A most remarkable feature of HIV is its ability to replicate continuously and unrelentingly despite apparently strong antibody and cytotoxic T-lymphocyte (CTL) responses. Here, Ronald Desrosiers reviews the properties that allow the virus to evade ongoing immune responses and that present formidable obstacles to those seeking long-term control of HIV.

Strategies used by human immunodeficiency virus that allow persistent viral replication

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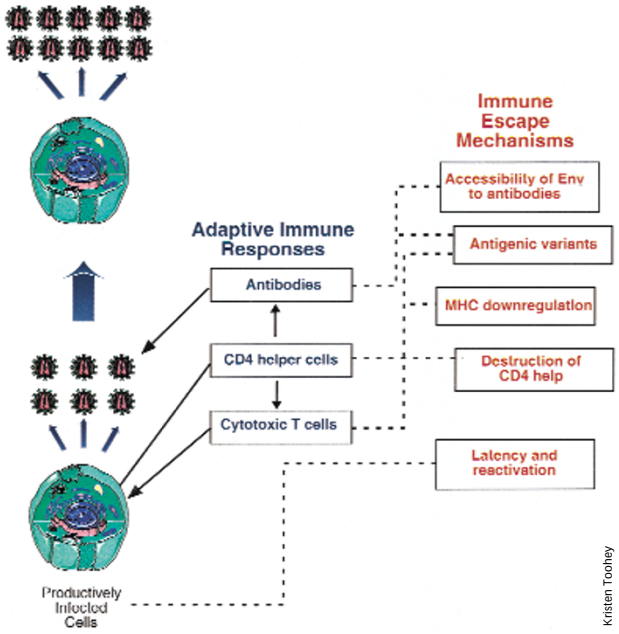
That HIV replication is continuous and ongoing was not entirely appreciated during the early stages of the epidemic. In fact, 'latency', 'dormant period' and other such terms were used often in the 1980s to refer to the long period of clinically inapparent infection that follows primary infection. Now, however, there is no doubt that virus replication is continuous and ongoing during all stages of HIV infection. Viral dynamic studies have shown unequivocally that large numbers of virus particles and infected cells are created and turned over every day[1,2]. Cells within the lymphoid tissues of SIV-infected macaque monkeys[3,4] and HIV-infected people[5] express viral products not only during primary infection but also during clinically inapparent and clinically apparent stages of infection long after the primary infection. This occurs despite the presence of readily measurable antiviral antibodies and antiviral CTLs. Antibodies that can bind viral proteins are typically present at titers in the tens of thousands and antibodies specific for HIV envelope typically constitute 5% or more of the total IgG (ref. 6). Recent data indicate that $CD8^+$ cells are important in at least limiting the amount of viral replication[7]. The numbers of $CD8^+$ CTL effector cells to a single HIV epitope have been measured using precise tetramer technology and found to constitute as much as 1–5% of all CD8 cells in the peripheral blood[8]. How can it be that cells are newly infected every day, express viral RNA and protein and make virus particles anew in the face of such high levels of antiviral antibodies and antiviral CTLs?



HIV uses a variety of strategies to avoid both humoral and cellular immune responses.

Integration

HIV and SIV, like other retroviruses, integrate DNA copies of their genetic information into the host cell genome. Expression of the viral genome in $CD4^+$ T lymphocytes depends on the state of activation of the infected cells. Activation of selected populations of $CD4^+$ T lymphocytes is a normal response to the recognition of foreign antigen. Many $CD4^+$ T lymphocytes in the body are not activated, or are only minimally activated, and are not ideal substrates for the replication of HIV and SIV. Cellular activation increases, among other things, nucleotide pool sizes and the concentration of specific transcription factors. Without a proper state of cellular activation, integrated proviral DNA may not be substantially expressed, essentially laying dormant until cellular activation can induce viral gene expression at a later time. Thus, although virus replication is continuous and ongoing at all times after primary infection, this does not mean that all infected cells are in the process of active virus production or reproduction. In fact, the numbers of cells containing proviral DNA are far greater than the numbers of cells that can be detected actively expressing viral RNA or protein[5]. This dormancy may be an important factor in the retention of residual levels of infectious cells long after continuous, highly active anti-retroviral therapy (HAART)[9].

Latency may facilitate viral persistence by allowing long-term storage of viral genetic information, essentially archiving genes and epitopes that can reemerge at a later date to test immune surveillance. However, it is important to emphasize that the ability of HIV to integrate its genetic information and the capacity for integrated proviral DNA to remain dormant in cells for prolonged periods cannot explain the persistent viral replication. Other factors must be involved in allowing HIV to evade ongoing immune responses.

Emergence of antigenic variants

The best estimate for the error rate of HIV reverse transcriptase is about 1 in 10,000 nucleotides[10]. This means that each newly infected cell will on average introduce about one mutation into the viral genome. Given that millions of newly infected cells are generated each day, the potential for genetic variation is enormous. Any genetic change that results in a growth advantage will be quickly selected for. In particular, any mutation that confers resistance to existing antiviral immunity without having debilitating effects on the inherent ability of virus to replicate will have an obvious selective advantage. In fact, mutations that reduce inherent replicative capacity may still be selected *in vivo* if they increase the effective replicative capacity sufficiently by avoidance of immune recognition. Selective advantage may also result from other pressures: the ability to replicate in certain types of cells or tissues in which the virus may reside or the ability to replicate in a new host with a different genotypic background. What is remark-

able about HIV, SIV and other lentiviruses is not just the rate at which they can accumulate mutations but also their ability to tolerate these mutations, particularly in the envelope surface protein[11]. Amino acid changes become fixed in the viral-encoded surface protein population at a rate of about 15 per year[12] and individual envelope proteins of independent HIV-1 isolates may vary by as much as 35% in their amino acid sequence[13].

The emergence of mutant forms of virus that result in escape from neutralizing antibody responses and escape from CTL responses during the course of infection of a single individual has been well documented. Monkeys infected with SIV derived from cloned DNA of defined sequence accumulated nucleotide changes in gp120 envelope over time at a rate of about 1% per year[12]. Infected monkeys mounted antibody responses that could neutralize the incoming cloned virus, but the sequence variants that emerged were also neutralization escape variants[14]. Escape from CTLs can occur by altering the ability of foreign peptide to be anchored in MHC or by altering recognition by the T-cell receptor. Borrow *et al.*[15] studied a patient with a strong, early, B44-restricted CTL response to an immunodominant epitope in Env. Genetic changes appeared in the predominant population of virus by 30–72 days after primary infection, and these resulted in escape from recognition by the CTLs (ref. 15). Similar results have been described in six B27-positive patients; however, in these studies the time to the appearance of escape mutants was considerably longer[16].

Destruction of CD4 helper cell activity

The dearth of anti-HIV CD4 helper cell activity in HIV-infected people has been known for more than a decade[17,18]. However, the importance of this observation has only recently come into clearer focus[19]. Individuals who seem to be controlling their HIV-1 infections remarkably well and who are long-term non-progressors quite uniformly show strong, HIV-specific CD4 proliferative activity[20]. These individuals typically maintain low or undetectable viral loads in the absence of anti-retroviral therapy. The strong HIV-specific CD4 proliferative activity in these unusual non-progessors contrasts with the absent or lower proliferative activity seen in typical progressors[20,21].

CD4 cells respond to sites of infection in the body through specific, T cell receptor-mediated interactions with foreign peptide on MHC. Responding CD4 cells become activated and facilitate CTL and antibody responses principally through the release of specific chemokines and cytokines. The picture that emerges is one of a battle during the initial weeks after HIV-1 infection between the virus trying to replicate in CD4 cells and CD4 cells trying to respond to sites of viral replication. Unfortunately for the host, these activated CD4 lymphocytes trying to respond to the infection are the ideal targets for replication of the virus. Most of the time, HIV ends up winning the battle, leaving the host without HIV-specific CD4 cells to provide help for the B-cell and CD8 CTL responses. Losing this essential battle early in the war also results in loss of the war, at least in the absence of anti-retroviral therapy. Only those individuals who for whatever reason are able to maintain substantial CD4 helper cell responses end up controlling HIV long-term. Infection by an attenuated virus may be one factor that can shift the balance in favor of the host CD4 response[22–24].

This emerging picture, still in need of more experimental verification, has enormously important implications for the future of drug treatment and vaccine development. Physicians are now facing serious difficulties in keeping their patients on HAART for 2–5 years or more. The 'holy grail' of drug treatment is to define conditions that will allow patients to discontinue HAART while still maintaining suppressed levels of HIV. This effort is now clearly focusing on regimens or treatments that will allow strong CD4 helper cell activity when HAART is discontinued. This emerging picture also suggests that if a vaccine does not create a sterilizing barrier, it will likely need to induce strong, HIV-specific CD4 helper cell activity that can be present at the time of HIV exposure.

Accessibility of envelope protein on virions

HIV-infected people and SIV-infected rhesus monkeys make high levels of antibodies reactive with the viral-encoded envelope proteins gp120 and gp41. These antibodies react very well and with high affinity to soluble, monomeric envelop protein. However, they react poorly, not at all, or only with low affinity to the native, oligomeric envelope protein as it exists on the surface of virions[25–28]—an important observation generally underappreciated even in the AIDS research community. The inaccessibility of envelope protein on virions is especially true of primary isolates, whose difficulty to neutralize has been well documented[28,29]. The ability of antibody to neutralize viral infectivity has been directly correlated with ability to bind to native, oligomeric protein on virions or on the surface of infected cells[25,26]. In contrast, most antibodies that can react with envelope protein do not neutralize and do not react appreciably with the oligomeric form. Antibodies that can bind virions and neutralize infectivity typically do so only in a very strain-restricted manner. This inaccessibility of virus to antibodies occurs despite the presence of binding sites on the surface glycoprotein for two different receptors (CD4 and CCR5). Virions also seem to incorporate cellular proteins that make them resistant to complement-mediated virolysis[30]. Thus, although high levels of antibodies against env are produced during the course of infection, most of the antibody specificities seem to be ineffective.

An antibody may have difficulty accessing the envelope protein on the surface of virions at least in part because of the way the protein folds upon itself in oligomers[31–34]. Another contributing factor is the presence of extensive carbohydrate. In fact, approximately 50% of the mass of gp120 is carbohydrate, making it one of the most extensively glycosylated proteins on record. Mutated versions of SIVmac239 missing two N-linked glycosylation sites within variable region 1 (V1) were much more immunogenic (that is, better at eliciting neutralizing antibodies) and much more antigenic (a more sensitive target for neutralization) than the parental strain from which they were derived[35]. This was true for any pairwise combination of the fourth, fifth and sixth glycosylation addition sites of the 24 sites present in gp120. Although a mutated virus missing five N-linked sites in the V1–V2 regions replicated normally in monkeys for the first 2 weeks, it was effectively neutralized and controlled at low or undetectable levels for more than 2 years[35]. Elimination of selected carbohydrate attachment sites is therefore a promising new avenue for improvement of antibody responses by candidate vaccines. Another is the ability of env-expressing cells locked into a 'fusion-competent' state to induce broadly neutralizing antibodies[36].

Other RNA viruses with error-prone polymerases, such as poliovirus, seem nowhere near as difficult to neutralize and nowhere near as malleable as HIV. The basis for these differences may lie in the inherent strategies used by persisting and non-persisting viruses. Any virus must depend for its survival on the ability to spread through the population. For a non-persisting virus such as poliovirus, transmission is limited to the several weeks after the time of primary infection. Resistance to antibodies, once they appear, may not be as important to such a virus. Instead, it

is important to replicate maximally for the brief period in order to maximize the likelihood for transmission. HIV, SIV and other lentiviruses, in contrast, help ensure their transmission by greatly lengthening the period during which transmission can occur. Thus, these viruses may sacrifice inherent replicative capacity in the short term to allow persistent viral replication in the long term.

Downregulation of MHC

Many viruses seem to have evolved strategies to minimize antigen presentation to the host immune system (review, ref. 37). For example, transforming strains of adenovirus encode a protein (E3) that prevents MHC class I molecules from reaching the cell surface[38,39]. Herpes simplex virus encodes a protein (ICP47) that inhibits the peptide transporter TAP (ref. 40); TAP is needed to transport peptides to the MHC molecules that will present them as foreign.

The Nef proteins of HIV and SIV interact with and modify the endocytic and sorting pathways of the cell[41]. One result of this interaction is the downregulation of MHC class I molecules from the surface of the cell, first shown by Schwartz *et al.*[42] Target cells from which MHC class 1 molecules have been internalized by expression of Nef become resistant to the cytolytic activity of MHC-restricted CTLs (ref. 43). Although it is easy to see how this could contribute to immune evasion by the virus, the physiologic relevance of the observation is not entirely clear. MHC downregulation occurs at the highest levels of Nef expression[43] and no one has shown whether infected cells as they exist in the host have downregulated their MHC class I molecules.

Conclusion

Further advancement of therapeutic regimens and development of effective vaccines against HIV must deal with the strategies of immune evasion summarized in this review. Dealing with any one alone would be difficult. Dealing with all of them will be heroic. The most promising tactics may be those that take advantage of the natural immune control that can sometimes occur. Drug regimens that facilitate the ability of the immune response to do its job are certainly the wave of the future. For vaccine development, immunologic memory induced by non-persisting antigens and vectors is not likely to be sufficient. Future vaccine research will probably need to focus more on vehicles that allow persistence of antigen or persistence of antigen expression.

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