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Virus-Host Interactions in HIV Pathogenesis: Directions for Therapy

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ABSTRACT

The challenge of controlling HIV infection involves an understanding of the heterogeneity of the virus, its wide cellular host range, its primary routes of transmission, and the immunologic and intrinsic cellular factors that can prevent its transmission and replication. Identification of HIV-infected individuals who have survived more than 10 years without signs of the infection and without therapy encourages studies examining the natural mechanisms for resistance to infection and disease. Within the immune system, emphasis should be given to the innate or natural response that appears within minutes of the infection and offers the optimal time for controlling HIV. All these parameters in HIV pathogenesis underline the information needed to develop optimal anti-HIV therapies and an effective AIDS vaccine.

INTRODUCTION

In the 25 years since the discovery of HIV, much progress has been made in our understanding of the virus and in recognition of the importance of the infected host's response to HIV, which can determine the clinical course (Levy, 2009). While the wide dispersion of HIV throughout the world has now affected people in all countries, its ability to cause the acquired immune deficiency syndrome (AIDS) has been greatly curtailed through the development, over the past decade, of anti-retroviral drugs. They can keep the virus in check by interfering with virus entry and replication. Thus, infected persons can have a nearly normal life for many years. Nevertheless, over time, resistance to these drugs occurs, and infected individuals can develop AIDS or toxic complications from the use of these drugs. In this article, I will review the known characteristics of HIV and various aspects of its interaction with cells. The anti-viral immune responses of infected individuals will be considered. These features have uncovered novel anti-HIV therapies and, it is hoped, will also result in an effective AIDS vaccine.

HIV HETEROGENEITY

One of the first observations made in the evaluation of various HIV isolates was the viral heterogeneity involved with the ability to infect and replicate in primary CD4+ T-lymphocytes and macrophages (Levy, 2007). Moreover, whereas all viruses could grow in most primary CD4+ T-cells, differences were appreciated when HIV isolates were added to primary macrophages or to established CD4+ T-cell lines. Some grew well in macrophages and others in the T-cell lines. Those growing in macrophages generally did not induce cytopathology or multinucleated syncytial cell formation; those growing in T-cell lines were syncytium-inducing (SI) viruses (Cheng-Mayer *et al*., 1988; Tersmette *et al*., 1988).

These early observations were later explained by the recognition that HIV, after binding to CD4, uses secondary cellular receptors for attachment to and subsequent entry into cells (Berger *et al*., 1999). These virus co-receptors are important binding sites for chemokines that recruit immune cells to the location of their secretion. In high concentration, these chemokines (*e.g*., MIP-1α; MIP-1β, RANTES) can compete for virus attachment to the chemokine receptor (Berger *et al*., 1999). The NSI viruses primarily use the CCR5 receptor and are known as R5 viruses; the T-cell line viruses use the CXCR4 receptors and are known as X4 viruses (Levy, 2007). Some HIV isolates can use both receptors and are called dual/tropic or R5/X4 viruses. Generally, during acute virus infection, an R5 virus emerges as the dominant type and over time, in many cases, becomes dual/tropic (Singh and Collman, 2000). Later, in about half of the cases, an X4 or more cytopathic strain emerges (Asjo *et al*., 1986; Cheng-Mayer *et al*., 1988). The X4 viruses are associated with a more rapid progression to AIDS (Levy, 2009).

Recognition of the co-receptors for binding sites of HIV has led to various approaches to block this interaction through specific drugs. Maraviroc prevents HIV infection *via* CCR5 (Deeks, 2006); other drugs in development target CXCR4 (Moyle *et al*., 2009). The therapies directed at CCR5 were not considered to be detrimental to the immune system, because about 1% of the human population lack CCR5 expression (CCR5-) and have no obvious immune abnormalities (Berger *et al*., 1999). These individuals are much less likely to be infected by HIV unless they encounter an X4 virus. However, these CCR5-negative people
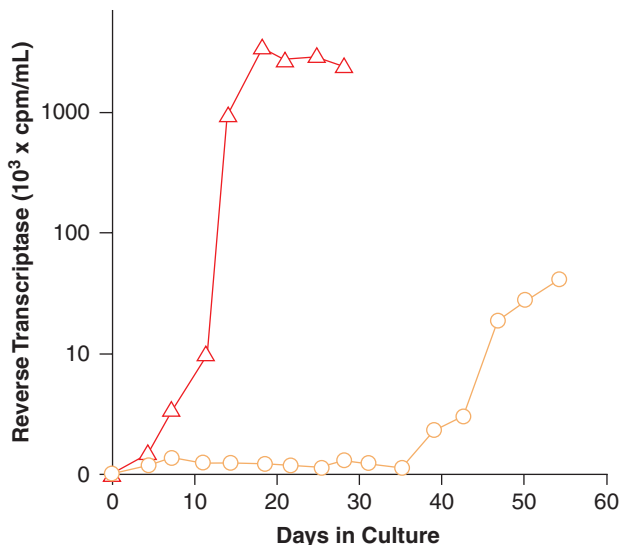
Fig. 1. Replication of early and late HIV-1 isolates from the same individual. Replication of 2 strains of HIV-1 recovered from the same individual over time. HIV-1SF2 (0), isolated early in the course of infection, replicates slowly and to low titer in the established HUT 78 T-cell line. In contrast, HIV-1SF13 (Δ), recovered when the patient had AIDS, grows rapidly and to high titer in the same cells. These and other biologic differences between these 2 strains have been used to distinguish viruses in culture as non-virulent and virulent strains. Reprinted from Levy (2007) with permission.

have been shown to have a greater chance to develop severe disease from West Nile virus (Glass *et al*., 2006). It is less certain what a drug against CXCR4 would do to compromise the immune system. Conceivably, it could lead to the emergence of HIV strains that use other co-receptors. Currently, there are at least 9 chemokine co-receptors that can be used by HIV for attachment to various cells of the body (Berger *et al*., 1999; Levy, 2007).

This heterogeneity of HIV, which is evident biologically, can also be appreciated genetically. The 2 types of HIV, HIV-1 and HIV-2, exist and differ by about 40% (McCutchan, 2000). In addition, based on genetic variations, 3 groups of HIV-1, differing by about 30% (M, N, and O) have been identified, and 8 groups of HIV-2 have been recognized (A-H), for which A and B are the most common HIV-2 groups in circulation (Marx, 2005). The largest number of HIV infections has occurred with group M HIV-1 strains, which can be further subdivided into 9 subtypes or clades differing by at least 15% (Robertson *et al*., 2000; Peeters *et al*., 2003). Clade B is the most common virus in the United States. It is also found in China, but has emerged as well as a recombinant between clades B and C viruses (Liu *et al*., 2008) (see below).

This viral heterogeneity can also be appreciated in the change in HIV properties over time in the same individual. Early viruses, as noted above, are usually the R5 type, which grow slowly to low titer in CD4+ T-cell cultures. Then, when the individual develops symptoms or AIDS, a related X4 virus emerges, which replicates rapidly to high titer in culture and is cytopathic (Fig. 1). This virus can then lead to AIDS much more quickly than R5 viruses, although R5 viruses have also been associated with 50% of AIDS cases (Kwa *et al*., 2003).

VIRUSES IN DIFFERENT TISSUES

Another noteworthy finding is that when viruses are isolated from different tissues of the same individual (see below), variations in their biological property can be found. For example, a virus coming from the blood may have the ability to infect peripheral blood mononuclear cells (PBMC) and to down-modulate CD4 expression, whereas those from the brain can be quite infectious for macrophages and not affect CD4 expression (Cheng-Mayer *et al*., 1989). This observation indicates that the same virus transmitted to an individual can, through multiple replicative cycles, become selected in various tissues as a virus related to the transmitted virus but having different biologic properties. It may be responsible for the pathology observed in the infected tissue.

Importantly, as cited above, when more than one virus infects the same cell (perhaps at the same time, when the CD4 receptor has not yet been down-modulated), interactions between the 2 RNA strands of each virus can take place. Recombination can then occur (Robertson *et al*., 1995). These recombinant viruses contain genetic information from 2 or 3 different viruses. Some may have genetic regions from up to 7 different subtypes (Levy, 2009). This observation has suggested that, over time, recombinant viruses will become more common and may take on different biologic properties, including resistance to anti-viral drugs or ability to infect many cell types. For this reason, recombination among HIV strains represents a newly emerging challenge in HIV pathogenesis.

VIRAL-HOST CELL INTERACTIONS

Within 2 days after transmission, the HIV isolate, often a single strain (Keele *et al*., 2008), enters the bloodstream and can infect a variety of different tissues depending on its different biologic properties. The number of cell types reported to be susceptible to infection is quite large and includes cells of the brain, the GI tract, and the kidney, and as well as oral keratinocytes and epithelial cells.

This infection of different cells occurs not only with the free virus, but importantly also with virus-infected cells. The latter results during the replicative cycle, when HIV integrates its proviral DNA into the chromosome of the cell. These cells, present in blood and genital fluids, can be a major source of transmission by interacting with cells of the immune system and mucosal tissues in the body. When infected cells interact with mucosal cells, HIV can be transferred readily to the individual. Such virus-infected cells can be found at levels of 50,000 cells in some genital fluids, and the efficiency for infected cells to transmit the virus is often better than that of the free virus (Levy, 1988). Moreover, by time-lapse photography, virus-infected cells have been shown to transfer HIV to many different cultured cervical or rectal epithelial cells (Phillips and Bourinbaiar, 1992). These same cells are often resistant to direct infection by HIV. Infection most likely occurs by cell-to-cell contact.

HIV transmission *via* the oral cavity occurs rarely. The amount of infectious virus in the saliva is quite low, and virus-infected cells are not commonly found (Table 1) (Levy and Greenspan, 1988). This lack of infection can also be reflected by the large number of anti-viral substances found in saliva (Table 2)

Table 1. Isolation of infectious HIV from Saliva [data from Levy (2007) and Levy and Greenspan (1988)]

	Virus Isolation	Estimated Quantity of HIV
Fluid	3/55	< 1
Cells	4/11	< 0.01

Table 2. HIV Transmission *via* the Oral Cavity [see Levy (2007) for specific citations]

- HIV and virus-infected cells are at low levels in saliva*
- Innate inhibitory factors in saliva can prevent transmission
  - Secretory agglutinin
  - Secretory leukocyte protease inhibitor
  - Fibronectins
  - Glycoproteins
  - Mucins (*e.g.*, MG1)
  - β defensins
  - Thrombospondin 1

*Infectious HIV has been recovered from the throats of infected children.

Table 3. Major Characteristics of Long-term Survivors of HIV Infection

- Clinically asymptomatic for ≥ 10 years; no anti-viral therapy
- Normal CD4+ cell number
- Low virus load (measured by plasma viremia; infected PBMC)
- Low immune activation; normal T-reg function
- Elite controllers: undetectable plasma virus for 2-10 years

(Levy, 2007). They can block virus infection by both cells and free virus.

HOST IMMUNE RESPONSES

With this heterogeneity of HIV isolates and the ability of the virus to be transmitted readily through the mucosae and the blood, the existence of individuals who have survived a long time with the infection (> 10 yrs) without showing signs of the disease and without therapy is noteworthy (Levy, 2009). Among these long-term survivors (LTS) or long-term non-progressors, a subgroup, now termed 'elite controllers', has been identified. These people, for at least 2 and some for over 10 yrs, have not shown the presence of any virus in the plasma, although they remain infected (Saez-Cirion *et al.*, 2007) (Table 3). It is my impression that these individuals reflect optimal control of HIV among long-term survivors and probably possess the same processes of immune resistance as LTS, but their responses are more effective.

The anti-HIV immune activity of infected individuals is mediated by the innate or natural immune system and the adaptive or acquired immune system. Up until recently, the adaptive immune system was given most of the attention by the HIV scientific community. The role of the innate immune system, however, has now received a greater appreciation (Levy, 2001). Cells of the innate immune system include NK cells, NKT cells, plasmacytoid dendritic cells (PDC), and γδ T-cells. Circulating soluble factors are part of this system and include complement and mannose-binding lectin that can attach to HIV directly and inactivate it (Levy, 2001). The B- and T-lymphocytes are the most active components of the adaptive immunity. In both the innate and adaptive immune systems, dendritic cells and macrophages, as antigen-presenting cells, can play an important role. In a comparison of both immune systems, a key difference is that the innate immune system has a rapid immune response (from minutes to days), whereas the adaptive immune system takes days to weeks. Innate immunity does not need to be antigen-specific, but responds more to conformational structures (Levy, 2001). It can therefore interact quickly against viruses and bacteria as pathogens without recognizing a specific component of a virus or bacterium. The adaptive immune system is much more antigen-specific. Both immune systems have been conserved through evolution. Evidence suggests that the adaptive immune system evolved from the innate immune system, probably about 450 million years ago, when the shark developed a mandible. At that time, a transposon appears to have entered a primitive immunocyte and established the process of gene rearrangement, leading to the variations in T-cell receptors and B-cell antibodies (Travis, 2009).

In brief, when the innate immune system encounters a pathogen, it rapidly secretes cytokines that can have direct antimicrobial activities (*e.g.*, interferons) or play a role in increasing both NK (an innate immune cell) and adaptive T-cell responses. The latter activity involves increasing MHC expression and enhancing T-lymphocyte functions (Levy, 2009).

INNATE IMMUNITY: PLASMACYTOID DENDRITIC CELLS

Among the innate immune cells are dendritic cells (DC). The most common DC circulating in the blood is the myeloid dendritic cell, which is the primary antigen-presenting cell in the body. A more recently recognized DC is the plasmacytoid dendritic cell (PDC), which is present in very low levels in the blood (2-10 cells/μL) (Siegal *et al.*, 1999). It is the major producer of type 1 interferons; PDCs secrete 200-1000 times more interferon than any other cell type. The PDC expresses CD4 as well as the CCR5 and CXCR4 co-receptors for HIV. Therefore, they can be infected by the virus. The infection rate of these cells is about 1:1000 PDC in individuals who have circulating virus in their blood (Schmidt *et al.*, 2004). These cells also express TLR-7 and TLR-9, and it appears that both of these innate cell receptors are involved in the response of PDC to HIV (Beignon *et al.*, 2005; Schmidt *et al.*, 2005). Our laboratory has shown that HIV-infected cells are the most effective inducers of type 1 interferons by PDC (Schmidt *et al.*, 2005). Only very high concentrations of HIV can induce this interferon production. When IFN is released, virus replication within the infected cell is markedly reduced (Schmidt *et al.*, 2005).

When the relationship of PDC to the clinical state was examined, it was noted that long-term survivors have an even higher average number of PDC than healthy donors (Soumelis *et al.*, 2001). Moreover, in individuals who have been acutely infected with HIV (within 3 months of transmission), the amount of PDC
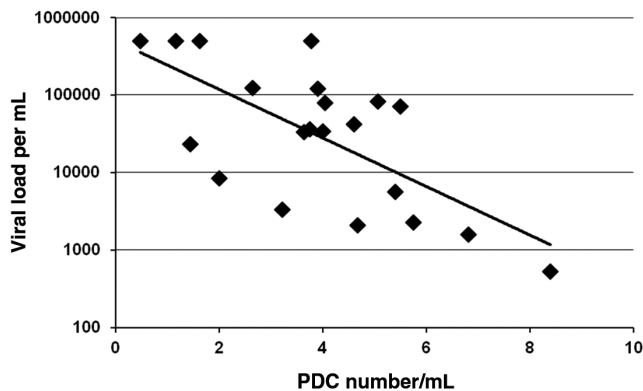
Fig. 2. Correlation of PDC number with viral load in acutely infected individuals. Individuals who have been acutely infected with HIV (within the preceding 3 months) were evaluated for viral load as well as PDC number before receiving any therapy. It is evident that those infected individuals with high PDC number had the lowest viral loads.

Table 4. Factors That May Protect High-risk Seronegative Individuals from HIV Infection

Genetic

- Reduced or lack of CCR5 expression
- Genes closely related to HLA Class I A2 alleles

Immune

- HIV-specific CD4+ T-cells
- HIV-specific CD8+ cell CTL
- CD8+ cell non-cytotoxic anti-viral response
- Increased NK cell function
- Reduced susceptibility of PBMC to infection
- Reduced immune activation
- IgA antibodies react to CCR5 in sera, saliva, and genital fluids
- Cervico-vaginal anti-gp41 antibodies that block transcytosis

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circulating in the blood correlates indirectly with the viral load (Killian *et al.*, 2006). The highest number of PDC has been observed in persons with the lowest viral load (Fig. 2).

In this regard, we have noted that the average PDC number among healthy human populations ranges from 2-18 cells/µL. This finding suggests that, if infected, individuals who naturally have higher numbers of PDC will become long-term survivors, protected by this immune response. Anti-retroviral therapy helps in bringing more PDC into the blood. The number of PDC is restored even sooner than that of CD4+ T-cells (Siegal *et al.*, 2001). Certain substances can also enhance circulating PDC levels (*e.g.*, Flt 3 ligand, G-CSF, thrombopoietin) (Levy, 2007).

INNATE IMMUNITY: THE CD8+ CELL NON-CYTOTOXIC ANTI-VIRAL RESPONSE

Many years ago, our laboratory began studying HIV-infected individuals who remained healthy and from whom we could not isolate virus readily from the blood. We discovered that these individuals had CD8+ T-cells that suppressed virus replication in target HIV-infected CD4+ cells without killing the cells (Walker *et al.*, 1986). This CD8+ non-cytotoxic anti-viral response (CNAR) is mediated by a CD8+ cell anti-viral factor (CAF), which blocks viral transcription (Mackewicz *et al.*, 1995; Levy, 2003). This immune activity therefore differed from the classic adaptive cytotoxic T-cell response (CTL), which kills virus-infected cells. CNAR acts against all HIV-1 and HIV-2 strains tested, as well as SIV isolates. It is not restricted by Class I or Class II molecules, and is a rapid response mediated by a cytokine. Therefore, CNAR has characteristics of an innate immune response (Levy, 2001). Its clinical relevance was obvious when we noted that long-term survivors had up to 3 times greater CNAR activity than those who progressed to disease (Barker *et al.*, 1998).

We believe that CNAR is extremely important in protecting individuals from advancing to disease and can prevent infection, as noted below with HIV-exposed seronegative people. We have

also shown that this anti-HIV activity appears to be lost first over time, then viral replication returns in the infected individual, followed by a reduction in CD4+ cell number (Levy, 1993, 2007). In many studies, CNAR can be restored with the use of a variety of cytokines (*e.g.*, IL-2, IL-15, interferon α), as well as the interaction of the cells with mature dendritic cells or antibodies to CD3 and CD28 (Levy, 2007). These types of approaches, if feasible in therapy, could be helpful in maintaining long-term survival for infected individuals.

HIGH-RISK EXPOSED SERONEGATIVE INDIVIDUALS

In examining individuals who had been exposed on many occasions to HIV without being infected, we found that CNAR was also present (Stranford *et al.*, 1999). For example, in wives of HIV-infected hemophiliacs, this innate activity was observed if exposure had occurred within the preceding 12 months. After that time period, CNAR was not detected. Our conclusion is that exposure to the virus is needed to maintain this anti-viral response. This is a general characteristic of innate immunity: Memory responses are usually not found (Levy, 2001), although more recently, memory responses have been reported for NK cells (O'Leary *et al.*, 2006). There are several genetic and immune factors that characterize these high-risk exposed seronegative individuals who appear protected from HIV infection (Levy, 2007, 2009) (Table 4).

INTRACELLULAR INTRINSIC ANTI-HIV FACTORS

Most recently, natural intracellular resistance to HIV replication has been described. This type of protection includes host cell proteins that inactivate the viral/proviral DNA, such as APOBEC 3G and F (Levy, 2007, 2009). In addition, TRIM 5α blocks the viral capsid from uncoating, and tetherin and a calcium-modulating protein prevent virus budding (Levy, 2009) (Table 5). These cellular processes offer avenues for novel therapies. It is noteworthy that APOBEC 3F and 3G, as well as tetherin, can be countered by the viral proteins Vif and Vpu, respectively (Levy, 2009).

Besides the type of virus infecting an individual, the intracellular resistance factors, and the host immune response to HIV,

Table 5. Natural Intracellular Resistance to HIV Replication

Cellular Protein	Mechanism of Action*
APOBEC3G	Cytosine deaminase†
Trim5a (Lv-1/Ref-1)	Blocks HIV-1 capsid uncoating; affects reverse transcription
Lv-2	Blocks nuclear entry of HIV-2
Tetherin	Inhibits virus release**
Murr-1	Inhibits degradation of NFκB

*Other activities may be involved.
†Countered by Vif.
**Countered by Vpu.
See Levy (2007) for specific citations.

the HLA type of the individual can correlate with the extent of HIV disease progression (Levy, 2009). Whether this observation relates to an adaptive immune response (*e.g.*, CTL function) or to an as-yet-unknown correlation of HLA type to other immune responses, perhaps innate, merits further study. In this regard, CTL activities could have both beneficial and detrimental effects during HIV infection (Zinkernagel and Hengartner, 1994). Our laboratory emphasizes what appears to be primarily beneficial effects of innate immunity (Levy, 2001).

CONCLUSIONS

This brief review of virus-host interactions describes directions that can be developed for anti-HIV therapies. These approaches include targeting the cell-surface receptors (*e.g.*, CCR5), increasing the expression of intracellular anti-viral factors, and enhancing innate immune responses mediated by soluble products (mannose-binding proteins) or through PDC and CD8+ cell activities. Treatments could include the manipulation of various cytokines, which can help the DC and CD8+ cell responses—for example, Flt-3, G-CSF, CpG, and thrombopoietin for PDC activity, and IL-15, IL-2, IFN-α, and CAF for CD8+ cell function. Nevertheless, it is well recognized that each cytokine can have a pleiotropic effect and influence many different cellular factors. Just emphasizing the presence of one may not provide the optimal solution for a therapeutic strategy.

In terms of adaptive immune activity, the induction of neutralizing antibodies, but not enhancing antibodies, is a major objective, both for vaccines and perhaps in post-infection immunization. Enhancing antibodies are a mechanism by which an antibody can bind less avidly to a virus and bring the virus into T-cells or macrophages *via* the Fc or complement receptor. Our group has shown that enhancing antibodies are present in individuals as they advance to disease (Homsy *et al.*, 1990).

Finally, the existence of CD4+ T-regulatory cells (and perhaps CD8+ T-regulatory cells) is becoming better appreciated (Fazekas de St Groth and Landay, 2008). These cells can decrease immune activation, which appears to be a major mechanism for pathogenesis in HIV-infected individuals (Sodora and Silvestri, 2008). At the same time, the cells may reduce the immune anti-viral activities that are needed to control HIV infection. Therefore, as we begin to look at the interplay between immune activation and immune response, a balance in the activities of human T-regulatory cells must be considered (Levy, 2009).

In summary, long-term survivors provide encouragement for a natural control of HIV infection (Table 3). Several of their anti-viral characteristics have been known for some time and should also include 2 innate immune functions that can help them ward off AIDS: PDC and CD8+ cell non-cytotoxic activities. While anti-retroviral therapies are very effective in blocking new infections and inactivating viruses, it is the immune system that can control the virus-infected cells that remain as reservoirs in the body. Importantly, since so many tissues in the body harbor these cells, a cure cannot be achieved merely by eliminating HIV from the immune system—which is alone a formidable risk. Approaches to removing these cellular reservoirs remain a major priority in therapy. It is imperative that we put more emphasis on the development of drugs that will enhance, in the appropriate way, both innate and adaptive anti-HIV immune responses. In such a manner, we can direct their functions to bring long-term survival (and perhaps cure) to all HIV-infected individuals.

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