Quasispecies Structure and Persistence of RNA Viruses

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Viral quasispecies are closely related (but nonidentical) mutant and recombinant viral genomes subjected to continuous genetic variation, competition, and selection. Quasispecies structure and dynamics of replicating RNA enable virus populations to persist in their hosts and cause disease. We review mechanisms of viral persistence in cells, organisms, and populations of organisms and suggest that the critical interplay between host and viral influences (including in some cases the quasispecies organization) is the main driving force for long-term survival of viruses in nature.

The emergence and reemergence of infectious diseases is influenced by the genetics of the infectious agents, the genetics of their hosts and potential new host species, and a considerable number of environmental factors (1-3). The current view proposes a strong stochastic (chance) component regarding the time, place, severity, and epidemiologic impact of infectious disease emergences (2,3). For RNA and possibly some DNA viruses, increasing evidence suggests that genetic variation (mutation, recombination, and genome segment reassortment in the case of multipartite genomes) affects adaptability to environmental changes (4-9). Since some types of adaptation involve changes in host cell specificity (5,6,10), genetic variation of viruses may be involved in the emergence of pathogenic viruses from apathogenic ancestors. Many studies over the last 2 decades have documented the unpredictability of genetic variation in viruses (2,5-7).

In this report, we consider viral persistence in connection with the population structure of RNA viruses, specifically, the extension (in space and time) of the pool of replicating genomes, which are a potential source of variant viruses with altered biologic features. For example, hantaviruses are apathogenic and endogenous to several rodent species. In several geographic areas of the American continent, the unusually mild and wet (El Niño effect) spring seasons of 1992 and 1993 resulted in abundant food and coverage for deer mice, increased numbers of infected deer mice, and increased risk for human infections. These factors led to the newly recognized severe pulmonary syndrome of humans in 1993 (11). Like many pathogenic RNA viruses, hantavirus evolved in humans, and recent epidemiologic evidence suggests human-to-human transmission (12); whether human-to-human transmission is exceedingly rare or is an unusual property associated with Andes virus is not known. The number of carriers, their ability to transport virus (because of their mobility and absence of severe symptoms), and the viral load (number and concentration of infectious particles) in each carrier must be involved in viral emergences and reemergences (2-4,6,13).

Population Complexity of Replicating Genomes: Viral Quasispecies

The viral load in an infected host is both static and dynamic. The static component can be divided into the total number of particles and the numbers and types of mutant viral genomes present in that total. Rather than being homogeneous, RNA virus populations consist of complex distributions of mutant (and sometimes also recombinant) genomes, in a type of population structure known as quasispecies (6,14-16). Assuming a random distribution of

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mutations among genomes, the number of variant genomes in a viral quasispecies increases dramatically with population size (15,17). For example, in a genome distribution with an average of five mutations per genome, the expected number of genomes with 20 mutations is 26 when the population size is $1x10^8$ viral particles but reaches 2,600 when the population size is $2x10^{11}$ particles, as calculated from the Poisson distribution (17). Fitness variations of the individual mutants modify the actual number of genomes in each mutation class (6,14-17). In a horse infected with epizootic Venezuelan equine encephalitis, viral titers in blood reach a high of 10^8 infectious units (i.u.)/ml, or a total of approximately $3x10^{12}$ i.u. These high titers are probably needed for efficient transmission of the virus to insect vectors and for completion of the arbovirus life cycle (18). High titers are also reached in acute infections with HIV-1, hepatitis B virus (HBV, a hepadnavirus replicated by an error-prone polymerase through an RNA intermediate), hepatitis C virus (HCV), influenza virus, the animal foot-and-mouth disease virus (FMDV), and probably many others (7,8).

Increasing evidence indicates that quasispecies evolution may lead to the selection of virulent viruses and to the emergence of new viral pathogens (1-7,10,17,18). For example, a single site at the 5'-untranslated genomic region of coxsackievirus B3 was associated with its cardiovirulent phenotype. Swine vesicular disease, not reported before 1966, may represent a human coxsackie B5 variant adapted to swine. Specific mutations in the RNA of lymphocytic choriomeningitis virus can confer a viral tropism for neurons or for cells of the immune system. Evolution of measles virus can lead to hypermutated forms that have been associated with subacute sclerosing panencephalitis. Major human influenza pandemics have been associated with antigenic shift resulting from genome reassortment between a human influenza virus and an animal influenza virus. Some small DNA viruses possess considerable genetic heterogeneity within infected hosts. Canine parvovirus was probably derived from a feline parvovirus as a result of two amino acid replacements in the viral capsid (1-7,10,17,18).

The viral load during RNA virus replication also has a strong dynamic component. In persons infected with HIV-1, HBV, or HCV, an estimated 10^{10} to 10^{12} new virions are produced each day. For some microorganisms (not only viruses), in what has been termed short-sighted evolution of pathogenic microorganisms (19), virulence may be an inadvertent consequence of mutation and selection in the parasite population. Models of HIV-1 pathogenesis based on the continuous production of antigenic variants have been proposed (8,19,20). As the infection progresses the complexity of antigenically distinct mutants may overwhelm the immune system, leading to AIDS (20). Models of HIV-1 pathogenesis, based on the stimulation of infected T-lymphocytes by secondary antigens (from opportunistic infections), have also been proposed; the progression of HIV infection to AIDS is still poorly understood. In HIV, and many other infections, the evolving viral quasispecies are exploring new mutant variants at astonishingly high rates. The balance between mutation rates and replication rounds is one of the reasons for the great adaptability of RNA viruses (6,7,14-17).

Types of Viral Persistence

"Persistence," which refers to long-term survival of viruses in their hosts, has been described in at least three ways. 1) Long-term survival of virus within a viable cell population occurs when cell disease and destruction are limited and viral genomes replicate in balance with the multiplication of the host cells. 2) Survival of viruses in entire organisms can rarely be reduced to persistence in one cell type since organisms are built as sets of interconnected mosaics of cell types and cell protein effectors; persistence of viruses in organisms often means coping with multitudes of selective forces and defense reactions while allowing the host to survive. 3) A virus can be maintained in nature by the continuous infection of susceptible host organisms, with or without persistence in cells or organisms and with or without long-term stability of viruses as free particles. Genetic, ecologic, and environmental factors exert different influences on these types of persistence. The quasispecies structure of RNA viruses plays an obvious, positive role in some persistence mechanisms. In others the role is more subtle, marginal, or nonexistent. The observations summarized here suggest, however, that persistence is always the result of interactions between viral and host determinants.

Persistence of Viruses in Cells

A variety of mechanisms enable viral genomes to replicate in balance with host cell multiplication. Most of the well-studied mechanisms of persistence of RNA viruses in primary cell cultures or established cell lines involve genetic variation of the virus, the cell, or both. One way to limit cell death is by generating and accumulating defective genomes. Defective genomes depend on a complementing, standard virus for replication; yet they may compete with that standard virus for cellular and viral gene products (which the virus needs to complete its life cycle and kill cells), thus increasing cell survival (21). A classic example is provided by defective interfering (DI) particles of vesicular stomatitis virus (VSV), and related viruses (22). During serial passage of VSV at high multiplicity of infection, DI particles accumulate in a cyclic pattern (23). Standard VSV and DI particles alternate in their dominance in a continuous process of mutant generation, competition, and selection. Excess virus leads to generation and accumulation of DI particles. With excess DI particles, mutant viruses able to escape interference rise to dominance (5). A feedback mechanism is established, which underlines the biologic relevance of the rapid evolutionary potential of RNA genomes (5).

Cell disease can also be limited by infecting cells with limited permissivity for the virus or by selecting noncytolytic variant viruses. In a number of cell-virus systems, cells and viruses coevolve, as documented for reovirus persisting in L cells (24) and for other RNA and DNA viruses (25-28). FMDV illustrates how the quasispecies structure initiates and maintains persistence (26,29). The presence in the FMDV quasispecies of variants with decreased ability to kill BHK-21 cells was not the mechanism (at least the prevailing mechanism) responsible for persistence. Rather, these experiments (29), which measured the proportion of cells that survived an initial cytolytic infection, indicated that a rapid variation of the cells initiated persistence. Indeed, the cells rapidly became more resistant to the infecting FMDV, and the virus became more virulent for the host BHK-21 cells (29). The quasispecies structure and consequent adaptability of RNA genomes do not justify any generalization on the participation of quasispecies, rather than the seemingly more static cellular DNA genomes, in initiation of viral persistence (29). However, the rapid evolution of FMDV toward virulence was very likely facilitated by mutant generation and was essential to sustain persistence (26,29). When the carrier cultures were challenged with FMDVs of distinct degrees of virulence (a population replacement experiment), the endogenous persistent virus was replaced by the externally added virus only when the latter displayed a higher virulence for BHK-21 cells. Thus, virulence can be a positive trait in viral persistence (30), and virulent variants present in the FMDV quasispecies helped maintain persistence when the triggering cellular event had occurred (29,30).

Persistence of Viruses in Organisms

Viral persistence in organisms requires a supply of susceptible cells replicating at the same pace as the virus and the ability to survive the host immune response. With RNA viruses, the continuous production of mutant viruses (inherent to the quasispecies dynamics [14,15]) contributes to virus survival (2,5-7,15-17). Viruses often use alternative receptors and coreceptors, and one or a few amino acid substitutions at exposed surface sites may trigger a shift in receptor specificity (8,10,31). For HIV-1, amino acid substitutions at the surface glycoprotein may effect shifts in receptor use (10,31), and mutations at several genes may promote escape from antibodies or cytotoxic T lymphocytes (CTLs) (8,10); the generation of 10^9 to 10^{10} viral particles per day undoubtedly facilitates escape (8,20). Although evidence of positive (Darwinian) selection of escape mutants is firm, the quasispecies structure and dynamics predict genetic variation in the absence of immune selection (5,32). The two mechanisms are compatible.

Some viruses (HBV, HIV-1, FMDV, measles virus, herpesviruses) may persist after an acute infection, and the dose of infecting virus often determines either clearance or long-term persistence (8,33). Viruses transmitted vertically may induce immune tolerance and persist in adults (33). Viruses may also persist by being sequestered in some privileged sites of an organism, such as the central nervous system, partially hidden from immune attack (33). Ineffective antibody responses may be due to tolerance, immunosuppression (as a result of some infection, genetic disease, or immunosuppressive treatments), production of nonneutralizing antibodies, or cell-to-cell spread of virus not exposed to immune recognition (33). Viruses that infect lymphocytes or macrophages (HIV, cytomegalovirus, measles virus) may alter immune responses and thus facilitate their own persistence (8,33).

Persistence of Viruses in Nature

All viruses have developed common functional and adaptive strategies; however, the strategies used by DNA and RNA viruses to evade host defenses have distinct features. RNA viruses often exploit mutation to achieve changes in host range and escape antibody and CTL responses (8,10,31,32). Because of their limited genetic complexity (which can be equated with the size of their genomes [of 3 to 30 Kb]), RNA viruses are generally tolerant to high levels of mutagenesis (6,14,16). In contrast, large DNA viruses (of the herpesvirus family, poxviruses, iridoviruses, adenoviruses) have complex genetic information; the need to maintain this information limits their tolerance to mutation. That simple genomes are generally more tolerant to mutagenesis than more complex ones can be argued on the basis of the higher mutation rates observed in simple replicons and the evolution of replication to include proofreading and postreplicative repair functions for the replication of DNA of cells and of at least some of the complex DNA viruses (5,6,14,16). Although antibody and CTL-escape mutants (as well as drug-resistant mutants) have also been described for DNA viruses, the latter have evolved alternative mechanisms to counteract host defenses (34,35). As examples, the adenovirus proteins E3/19K and E1a suppress surface molecules (MHC class I, class II, adhesion molecules) required for T-cell recognition. The Epstein-Barr virus BCRF1 protein is a host interleukin (IL)-10 homologue that activates the IL-10 receptor. Human cytomegalovirus encodes a protein structurally resembling the macrophage inflammatory protein 1α/RANTES receptor. Cytokines regulate immune and inflammatory responses and may trigger antiviral responses in organisms. It is not surprising that DNA viruses causing either persistent or acute infections have evolved to encode homologues of the extracellular binding domains of cytokine receptors (34,35).

Phylogenetic analyses of herpesvirus genomes infecting a broad range of host animal species (the complete genomic nucleotide sequence of 18 herpesvirus genomes is known) suggest a possible cospeciation with their host organisms (36). The capture of cellular genes (and gene assemblies) by DNA viruses to counteract host defense responses agrees with the proposal of a modular origin of viruses (37,38) and has opened a new approach for analyzing new functions related to the immune response in differentiated organisms (35). The selective forces imposed by viral parasites may have contributed to a more rapid diversification of cellular proteins involved in host defense (34). In turn, coevolution may have relaxed the specificity of viral analogues of cellular effectors: the viral chemokines vMIP I and vMIP II of herpes simplex virus-8 bind to a broader range of cellular chemokine receptors (although with lower affinity) than their cellular homologues (39). Genes that have strong sequence identity with cellular counterparts are also encoded by RNA viruses (e.g., sarcoma and leukemia viruses) (34) that tend to exchange genetic material with their hosts. However, evasion strategies based on gene capture and protein mimicry are dominant in DNA viruses, and strategies based on mutant production are widespread among RNA viruses (6-8,16,17,34,35).

Persistence of Viruses at the Population Level

To be maintained in nature and avoid extinction, viruses must have susceptible hosts as well as adaptability to a range of biologic environments (6). Even persistence in an individual host would not help long-term persistence of a viral pathogen in nature, without a number of additional influences (13,19,40), such as the possibility of transmission within the same host species (sexual, perenteral, or respiratory routes) or to a different host species (13,40). Human rhinoviruses do not persist in their hosts and succeed (supported by the high frequency of common colds) in continuous reinfections through aerosols transmission (or other contacts). In the other extreme of life cycle complexity, arboviruses sequentially infect a number of disparate hosts. As an example, insects can transmit Venezuelan equine encephalitis virus between horses and other mammalian species and can also infect humans, the dead-end point of a complex infectious cycle (18). Evidence indicates that a few point mutations in the viral genome may be sufficient to upset the balance of viral loads in enzootic cycles, render the virus epizootic, and cause severe outbreaks (18). In La Crosse virus, an important cause of pediatric encephalitis, the virus persists in cells of the midgut epithelium of *Aedes triseriatus*. The virus is transovarially transmitted and survives transeasonally in the diapausing mosquito embryo. In quiescent ovaries there is reduced viral replication with limitations in the host-derived 5'-mRNA sequences that prime viral transcription (41). Long-term survival at the population level is associated with persistence and limitation of virulence in the vector mosquito. The complex arboviral life cycle appears to require the fine tuning of a number of factors: the amount of virus in viremia and the duration of viremia, which are likely to contribute to the efficient uptake of virus by the vectors (18), and replication and stability of the virus in the vectors to ensure infection of the mammalian host (18). Perturbations in these, and probably other factors, could lead to viral extinction. What prevents viral extinction? Again, all evidence points to genetic factors of the virus and the hosts together with environmental and ecologic influences (1,3,5-7,17,18). Virus variants unable to fulfill the required processes with the correct timing may be generated, but they would be selected against. We can study only successful examples. Virus variants that do not complete a complex life cycle are yet another example of negative selection (elimination of suboptimal viruses). Negative selection is one of the forces preserving virus variants that are fit in relation to the interactions with their hosts (6,7,14-17,32), a force we believe is responsible for maintaining (at least to some extent) the identity of RNA viruses as disease-causing agents.

The stability of virus particles may also play a relevant role in successful transmission, as documented in aerosol transmission, airborne spread, or mechanical transport of viruses by insects (42). An example is provided by FMDV. In spite of its lability at mildly acidic pH and at moderate temperatures, FMDV is resistant to desiccation and can be transported on dust particles over long distances. Most spectacular is the case of the highly complex and host-specific baculoviruses. They form rod-shaped virions occluded in large capsules made of a viral-coded matrix protein termed polyhedrin. Capsules are uptaken by the insects (of the orders *Lepidoptera* and *Hymenoptera*) and are dissolved by midgut epithelial cells. Thus, capsule formation is responsible for the spread of the virus in the insect population. Again, a great diversity of mechanisms (as varied as those seen at the level of individual organisms or of the cells) operate to ensure viral persistence at the population level.

Basic Issues

The mutant distributions that compose viral quasispecies are the raw material on which selective forces and random sampling events act in the molecular evolution of RNA viruses (5-7,14-18,32,38). In addition to constituting a basic adaptive strategy, the quasispecies genetic organization has a number of biologic implications (5-9,16,32,43), some of which have a direct bearing on viral persistence. It has been wrongly argued that if quasispecies distributions were involved in virus persistence, all RNA viruses would establish persistent infections but that on the contrary, only a minority do. We hope to have shown that persistent infections are unavoidably, necessarily, and evidently the result of an interplay between viruses and their hosts (44). Thus, a quasispecies structure does not imply necessarily that the virus will produce a persistent infection. A potent CTL response may clear a virus infection provided that the viral load has a size amenable to clearing, and this may occur whether the virus is a complex quasispecies or not. In contrast, a similar CTL response confronting a high viral load may frequently fail to clear the infection; in this failure, the presence (in a dynamic quasispecies) of CTL-escape mutants (and many other types of mutants with biologically deviant properties) may be crucial for virus survival, including the establishment of persistence or chronicity. Also, increasing evidence suggests that viruses thought not to persist, such as poliovirus, may actually do so; late postpoliomyelitis syndromes may be one consequence (33,42). Even with the available analytical technology, total clearing of an infecting virus from an organism cannot be guaranteed.

The issue is clear: either we design new antiviral strategies that take into consideration the quasispecies structure of RNA viruses (6,9,43), or viral diseases (classical, new, or reemergent) will remain difficult to control.

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