologous gene sequences from modern-day species that diverged millions of years ago, and statistical methods are used to calculate the rate of evolution across a phylogenetic tree. This method has shown that many human restriction factors have been evolving under episodic positive selection throughout primate evolution (TABLE 1). Other methods in addition to the calculation of dN/dS can also be used to identify positive selection across different timescales31, and additional background on the relevance and use of measures of positive selection in human evolution can be found in other reviews32.

How do hosts keep up in the arms race?

If single nucleotide changes were the only effector mechanism in the co-evolution of hosts and viruses, the host would be at a seemingly enormous disadvantage, because RNA viruses and some small single-stranded DNA viruses have nucleotide substitution rates that are 1,000 times faster than those of their hosts33–37. How then does a host restriction factor ever win an arms race with a virus, especially considering that a host might be simultaneously challenged by many different types of virus? The answer lies in the types of genetic landscape that viruses and hosts can explore.

Limitations on viral evolution. RNA viruses maintain densely packed genomes that include overlapping reading frames and RNA hairpin structures involved in genome packaging and replication. The limitations on the
Box 2 | Selective sweeps are the mechanism of adaptation

An important aspect of the positive selection of restriction factors is that the selection (and therefore evolution) of an advantageous mutation acts on a population level. The cost of a viral infection must affect the ability of the population to reproduce before it will exert a selective pressure on the population to evolve. During a population-wide infection, some individuals may carry a previously neutral genetic mutation that now confers those individuals with a reproductive advantage in the face of infection, and this advantageous genotype can rapidly rise in frequency until the mutation reaches a frequency of 100% (known as fixation). In a classic selective sweep, surrounding regions of the genome are inherited together (known as hitchhiking) with the genomic sequence that confers the fitness advantage, thus decreasing genetic diversity near the region of the genome under positive selection (reviewed in REF 90–92). Therefore, genomic loci that are under positive selection in a population are predicted to have skewed allele frequencies across long genetic distances surrounding the selected locus. Eventually, if a mutation reaches fixation within a species, between-species comparisons will reveal an excess of non-synonymous mutations in this region relative to the number expected under neutral selection93.

dN/dS
The ratio of the rate of non-synonymous mutations to the rate of synonymous mutations. Values greater than one are indicative of positive selection; values less than one are indicative of negative selection, and values near one are indicative of neutral selection.

Selective sweep
A decrease in diversity in the genomic region surrounding an allele under positive selection.

Hitchhiking
Genetic linkage between loci under positive selection and nearby loci not under positive selection.

Balancing selection
Selection to maintain polymorphism owing to frequency-dependent selection or heterozygote advantage.

Frequency-dependent selection
Selection to maintain mutations at an intermediate frequency to confer a fitness advantage.

Host heterozygosity
Genetic polymorphisms in genes encoding host restriction factors can be maintained as a result of population-level adaptation against viruses. Balancing selection in restriction factors may result when multiple viruses co-infect a population, such that different host haplotypes are advantageous against different viruses, essentially maintaining polymorphism within the population (known as frequency-dependent selection). Several of the best-known examples of genes under balancing selection are genes involved in immunity. These include: MHC genes49, which maintain multiple alleles that present a variety of antigens and therefore protect against a variety of pathogens; the glucose-6-phosphate dehydrogenase gene41, a housekeeping gene that contains polymorphisms that are associated with clinical disorders and also with malaria resistance; and TRIM5, which has been suggested to be under balancing selection in Old World monkey populations42.

Heterozygosity for a restriction factor may be advantageous to a population on a short timescale, as host polymorphisms would force a virus to evolve the ability to target multiple alleles of a given host factor. This was recently suggested for the restriction factor APOBEC3G in African green monkeys43, a primate species naturally infected with simian immunodeficiency virus (SIV). APOBEC3G is polymorphic in African green monkeys, and some individuals have a single amino acid change that renders APOBEC3G resistant to its viral antagonist, Vif. In an experimental infection of African green monkeys with SIV, the virus from a monkey that was heterozygous for the Vif-resistant allele of APOBEC3G was unable to evolve the ability to antagonize APOBEC3G, whereas the virus from a monkey that was homozygous for the Vif-resistant allele was quickly able to evolve the ability to antagonize APOBEC3G. This suggests that maintaining polymorphism in a restriction factor can be functionally beneficial.

Gene duplication and innovation. The duplication of restriction factor genes is another evolutionary strategy for accelerating host adaptation to a virus. By duplicating a restriction factor, the host can simultaneously explore multiple evolutionary trajectories. For example, in primates, artiodactyl (cloven-hoofed mammalian), canine and feline species, the APOBEC3 repertoires include many more paralogue sets than in rodents. In these lineages, the ancestral mammalian state — which was a single APOBEC3 gene — has been expanded to a family of APOBEC3 genes44–48. Most primate genomes now encode seven APOBEC3 gene paralogs, which vary in terms of their antiviral activities and retroelement targets, suggesting that they are adapted to different viruses. Several APOBEC3 genes (including APOBEC3D, APOBEC3G and APOBEC3H) show evidence of positive selection in primates49,50, but the specific residues that are under positive selection vary between the APOBEC3 genes, further supporting the idea that each paralogue has evolved to target different viruses. In this way, increasing the copy number of a given restriction factor probably gives the host the flexibility to rapidly evolve in response to several different viruses, leading to large families of related restriction factors. Other restriction factor families that are the result of gene duplications include the Mx1 gene family, which comprises two paralogs in some mice51; the IFITM gene family, which comprises at least four paralogs in humans and five paralogs in mice52; and the Trim5 gene family, which comprises eight paralogs in mice and cows and three paralogs in rats53.4

Alternatively, multiple members of a restriction factor family could evolve to target the same virus in different ways, thereby constraining viral evolution such that the virus must maintain multiple defence strategies. An example of this is the pair of human paralogs APOBEC3F and APOBEC3G, APOBEC3F and APOBEC3G deaminate cytosine bases in the viral genome within different preferential sequence contexts53. Thus, primate lentiviruses (such as HIV-1) have had to evolve multiple mechanisms to antagonize these APOBEC3 proteins; for example, Vif binds to APOBEC3F and APOBEC3G using distinct domains54–56. In this way, the host limits the ability of the virus to evolve while increasing antiviral activity.

Although TRIM5 is not duplicated in primates, it has undergone gene innovation in macaques and owl monkeys, which have independently gained additional exons through the insertion of a cyclophilin A gene (CYP4A; also known as PPLA) into non-coding segments of the TRIM5 gene57,58. In both species, a TRIM–CYP fusion protein with potent antiviral activity is produced, although the viral targets are not identical. Moreover, TRIM–CYP and TRIM5α can both be expressed by the same individual, allowing for the restriction of multiple lentiviruses. By restricting viral replication in this manner, the host also slows down the evolution of the virus.
Paleyovirology is the study of ancient, extinct viruses (paleoviruses) and their effects on modern-day host–virus interactions. We know that ancient retroviruses infected primates because there are remnants of viral sequences in primate genomes. However, many other retroviruses did not become endogenous in the host genome, and so we have no direct evidence of their existence. In fact, no endogenous lentiviral sequences have been found in primate genomes except in a single genus of prosimians. However, by identifying signatures of positive selection in host restriction factors, we can infer the existence of many additional paleoviruses, as well as the historical timeframe and species in which the infection took place. By combining evolutionary analyses with functional tests, we can determine the type of virus that is likely to have driven selection in the host (FIG. 5).

One of the clearest examples in which a paleovirus was identified by examining positive selection comes from the analysis of APOBEC3DE, a member of the APOBEC3 family in primates that restricts endogenous retrotransposons. APOBEC3DE has rapidly evolved in primates, particularly in the chimpanzee and bonobo lineages. Since its divergence from the human gene, APOBEC3DE in the chimpanzee lineage has accumulated 24 mutations, of which 23 are non-synonymous changes. These changes have broadened the antiviral activity of chimpanzee APOBEC3DE to include the ability to restrict lentiviruses. Human APOBEC3DE, by contrast, has not evolved the ability to restrict lentiviruses. Therefore, by identifying the adaptive consequences of rapid evolution in chimpanzee APOBEC3DE, we suggest that a lentivirus infected the common ancestor of chimpanzees and bonobos in the past; this infection probably occurred approximately 2–5 million years ago, after the chimpanzee–bonobo ancestor diverged from humans. Similarly, the acquisition of a TRIM–CYP fusion gene with antileptiviral functions in owl monkeys 2–6 million years ago strongly argues for such a challenge occurring in this lineage of primates, which is both phylogenetically and geographically distinct from the primates that are known to be infected with lentiviruses currently. By studying the evolution of restriction factors, we can form a more accurate picture of the ancient history of retroviral infections in primates.

Lessons from the evolution of restriction factors

Studying the evolution of restriction factors can help us to understand why humans are susceptible to viruses that exist today, as our immune responses to contemporary viruses have been shaped by our evolutionary responses to previous infections. The modern innate immune system is generally not yet optimized against modern viruses, but rather was selected for by previous rounds of co-evolution with ancient viruses. By determining the types of viral infection that occurred in the past and how they were eliminated, we can form new ideas about how to manipulate the immune system to our advantage in the ongoing battle against viruses.

Identifying previous viral infections. Paleyovirology is the study of ancient, extinct viruses (paleoviruses) and their effects on modern-day host–virus interactions. We know that ancient retroviruses infected primates because there are remnants of viral sequences in primate genomes. However, many other retroviruses did not become endogenous in the host genome, and so we have no direct evidence of their existence. In fact, no endogenous lentiviral sequences have been found in primate genomes except in a single genus of prosimians. However, by identifying signatures of positive selection in host restriction factors, we can infer the existence of many additional paleoviruses, as well as the historical timeframe and species in which the infection took place. By combining evolutionary analyses with functional tests, we can determine the type of virus that is likely to have driven selection in the host (FIG. 5).

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Predicting viral pathogenicity. In the virus–host arms race, positive selection occurs when the reproductive fitness of either party is challenged. If a virus is not pathogenic to the host, it is not likely to exert a selective pressure on the host. Therefore, adaptive changes in host restriction factors would not be expected to occur during a non-pathogenic infection. Mildly pathogenic viruses would be expected to impart weak selective pressures that might increase the allele frequency of a
selected mutation but would not drive polymorphisms to fixation\textsuperscript{71}. For example, simian foamy viruses (SFVs) are considered to be non-pathogenic in their natural hosts\textsuperscript{72,73}. Interestingly, SFVs have co-evolved with their hosts for more than 30 million years\textsuperscript{74}, demonstrating that there might not be a selective pressure to stop SFV replication. Furthermore, the rate of evolution of SFVs is many times slower than for other RNA viruses\textsuperscript{75}, which suggests that the arms race between virus and host has slowed down considerably in this case.

Natural infection of African green monkeys by SIV is also thought to be non-pathogenic, as infection does not cause immunodeficiency despite high viral replication levels\textsuperscript{76}. Surprisingly, polymorphisms in the African green monkey APOBEC3G gene that allow evasion from antagonism by host-specific SIV Vif proteins were found in the grivet and sabaueus subspecies\textsuperscript{77}, suggesting a recent selective pressure on APOBEC3G. Furthermore, the SIV strains that circulate in these subspecies have regained the ability to antagonize APOBEC3G. This suggests that there is an arms race between SIV and African green monkeys that implies some degree of SIV pathogenesis in African green monkeys. For example, SIV might formerly have been pathogenic to African green monkeys, or pathogenesis might be present even now in an unmeasured or mild form. In this manner, the evolution of a host restriction factor and the reciprocal viral evolution can inform our views of viral pathogenesis.

\textbf{Explaining why humans are susceptible to modern-day viruses.} HIV-1 and HIV-2 are the result of multiple cross-species transmission events of SIV from chimpanzees and sooty mangabeys, respectively, into humans\textsuperscript{78}. Primate restriction factors have been shown to have an important \textit{in vivo} role in preventing lentiviral cross-species viral transmission events. For example, experimental infection of rhesus macaques — which are not infected with SIV in the wild — with HIV or SIV can mimic a cross-species transmission event. During experimental HIV-1 infection, rhesus macaque TRIM5α and APOBEC3G completely restrict viral replication\textsuperscript{79,80}. Furthermore, naturally occurring polymorphisms in rhesus macaque TRIM5 attenuate viral replication by 100- to 1,000-fold during experimental infection with SIV from sooty mangabeys\textsuperscript{81}. These host genes involved in susceptibility or resistance to SIV infection may help to explain the dynamics of lentiviral zoonoses.

The four groups of HIV-1 — which are each the result of an independent cross-species transmission event to humans from chimpanzees infected with SIV — differ in their global spread, with group M representing the pandemic strain. It has recently been shown that the adaptation of HIV-1 to human-specific mutations in the restriction factor tetherin was achieved only by group M and N viruses and not by the non-pandemic group O and P strains\textsuperscript{82,83}. Clearly tetherin did not prevent any of the four cross-species transmission events, but it has been suggested that overcoming tetherin-mediated restriction was necessary for the efficient replication of HIV-1 group M in humans and therefore for pandemic spread (reviewed in REF. 79).

In studies of humans, the effects of restriction factor expression levels and polymorphisms on HIV-1 susceptibility and disease progression have not yielded a consensus viewpoint (reviewed in REF. 80). However, our immune system may be better at preventing cross-species
viral transmissions than intra-species viral transmissions because viruses that have crossed the species barrier have already partially adapted to the host. Perhaps for this reason, the evidence for the effects of restriction factors on intra-species viral acquisition is less clear.

**Identifying host-virus interaction domains and implications for treatment.** The interactions between a virus and a host restriction factor can be mapped down to distinct protein–protein interfaces and, in some cases, to single amino acid residues. Because these interaction domains are directly engaged in genetic conflict, they often contain the residues that are most rapidly evolving. By looking at genetic signatures of positive selection, the sites involved in protein–protein interactions can be predicted and then tested functionally, as was recently done with remarkable accuracy for SAMHD1 (Refs 81, 82). Without knowing anything about the domains of SAMHD1 that are required for antagonism by the lentiviral Vpx, two groups carried out positive selection analyses of SAMHD1 using the dN/dS test and identified two different regions of SAMHD1 that have evolved very rapidly in primates. When functionally tested, these two regions of SAMHD1 were shown to be required for its degradation by Vpx proteins from different lentiviruses in a virus-specific manner. This information has helped to explain how lentiviruses and SAMHD1 have evolved on a molecular level. By mapping host–virus interactions, the constraints of the evolutionary arms race can be more fully understood.

Moreover, these protein–protein interactions between host restriction factors and viral antagonists provide tempting targets for small-molecule inhibitors. An ideal inhibitor of a viral antagonist would specifically disrupt the ability of the antagonist to bind to the host restriction factor or to other host machinery required for restriction. This would enable a host restriction factor to specifically inhibit viral replication, without any effect on the rest of the immune system of an individual. Inhibitors of viral antagonists could be used as therapeutic treatments in combination with other antiretroviral drugs. Several inhibitors of HIV-1 Vif have been identified (Refs 83, 84), and attempts have been made at disrupting Vpu function (85). Achieving inhibition of a viral antagonist without disrupting host functions might be difficult because many viral antagonists use or mimic host machinery for their activity. Also, the virus might be able to quickly evolve resistance mutations, as genes encoding viral antagonists often do not have as many functional constraints as more conserved viral genes.

**Conclusion**

Restriction factors are early, potent and specific cellular blocks against retroviral replication. They have clearly had an important role in innate immunity against viruses throughout primate evolution, and more work needs to be carried out to define how and when they are important in viral zoonoses, global epidemics and the progression to disease. In this way, characterizing the evolution of restriction factor antiviral activity will help us to understand why we are winning or losing current battles against viruses.

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