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Biology of CCR5 and Its Role in HIV Infection and Treatment

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Chemokine receptors are found on cell surfaces and promote cellular migration by chemotaxis. The CC chemokine receptor 5 (CCR5) is used by the human immunodeficiency virus (HIV) to infect cells. Strategies that target human CCR5 are therefore being developed to prevent and treat HIV infection. Antiviral strategies that target a host element necessary for viral replication may be predicted to interfere with the function of that element and may therefore adversely affect the host. We conducted a review of the literature between November 2005 and April 2006 with a focus on articles addressing the genetics and function of CCR5, the effects of CCR5 deletion in human and murine systems, and treatment strategies for HIV infection that target this coreceptor. English-language articles in the human and murine literature published between March 1996 and April 2006 were identified through a search of MEDLINE using *CCR5* as the search term. Relevant articles as judged by their titles and abstracts were reviewed in detail. In addition, based on our knowledge of the field and with permission, unpublished work was also reviewed. In this article, we explore the effects that targeting CCR5 may have on host defenses in individuals with immunity already compromised by HIV infection.

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As intracellular pathogens, viruses must use host cellular machinery for replication. Thus, targeting of host elements necessary for viral replication could limit the ability of the virus to replicate.[1] In other conditions such as inflammatory bowel disease, multiple sclerosis, malignancy, and rheumatologic disorders, strategies that target host immune elements such as proinflammatory cytokines,[2] costimulatory molecules,[3] B lymphocytes,[4] and integrins[5] have documented clinical efficacy. Interference with host defense elements is not without some cost, however, and is sometimes associated with an increased risk of infection or neoplasia.[6,7]

With the recognition that the human immunodeficiency virus (HIV) uses the CC chemokine receptor 5 (CCR5) for entry into human cells, strategies that target CCR5 are being developed to prevent and treat HIV infection. In this context, the multiple and overlapping interactions among chemokines and their receptors permit substantial tolerance of CCR5 deletion or blockade. This is reflected in the general good health of the many persons who are born homozygous for a deletion of 32 base pairs in the coding sequences for CCR5 (*CCR5*Δ32) that renders the protein dysfunctional. We review herein the biology of CCR5 and the effects of CCR5 sequestration or inhibition on host immunity, and discuss the potential effects these strategies may have on host immune defenses in health and in persons with preexistent immunodeficiency due to HIV infection.

We conducted MEDLINE searches between November 2005 and April 2006 to identify English-language articles in the human and murine literature published between March 1996 and April 2006 using *CCR5* as the search term. We focused on articles that addressed the genetics and function of CCR5, the effects of CCR5 deletion in human and murine systems, and treatment strategies for HIV infection that target this coreceptor. Articles determined to be relevant as judged by their titles and abstracts were reviewed in detail. In addition, we reviewed unpublished research based on our knowledge of the field and with permission.

Structure and Function of Chemokine Receptors

As indicated by their name, chemokine receptors are cellular receptors for chemokines—small molecules in the cytokine family that promote cellular movement by chemotaxis.[8] These receptors have a common 7-transmembrane structure (FIGURE 1) comprised of extracellular and intracellular loops separated by hydrophobic membrane-spanning domains, a characteristic that

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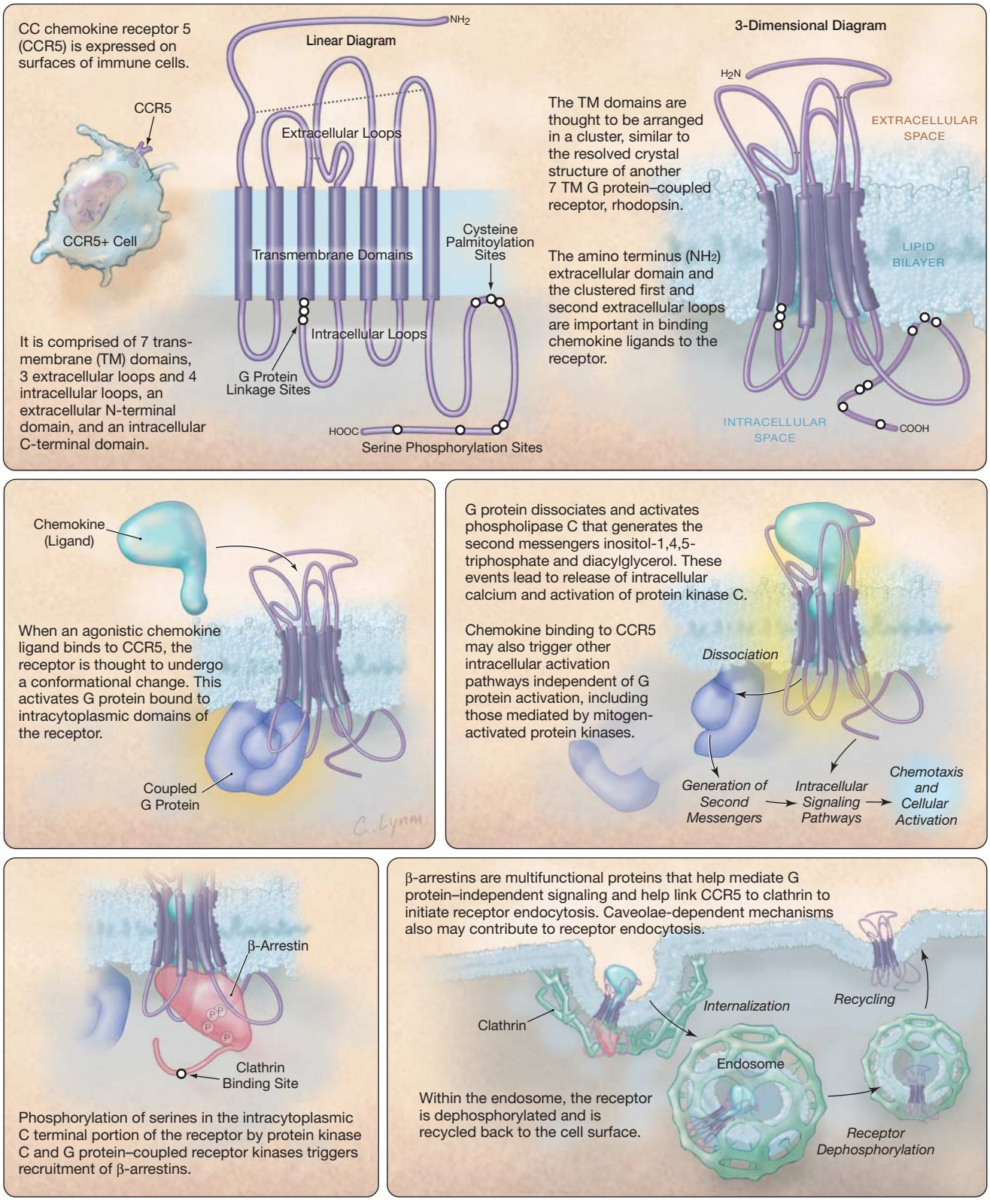
Figure 1. Structure and Function of CC Chemokine Receptor 5



makes them difficult to study in isolation unless they are expressed on a cell surface. Chemokine receptors have several other characteristics that help define their function. They are coupled with G proteins and can transmit signals to the cell via activation of these G proteins and also through other signaling molecules (Figure 1). When bound by their chemokine ligands, these receptors can become internalized, impairing their subsequent ability to bind their ligands. Once internalized, however, these receptors tend to recycle to the cell surface in time. Finally, many chemokine receptors share ligands and vice versa. Thus, a single chemokine receptor may bind and be activated by more than 1 chemokine and conversely, a single chemokine may bind to and activate multiple chemokine receptors (TABLE).

Table. Interactions Among Some Chemokines and Their Receptors*

Chemokine		Chemokine Receptor			
Consensus Name	Common Name	CCR1	CCR2	CCR3	CCR5
CCL3	MIP-1α	+			+
CCL4	MIP-1β	–			+
CCL5	RANTES	+		+	+
CCL7	MCP-3	+	+	+	–
CCL8	MCP-2	+	+	+	+
CCL11	Eotaxin		–	+	+
CCL14α	HCC-1	+			+
CCL16	HCC-4	+	+		+

Abbreviations: CCL, CC chemokine ligand; CCR, CC chemokine receptor; HCC, hemofiltrate CC chemokine; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normally T-cell expressed and secreted.
*The plus sign indicates activating interaction and the minus sign indicates inactivating interaction.

While the biologic rationale for preservation of these complex, overlapping, and possibly inefficient relationships might not be apparent, these networks can be viewed as interesting examples of how host defense mechanisms are often duplicative. This provides backup systems to ensure the effectiveness of host responses to microbial challenges and the means to overcome microbial counterdefenses because many microbes have developed strategies to block chemokines or their coreceptors or use them for pathogenesis.[8] In addition, chemokine ligands that bind the same receptor may have different biologic activities in vivo because they may be expressed by different cells or at different sites, they may signal through the receptor differently, and they may interact with different additional chemokine receptors. These complex, overlapping relationships could explain how deletion or blockade of a particular element can be tolerated without serious harm to the host.

CCR5 Structure and Function

The structure of CCR5 has not been resolved but is likely to resemble that of rhodopsin.[9] The loops of the receptor are likely arranged in a clustered orientation such that they are more proximal to each other than would be seen in a linear orientation (Figure 1).

Chemokine ligands are thought to bind to the amino terminus of CCR5 and then to regions of the first and second extracellular loops, although mutational analysis indicates that chemokines may differ in terms of their direct sites of interaction.[10] The free amino terminus of the chemokine is thought to activate receptor signaling via interaction with the bundled transmembrane domains of the receptor.[11] Chemokine binding to CCR5 promotes the dissociation of the receptor-bound G protein, which activates phospholipase C, generating the second messengers inositol-1,4,5-triphosphate and diacylglycerol that lead to the release of intracellular calcium and activation of protein kinase C.[12,13] In addition to the pathways activated by these intracellular mediators, G protein–independent pathways, such as those mediated through mitogen-activated protein kinases, may be activated after chemokine binds to its CCR5 receptor.[14,15] Phosphorylation of intracytoplasmic residues in the C terminal portion of the receptor promotes recruitment of β-arrestins, multifunctional proteins that prevent further G protein coupling, which attenuates signaling through the receptor, and that also may serve as a scaffold to recruit other signaling molecules. Importantly, β-arrestins facilitate the binding of the receptor to clathrin to initiate receptor internalization[16,17] and subsequent receptor recycling to the cell surface (Figure 1).

Several chemokines can bind, signal through, and promote internalization of CCR5, including macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and regulated upon activation normally T-cell expressed and secreted (RANTES), known also as CC chemokine 3 (CCL3), CCL4, and CCL5, respectively (Table). Other chemokines, such as monocyte chemoattractant protein 3, also known as CCL7, may bind to CCR5 without signaling and thereby may serve as an antagonist by interfering with binding of an activating (agonistic) ligand.[18]

CCR5 and Initiation of Immune Responses

Numerous host defense cells can express CCR5 (BOX), including immune effector cells (such as T cells, natural killer cells, and natural killer T cells) that can elaborate inflammatory cytokines or destroy infected cells, and antigen presenting cells (such as monocytes, macrophages, and dendritic cells) that can prime immune responses. When chemokine ligands bind to CCR5 expressed on these immune effector cells and antigen-presenting cells, they can be activated and induced to migrate. Among these CCR5-expressing cells, however, only those that coexpress CD4 (Box) are potentially susceptible to HIV infection.

Box. Human Leukocytes That May Express CCR5

Immune effector cells
- T cells
  - Effector/memory T cells*
  - T helper type 1 effector cells*
  - α4β7† gut homing T cells*
  - Activated T cells*
- Natural killer cells*
- Natural killer T cells*

Antigen-presenting cells
- Monocytes, macrophages*
- Immature dendritic cells*
  - Langerhans cells*

Basophils

*Can coexpress CD4; susceptible to HIV infection.
†A cell surface integrin that mediates immune cell homing.

At sites of microbial invasion, certain substances that are common to classes of microbial pathogens such as endotoxin, flagellin, peptidoglycan, single- or double-stranded RNA, and unmethylated DNA containing certain CpG motifs that are common in the bacterial genome, activate cellular toll-like receptors on or within macrophages, dendritic cells, and other cells to initiate the innate immune response (FIGURE 2).[19] In contrast to the enormous diversity and fine specificity of sequences and structures recognized by the adaptive immune system, a limited number of patterns comprise toll-like receptor–activating signals. Thus, cellular proliferation is not required to generate a robust response. The innate response therefore is rapid but not especially specific. This immediate response includes elaboration of cytokines and chemokines that attract other host defense cells to the site of microbial invasion. Immature epidermal dendritic cells called Langerhans cells expressing CCR5 are attracted to these endangered sites by high concentrations of CCR5-binding chemokines expressed by activated macrophages. These Langerhans cells efficiently ingest microbes and their products and while doing so, rapidly mature, losing CCR5 expression, increasing expression of costimulatory molecules, and gaining expression of CCR7, a chemokine receptor that promotes homing to lymphoid tissue. In lymphoid tissue, these matured dendritic cells can present ingested foreign antigens to naive T and B lymphocytes to initiate the more specific adaptive immune responses. Thus, the expression of CCR5 plays an important role in priming adaptive immune responses.

The expression of CCR5 also plays a role in the distribution of effector cells to sites of microbial infection where they may contribute to microbial control and/or elimination (FIGURE 3). At sites of inflammation, antigen-specific effector T cells as well as natural killer cells may release the chemokines MIP-1α, MIP-1β, and RANTES—CCR5 ligands—upon binding to infected target cells as a means to attract more effector cells to the site.[19] Release of these chemokines attracts immune cells that express CCR5 (mature T cells with effector function, monocytes, and natural killer cells), but not naive T cells or central memory cells, which do not express CCR5.[20,21] These chemokines can bind to glycosaminoglycans, which are polysaccharides abundantly distributed on the endothelial cell surface and in the extracellular matrix. Chemokines thus are concentrated at these sites and on the surface of nearby endothelial cells.[22] Circulating immune cells tend to roll across the endothelial surface by binding cell surface selectins to their weak carbohydrate-containing endothelial cell ligands.[23] Chemokine binding to CCR5 promotes T-cell activation and up-regulation of β2 integrins, transmembrane glycoproteins that promote cellular adherence to arrest the rolling.[24] CCR5 binding by a chemokine also promotes T-cell polarization and migration of the cell across the endothelial surface toward the inflammatory site.[24] There is also some evidence that T-cell surface CCR5 is accumulated at the immunologic synapse, the site at which the T-cell receptor interacts with the peptide/major histocompatibility complex on the antigen-presenting cell,[25] and this interaction also may enhance T-cell activation.

CCR5 and HIV Infection

Shortly after CD4 was recognized as necessary for HIV replication within host cells,[26] a series of experiments was performed to ascertain whether expression of human CD4 was sufficient to permit HIV replication. Expression of human CD4 on mouse cells did not permit HIV infection,[27,28] and experiments wherein permissive human cells were fused with nonpermissive mouse cells indicated that this was not related to an inhibitory factor in mouse cells but that failure of virus entry was related to the absence of an element in mouse cells that was necessary for early postbinding events.[29] The hunt for a HIV coreceptor commenced. In 1995 Cocchi et al[30] reported that MIP-1α, MIP-1β, and RANTES when applied together could prevent HIV entry into host cells. Within a few months, several groups identified the chemokine receptors CXCR4 and CCR5 as key coreceptors for HIV entry.[31-37] Since these observations were reported, other chemokine receptors have been identified that can facilitate HIV infection in vitro.[38] But it appears that CXCR4 and CCR5 are the critical coreceptors for HIV infection in vivo. Whereas CCR5 can bind and be activated by a number of chemokines, the only defined agonistic ligand of CXCR4 is stromal-derived factor 1; knockout of either CXCR4 or stromal-derived factor 1 is lethal.[8]

Host Cell Receptors and HIV Cellular Entry

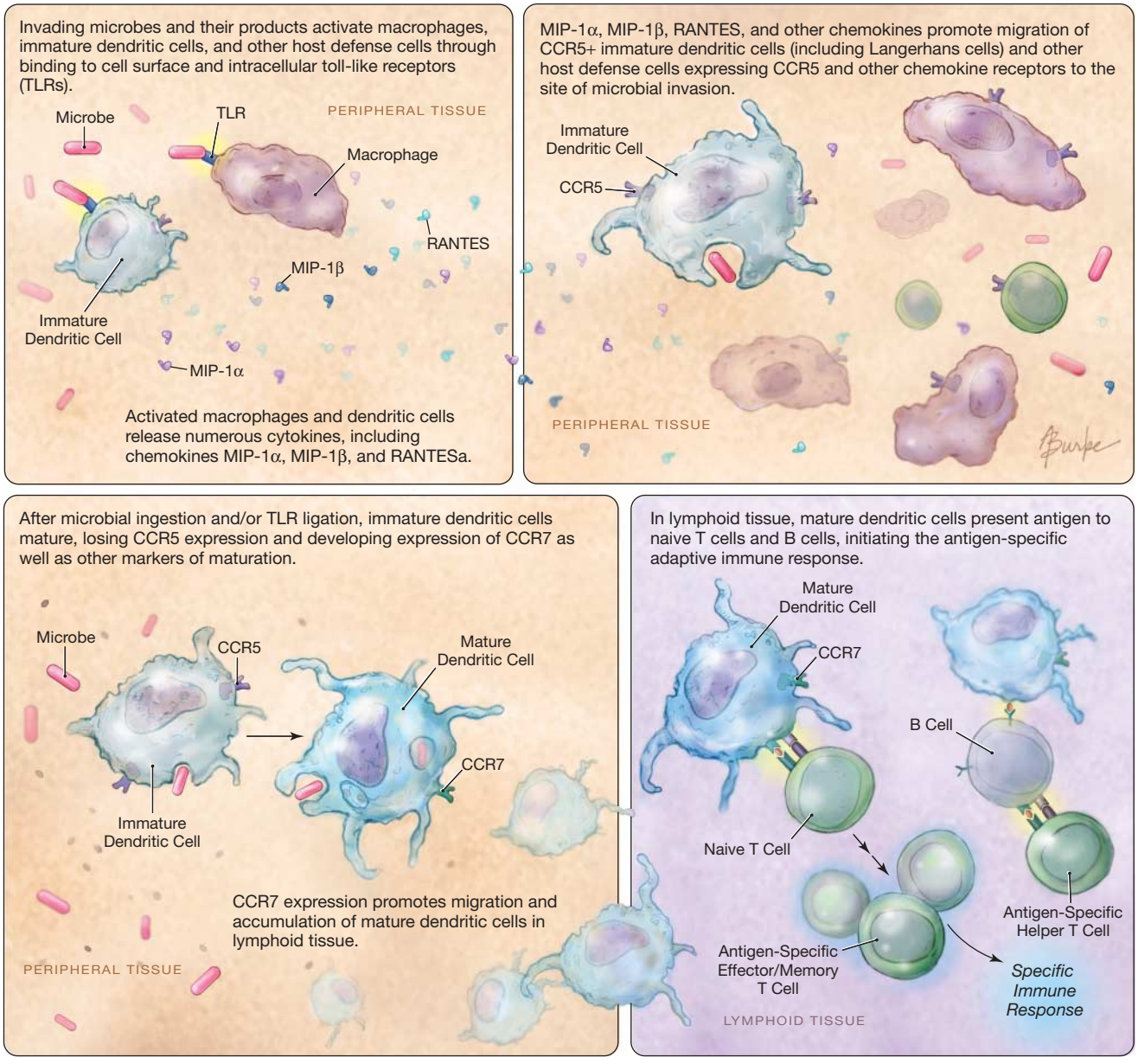
As is typical for other successful intracellular pathogens, HIV uses highly conserved host elements for cellular entry, in this case CD4 and either CXCR4 or CCR5. The mechanisms whereby HIV uses these elements for cellular entry are being progressively elucidated (FIGURE 4). The HIV envelope protein is comprised of 3 heterodimeric glyco-

proteins, each comprised of a transmembrane glycoprotein 41 noncovalently associated with glycoprotein 120. After glycoprotein 120 binds to cellular CD4, a conformational change in the envelope glycoprotein is induced that exposes previously inaccessible domains permitting binding to the CCR5 or CXCR4 coreceptor.[34,39,40] Once the envelope glycoprotein binds to the coreceptor, another major conformational change in the envelope complex is induced that uncovers the free amino terminal–fusion domain of glycoprotein 41 that is then embedded into the host cell membrane. This links the viral membrane to the host cell membrane. This new conformation now permits each of the 3 glycoprotein 41 molecules to zipper upon itself, forming a 6-helix bundle that brings the viral membrane and host cell membrane close enough together to promote their fusion and viral entry into the cell.

Loss or Sequestration of CCR5 and Protection Against HIV Infection

Shortly after CCR5 was identified as a key coreceptor for HIV entry, a deletion of 32 base pairs was identified in the *CCR5* gene open reading frame (coding region) in a few persons who

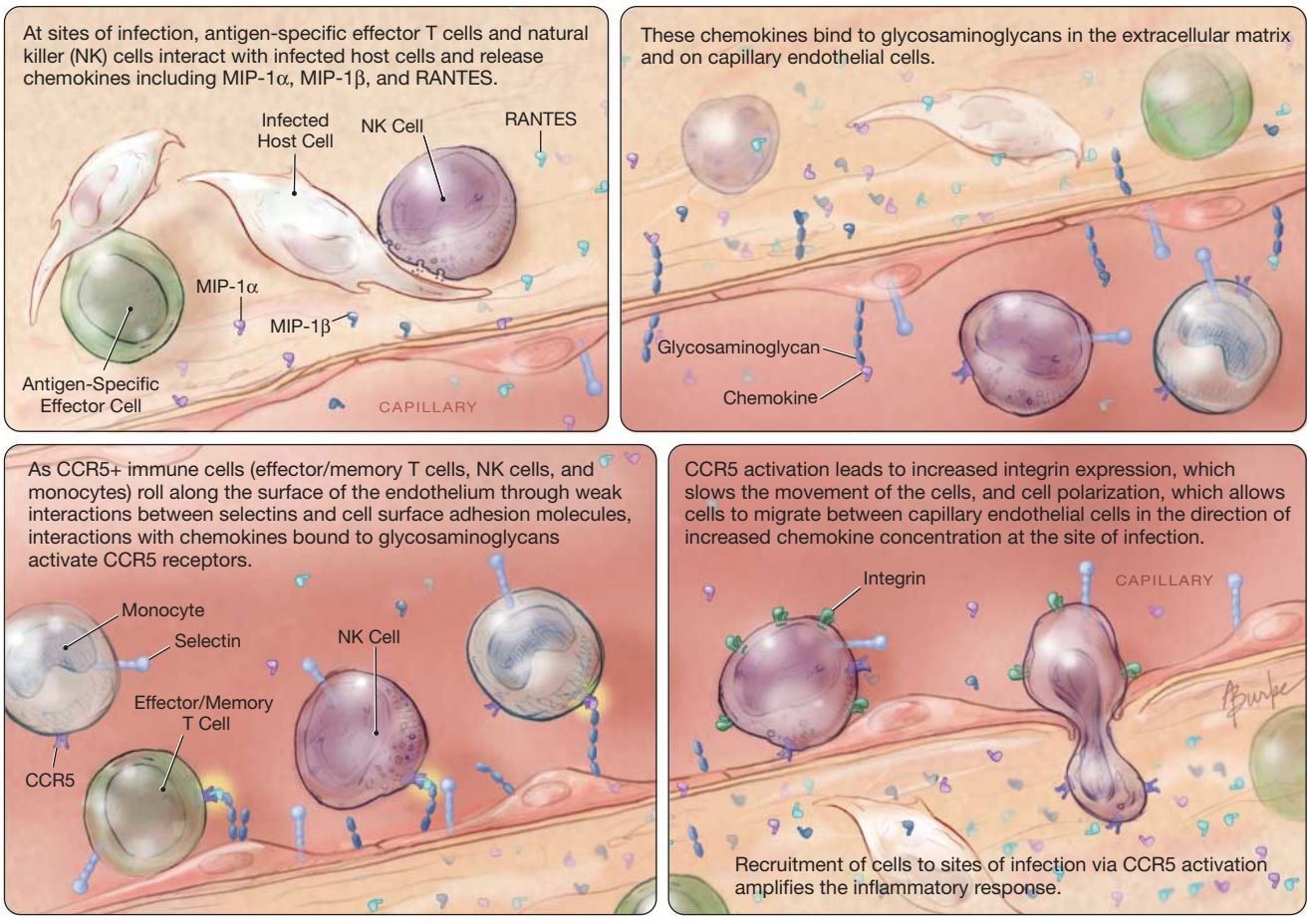
Figure 2. Potential Role of CC Chemokine Receptor 5 (CCR5) in Initiating Immune Responses



were at high risk for HIV infection but who had remained free of infection.[41] Importantly, their blood cells were resistant to infection with viruses that used CCR5 for entry. This deletion of 32 base pairs in the *CCR5* gene (*CCR5*Δ32) results in a truncated dysfunctional protein that does not get expressed on the cell surface. Surprisingly, this allele is common in the white population with a prevalence of 10% to 14%.[42,43] Approximately 1% of the white population has 2 copies of this mutant gene, and these persons are dramatically overrepresented in populations of high-risk HIV seronegative persons.[42,44] HIV infection in persons homozygous for *CCR5*Δ32 is extremely rare, and when it does occur, it is caused by viral strains that can use CXCR4 for viral entry.[45,46] Thus, congenital absence of CCR5 protects against acquisition of HIV infection. Factors limiting the establishment of infection by viruses that uniquely use CXCR4 for cellular entry are not completely understood and may reflect partial inhibition of CXCR4 tropic virus replication at multiple levels, which provides, in aggregate, a sufficient barrier that renders them poorly infectious.[47] Heterozygous individuals who have 1 *CCR5*Δ32 allele and 1 wild-type allele may be at marginally lower risk for HIV infection,[48,49] but when heterozygous individuals acquire infection, they have a somewhat attenuated course.[50,51] Lower levels of plasma viremia may be seen in these individuals.[50] As HIV disease progresses, approximately half of the infected persons may develop viruses that can use the CXCR4 coreceptor for entry (X4 viruses).[52] This is associated with an acceleration of disease course,[53] although it is not clear whether the emergence of X4 viruses is the cause or consequence of accelerated immune deterioration.[52]

Other rare mutations in CCR5 that result in a dysfunctional protein have been found in some high-risk seronegative persons.[10,54] In addition, there is some evidence that in different populations, the number of *MIP-1α* gene duplications inversely predicts the risk of HIV acquisition and rate of disease progression.[55] If, as suspected, more gene duplication results in greater expression of the CCR5 ligand MIP-1α, the resultant increased receptor occupancy and internalization may limit availability of the coreceptor and decrease the risk of HIV infection and viral propagation. Thus, the avail-

Figure 3. Potential Role of CC Chemokine Receptor 5 (CCR5) in Amplifying Tissue Inflammation

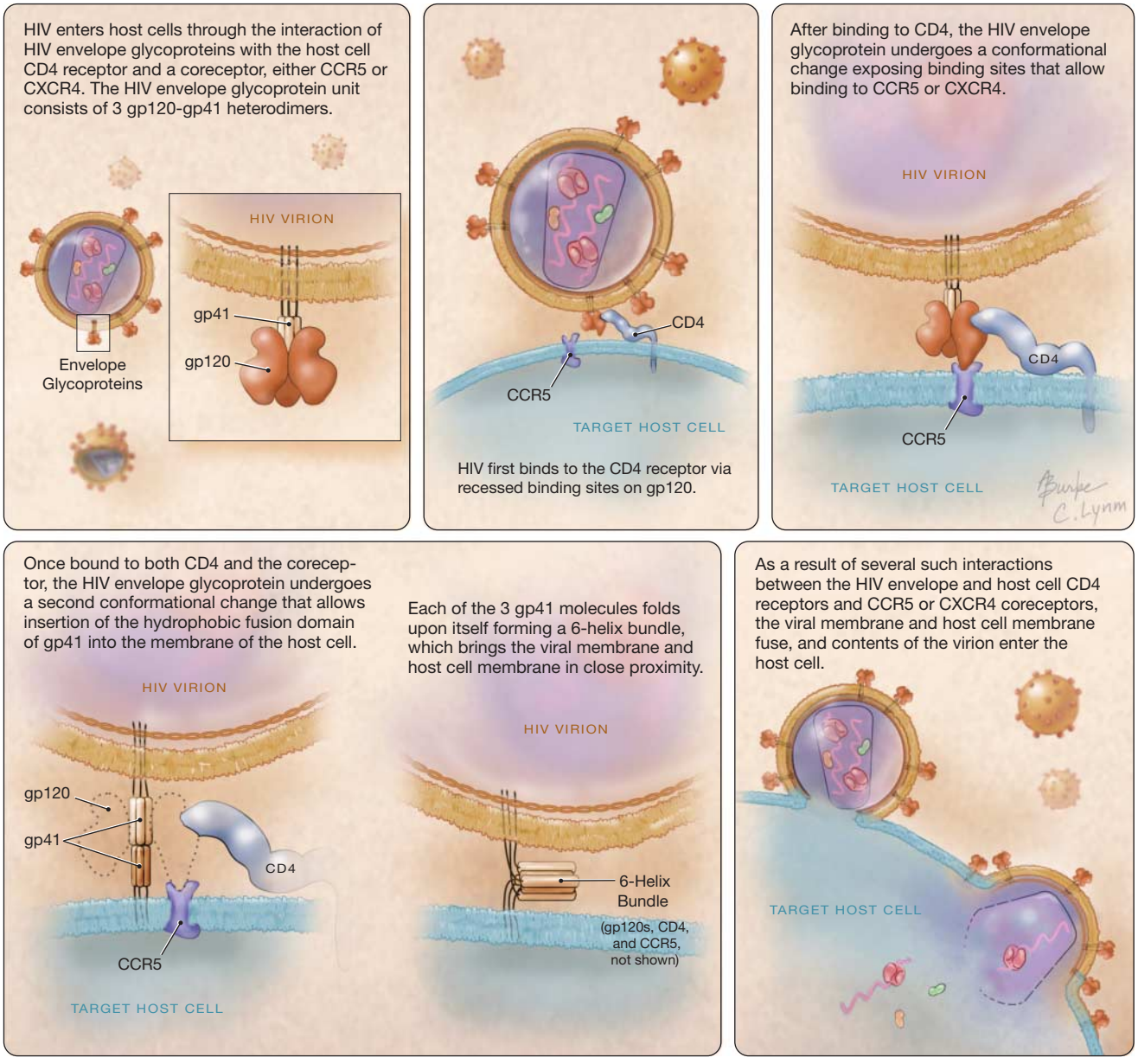


ability of cell surface CCR5 is a critical determinant of susceptibility to HIV infection and disease progression.

*CCR5*Δ32 Allele and Immune Function

As noted above, the *CCR5*Δ32 allele is common in whites but it is almost never seen in Asians or Africans,[43] indicating that it appeared in humans after these populations diverged. Recent studies suggest that it is at least 2900 years old.[56] Where it is prevalent, it exists in Hardy-Weinberg equilibrium suggesting that now on a population level, there is not an important advantage or disadvantage to the heterozygous or homozygous genotype. The high penetrance of this mutation in certain populations, however, suggests that at some time there must have been an important selective advantage provided to persons with this allele. Candidates for this selection pressure have included protection from plague caused by *Yersinia pestis*, although laboratory evidence for such an advantage is controversial at best,[56-58] and protection from smallpox due to Variola virus because CCR5 and other chemokine receptors may be used for entry by related poxviruses.[59] The restricted geographic distribution, the estimated age of the *CCR5*Δ32 allele, and

Figure 4. Model for HIV Entry



the apparent absence of HIV in humans before the last century[60] suggest that HIV had no role in the selection for this allele. In any event, persons who are homozygous for *CCR5*Δ32 seem to have a normal life expectancy and no overtly apparent alteration in risks for infectious or immunologic disorders. The apparent tolerance of this null phenotype (no CCR5 expression) is thought to be related to the redundancy of chemokines and their chemokine ligands as described above. As a result of these overlapping interactions, absence of CCR5 expression could be compensated for by the use of other chemokine receptors for directing cellular trafficking and cellular activation. Recently, however, there is evidence that given a sufficient challenge, there is a clinical phenotype associated with congenital absence of cell surface CCR5. For example, the survival of renal allografts without rejection of the transplanted organ among persons homozygous for *CCR5*Δ32 is apparently longer than among persons with the wild-type alleles,[61] consistent with experimental models wherein application of CCR5 inhibitors prolonged allograft tolerance in rodents.[62,63] Most recently, a retrospective analysis performed in the southwestern United States indicated that among persons with severe West Nile virus infection and especially among those with fatal infection, persons homozygous for *CCR5*Δ32 were overrepresented,[64] validating in humans the results of experimental studies in *CCR5* knockout mice.[65] In murine systems, knockout of *CCR5* results in heightened severity of some infectious processes[66,67] but not others,[68-70] yet for some infections, inflammation-related morbidity may be reduced.[71,72] While there is controversy regarding a possible beneficial effect of a single *CCR5*Δ32 allele on the outcome of infection with hepatitis C virus,[73-75] there is reason to believe that persons with the *CCR5*Δ32 allele are more likely to resolve infection with hepatitis B virus (M. Carrington, PhD, written communication, April 13, 2006). Thus, an interim conclusion that may be drawn until more data are generated is that the *CCR5*Δ32 homozygous state that confers high-level protection from HIV infection is largely well tolerated but certain challenges of sufficient magnitude may unmask an apparent immunodeficiency associated with this genotype. It remains to be seen if other infections or infection-related outcomes are managed more effectively by these hosts.

Inhibition of HIV Entry as Treatment for HIV Infection

Targeting viral entry holds promise as a strategy to treat or prevent HIV infection. Agents that interfere with HIV entry may target either HIV or host cell elements. A small number of human monoclonal antibodies against the HIV envelope glycoprotein have been developed that are capable of neutralizing HIV infectivity and preventing entry,[76-79] but administration of several of these intravenously was not associated with a durable antiviral effect.[80] Interference with the binding of CD4 and glycoprotein 120 by administration of soluble CD4 was ineffective in early studies,[81,82] but a polyvalent CD4-IgG fusion protein (PRO 542, Progenics Pharmaceuticals, Tarrytown, NY) has demonstrable antiviral activity in vivo after intravenous administration.[83] A small molecule that blocks the CD4 binding site on glycoprotein 120 (BMS 378806, Bristol-Myers Squibb, New York, NY) has antiviral activity in vitro[84] and is being developed as part of a topical strategy to prevent mucosal HIV transmission.[85-87] The first entry inhibitor approved for clinical use is a parenterally administered peptide containing sequences of HIV glycoprotein 41.[88] This peptide, enfuvirtide (Roche/Trimeris, Basel, Switzerland/Durham, NC), blocks the zippering of glycoprotein 41 (Figure 4) that brings the viral and cell membranes together to promote their fusion.

Inhibition of CCR5 for Treatment or Prevention of HIV Infection

With the appreciation that CCR5 is necessary for HIV cellular entry and that CCR5 is the key coreceptor for most HIV strains in infected persons, several strategies that target this element are in development for prophylaxis and treatment. It is important to understand that different strategies for CCR5 blockade may have different effects on the host and different interactions with HIV. Moreover, as HIV disease advances, increasing proportions of persons harbor replicating viruses that can use CCR5 and CXCR4 (dual tropic) or CXCR4 alone (X4 tropic) for cellular entry.[89] The treatment outcomes of such persons with regimens that include a CCR5 inhibitor have not been completely studied and may be successful if other treatment agents remain active against the X4 isolates. On the other hand, if the regimen is not completely suppressive of viral replication, X4 viruses may emerge. Careful follow-up in these instances is necessary to ascertain if the sustained replication of X4 strains accelerates disease progression. Baseline screening of patient viruses for X4 and R5 tropism is necessary to resolve these questions before initiation of treatment with CCR5 inhibitors.

CCR5 Agonists. As noted above, chemokine binding to CCR5 is an agonistic event that results in intracellular signal transduction and internalization of the coreceptor. A number of amino terminus–modified chemokine analogues have been developed that are substantially more active HIV inhibitors than the native RANTES.[90-92] These agents bind CCR5 and promote its internalization. Their potency is directly related to the magnitude and duration of receptor internalization, which for the most potent, N^{α}-(*n*-nonanoyl)-*des*-Ser^{1}-[L-thioproline^{2}, L-α-cyclohexylglycine^{3}] (PSC) RANTES, can be as long as 24 hours.[90] By promoting intracellular sequestration of the coreceptor, these agents are unlikely to promote the emergence of drug-resistant HIV isolates capable of using CCR5 for cell entry. Vaginal application of PSC-RANTES has provided high-level protection in rhesus macaques against infection by SHIV 162P3, a chimeric simian immunodeficiency virus that contains envelope sequences of HIV.[93] Although this strategy has not yet been

associated with evidence of local inflammation, this possible outcome must be kept in mind as this and related agents are developed. While ligand binding to CCR5 promotes both signaling and receptor internalization, these events may not necessarily be linked. Conceivably, agents that bind and internalize CCR5 without agonist activity can be developed. As an example, we have recently found that human β-defensin 3, a small cationic peptide, can antagonize the binding of ligand to the other HIV coreceptor, CXCR4, and promote its internalization without evidence of agonist activity.[94,95]

CCR5 Antagonists. There are 2 classes of CCR5 antagonists in development—a monoclonal antibody to CCR5 and several small molecule antagonists. The humanized monoclonal antibody HGS Ab004 (Human Genome Sciences, Rockville, Md) binds to the second extracellular loop of CCR5, thereby inhibiting both chemokine and HIV envelope binding.[96] Small molecule CCR5 inhibitors aplaviroc (GlaxoSmithKline, Philadelphia, Pa), maraviroc (Pfizer, New York, NY), and vicriviroc (Schering-Plough, Kenilworth, NJ) have been tested for activity in large-scale human trials. Each of these small molecules is likely to be an allosteric inhibitor that locks CCR5 into a conformation such that it is not able to bind HIV envelope protein. Each can function as a receptor antagonist, blocking to various degrees the signals induced by different receptor-binding chemokines.[97] None of these agents is thought to promote signaling and receptor internalization, therefore, these agents are not likely to promote inflammation as the agonists (above) might. Because CCR5 is maintained on the cell surface, however, viral escape mutants that can still use CCR5 for cellular entry may be selected.[98] These agents appear capable of prolonged receptor binding in vitro and in vivo.[99] By blocking CCR5 activity, they may block the cellular trafficking and activation that is mediated by CCR5. These agents have had some developmental challenges. Trials of aplaviroc have been halted because of liver toxicities[100]; 1 vicriviroc trial in treatment-naive patients has been terminated because of treatment failures,[101] and a salvage study of vicriviroc has been unblinded because of the unexpected occurrence of malignancies, including lymphomas.[102] Interestingly, in HIV-infected persons who are heterozygous (1 wild-type allele and 1 *CCR5*Δ32 allele) for *CCR5*, the occurrence of malignant non–Hogkins lymphoma appears to be lower than expected.[103] The relationship of vicriviroc treatment to the development of malignancies is still uncertain. Results from further studies, including ongoing trials of vicriviroc and maraviroc, are needed to determine the efficacy and safety of CCR5 antagonists for treatment of HIV infection.

Nonagonistic Nonantagonistic CCR5 Inhibitors

Pro-140 is a humanized monoclonal anti-CCR5 antibody (Progenics Pharmaceuticals) that inhibits HIV entry by binding to the second extracellular loop of CCR5 but neither signals nor blocks the function of the receptor at concentrations sufficient to block HIV entry.[104] This surprising selective activity may render this inhibitor less immunosuppressive than the antagonistic inhibitors.

Possible Consequences of CCR5 Inhibition

As noted above and depending on the strategy applied, blockade of CCR5 might result in induction of inflammatory responses through activation of the receptor or might block the trafficking and cellular activation mediated by CCR5 through antagonism. While compensation for the congenital absence of CCR5 by a redundant network of chemokine-ligand interactions may be reasonably well tolerated unless severely challenged, it is not clear whether acute effects of pharmacological or immunologic CCR5 blockade will be as well tolerated. Also, in the setting of immunodeficiency due to HIV infection, it is not clear whether CCR5 blockade will be as well compensated. Several hypotheses that explore potential consequences of CCR5 inhibition as a therapeutic strategy in HIV infection are outlined below.

Will CCR5 blockade block trafficking of effector cells to tissue sites of inflammation? This is a plausible consequence of CCR5 inhibition and may underlie the enhanced allograft tolerance and apparent heightened risk of serious West Nile virus infection in persons homozygous for *CCR5*Δ32. This effect might be even more profound in persons who have not experienced years of accommodation to the absence of the receptor or in persons with HIV-related immunodeficiency. To test this hypothesis, the influx of effector cells into tissue sites of inflammation could be examined by immunohistochemical analysis of cellular infiltration at biopsied sites of skin test antigen placement in persons receiving treatment with CCR5 antagonists and in controls.

A minority of persons with advanced HIV infection experience an immune restoration inflammatory syndrome after administration of suppressive antiviral therapies.[105,106] This morbid inflammatory syndrome has been attributed to the rapid ingress of effector lymphocytes to sites of unrecognized opportunistic infection. If this is the case, one might hypothesize that the occurrence of an immune restoration inflammatory syndrome might be lower among patients treated with a regimen that includes a CCR5 antagonist.

Will CCR5 blockade affect the reconstitution of gut-associated lymphoid tissue? Studies in humans and rhesus macaques indicate that CD4 lymphocytes within the gut-associated lymphoid tissue are rapidly and profoundly depleted in the acute phase of HIV or simian immunodeficiency virus infections.[107-110] These target cells are virtually all CCR5+. Although there is controversy as to whether this cell population is restored with antiviral therapies, one might hypothesize that administration of CCR5 inhibitors may block or delay this restoration if recruitment of these cells to these sites requires functional CCR5.

Will CCR5 blockade attenuate responses to immunization? This too is a plausible consequence of CCR5 inhibition because the accumulation of antigen-presenting cells at sites of antigenic challenge is thought to be mediated at least in part via CCR5 activation. This can be evaluated by examining T-cell and B-cell responses to vaccine administration[111,112] in persons treated with regimens including CCR5 inhibitors and in appropriate controls.

Will administration of CCR5 inhibitors increase the risk of opportunistic infections and malignancies in HIV-infected persons? This, of course, is a central safety question regarding this novel class of antiretroviral agents. While congenital absence of CCR5 is generally well tolerated, there is increasing recognition that with a sufficient challenge, persons with this genotype may have an altered immune response. How this will play out in persons who have not had a lifetime of accommodation to the absence of this receptor and who may also have varying degrees of HIV-related immune impairment remains to be seen. Close clinical and immunologic monitoring of clinical trials of CCR5 inhibitors is therefore warranted. This potentially valuable class of antiviral compounds also has the potential for subtle immune compromise. How these activities are balanced in persons at different stages of HIV disease and with different degrees of antiviral drug resistance[113] will determine the place of these agents for the treatment of HIV infection.

In conclusion, through interactions with its chemokine ligands, the chemokine receptor CCR5 helps to initiate immune responses and to distribute effector immune cells to sites of inflammation. CCR5 is also a key cellular receptor that is required for almost all instances of HIV infection. Strategies targeting CCR5 are therefore in development for prevention and treatment of HIV infection. Although deletion of CCR5 is generally well tolerated in mice and in humans, with sufficient challenge, a perturbed host immune response can be demonstrated in both. Whether pharmacological inhibition of CCR5 function will be as well tolerated or whether blocking this receptor will have special adverse consequences in persons with underlying HIV-related immune impairment remains to be seen.

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Study concept and design: Lederman, Penn-Nicholson, Cho.
Acquisition of data: Lederman.
Analysis and interpretation of data: Lederman, Mosier.
Drafting of the manuscript: Lederman, Cho, Mosier.
Critical revision of the manuscript for important intellectual content: Lederman, Penn-Nicholson, Cho, Mosier.
Obtained funding: Lederman.
Administrative, technical, or material support: Cho.
Study supervision: Lederman, Mosier.
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