VIRUS EVOLUTION IN THE FACE OF THE HOST RESPONSE

Esteban Domingo
Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain, and Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain.

Abstract: Microbial infections are highly dynamic. Viruses have evolved two main strategies against the host response: interaction or evasion. Interaction is typical of complex DNA viruses. Their genomes encode a number of proteins that exert modulatory functions that alter the immune response of the host. Evasion strategy is used mainly by RNA viruses, and is based on high mutation rates and quasispecies dynamics. The complexity of viral populations demands research on new antiviral strategies that take into consideration the adaptive potential of viruses, in particular RNA viruses.

1. INTRODUCTION

Microbial infections are highly dynamic processes in which the invading pathogen must counteract host responses to replicate and to find additional host individuals for long-term survival. Viruses are no exception and display a broad repertoire of strategies to cope with host defences. The presence of viral genomes or virus-like elements in the genomes of differentiated organisms suggests that cells and cellular organisms have undergone a long co-evolutionary process in which functional modules have been frequently exchanged: viruses have probably been active agents of horizontal gene transfer. For reviews of this topic, see Baranowski, Ruiz-Jarabo and Domingo (2001), and Bushman (2002).

In this co-evolutionary process there must have been strong selective pressures to maintain both viable hosts (otherwise this author would not exist to write this review) and infectious viruses (otherwise virus evolution would

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not be the topic of this review). The process can be imagined in simplified terms. Viruses and other agents of horizontal gene transfer (conjugative plasmids, mobile elements, etc.) contributed to cellular diversification, and possibly also to functional compartmentalization. In turn, viruses acquired specific functions to target subsets of cells. Viruses that killed cells massively would not survive. Cells that were totally resistant to viruses would be deficient in one of the several mechanisms that permit their exploration of new biological properties through genetic change. The result of eons of these interactive processes is a number of mechanisms evolved by viruses to overcome host defences and of hosts to limit the actions of molecular parasites. Dramatically, this evolutionary history is responsible for difficulties we encounter in virus disease prevention and control.

2.   TWO VIRAL STRATEGIES: INTERACTION VERSUS EVASION

2.1   The interaction strategy

Complex DNA viruses, such as the poxviruses and the herpesviruses, express a number of genes whose products contribute to evade detection and destruction by the host immune response. These immunomodulatory products include homologues of cytokines, chemokines and their receptors, as well as some that can modulate signal transduction, inhibit apoptosis, the activity of cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, complement, antibody production or down-regulate major histocompatibility complex proteins, among other activities (Alcami, 2003; Seet *et al.,* 2003; Campo, 2002). Interaction strategies extend to some DNA viruses of small genome size, such as the papillomaviruses, and even to some RNA viruses. The important human pathogen hepatitis C virus appears to exploit both interaction and evasion strategies to survive as one of the most successful viral pathogens worldwide (Isaguliants, 2003). By analysing the molecular basis of such interactive mechanisms much can be learned not only of viral infections but also of the host immune system.

2.2   The evasion strategy

In contrast to complex DNA viruses, RNA viruses exploit genetic variation as a means to escape from selective pressures intended to limit their replication. The molecular basis of this adaptive strategy is the high rate of occurrence of mutations during RNA genome replication (Batschelet,

Domingo and Weissmann, 1976; Drake and Holland, 1999). This is due largely to the absence of proofreading-repair activities that are usually associated with cellular DNA polymerases, and also to the absence of post-replicative mismatch correction pathways that can act on double stranded DNA but not on RNA. The result of these critical biochemical differences is that viral RNA replicases and retrotranscriptases display average error rates in the range of 10^{-3} to 10^{-5} misincorporations per nucleotide copied (Drake and Holland, 1999) while for cellular DNA polymerases involved in DNA replication average rates are around 10^{-10} misincorporations per nucleotide copied (Alberts *et al.*, 2002).

A consequence of high error rates is that mutant RNA genomes are generated continuously during replication, even in a single infected cell. Therefore, RNA virus populations consist of complex and dynamic distributions of mutant genomes, termed viral quasispecies (Eigen, 1996; Eigen and Biebricher, 1988). Multiple quasispecies co-exist in infected organisms, even within individual tissues and organs. Quasispecies was developed as a general theory of molecular evolution (Eigen and Schuster, 1979), and it has had a major impact for virology (Domingo, 2000; Domingo *et al.,* 2001). Here, only those features are emphasized related to the evasion strategy of RNA viruses (and, according to recent evidence, also some DNA viruses of small genome size (López-Bueno, Mateu and Almendral, 2003)) that represent a problem for disease prevention and control. In the mutant distributions of viral quasispecies, antibody- or CTL-escape mutants may be present or be generated with high frequency. Their selection may result in progression of infection despite an immune response, and may limit the efficacy of vaccines and other immunotherapeutical treatments. Likewise, inhibitor-escape mutants may be present or generated in mutant spectra, and their selection may result in failure of antiviral treatments. This has been reviewed by Domingo (2003).

High mutation rates may also favour reversion of attenuated virus to virulence, provided the number of genetic lesions involved in reversion is limited (that is, that virulent variants can be present in the mutant spectrum of the attenuated virus), and the virulent form has a selective advantage over the attenuated form in the environment in which replication takes place. More generally, mutants with increased virulence, altered host cell tropism or altered host range may be present or generated in mutant spectra. Their selection may result in more severe disease, or in disease with atypical symptoms, or in viral disease emergence or re-emergence. This has been reviewed by Baranowski *et al.* (2003). The last-named events are often conditioned to alteration in viral traffic prompted by a variety of sociological and ecological factors (Mahy, 1997; Murphy and Nathanson, 1994). Therefore a number of problems relevant to the emergence, prevention and

control of viral disease relate directly or indirectly to high mutation rates and quasispecies dynamics of RNA viruses.

3.   THE NEED FOR NEW ANTIVIRAL STRATEGIES

The evidence for implication of RNA genome plasticity in current problems related to the control of viral diseases and to disease emergence (Domingo, 2004) suggests that classical approaches to prevention and therapy must be carefully reconsidered. In principle, monotherapy, with either a single antiviral inhibitor or a single monoclonal antibody, is incompatible with the dynamics of rapid mutant generation, competition and selection in viral populations. This is because inhibitor- or antibody-escape mutants are likely to be rapidly selected (Domingo *et al.*, 2001). The frequent (in some case systematic) selection of human immunodeficiency virus (HIV) mutants resistant to antiretroviral agents constitutes a dramatic example. It is remarkable that the mutations that confer resistance to inhibitors have always been compatible with viral function. If resistance mutations entailed lethality, the problem of treatment failure would not occur. The author has speculated that, in their long evolutionary history, viral enzymes had to cope with metabolites that are related to the currently used inhibitors, and that they had to evolve to overcome inhibitory (or other perturbing) effects (Domingo, 2003). According to this proposal, there would be deep evolutionary determinants involved in the current difficulties encountered in suppressing viral replication, in addition to viruses using part of the cellular machinery to complete their replication cycles.

For similar reasons, vaccines that stimulate the host immune system to target a limited number of antigenic determinants of the virus are likely to fail to prevent progress of the infection, and may even promote the selection of antigenic variants of the pathogen (Domingo *et al.,* 2001). These limitations have been amply recognized, and strategies that include use of combination therapy (multiple inhibitors addressed to independent viral targets but administered simultaneously) and multivalent vaccines (with multiple, independent B-cell and T-cell epitopes) have been proposed (Domingo, 2003; Domingo and Holland, 1992). If implemented, these strategies would diminish the frequency of treatment failures. However obvious, these recommendations are not always followed.

Recently, a new antiviral strategy based on virus extinction associated with increased mutagenesis has actively been pursued (Eigen, 2002). It is based on the concept that for any given amount of genetic information to be transmitted during replication there is a maximum error rate compatible with maintenance of the information. Violation of the error threshold should

result in loss of virus infectivity, and this has now been documented with several virus-host systems (Domingo, 2000; Eigen, 2002; Graci and Cameron, 2002). This promising new antiviral strategy exploits high mutation rates of viruses for their destruction rather than for their survival.

4. CONCLUDING REMARKS

The picture that has emerged from studies of viral genomes at the population level is one of exceeding complexity, rendering classical strategies for viral disease prevention and control largely obsolete. For many important human and animal diseases, no effective vaccines or antiviral agents are available. Obviously, successful vaccines (to prevent infections by poliovirus, measles virus, influenza viruses, foot-and-mouth disease virus, among others) have had a great positive impact on human and animal health, and they will continue to be used, and will contribute to prevent disease. The key issue here is to make such effective vaccines available to people in need of health care, worldwide. Vaccine efficacy should not impede awareness that even these viruses that can now be controlled harbour enormous potential for genetic change and for causing forms of disease that cannot be anticipated. We know this from experience with the emergence of HIV-1, SARS virus and many other pathogens over the last decades. It is extremely important to view viral pathogens (and in reality any microbial pathogen) as a highly dynamic and flexible entity in its fine genetic identity as well as in its manifestations of an infection (Holland, de La Torre and Steinhauer, 1992). New approaches to treat and prevent viral diseases must be found that take into consideration (rather than ignore) the highly dynamic nature of the pathogens to be controlled.

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