The discovery that HIV requires a chemokine co-receptor to invade host cells has prompted many investigations into therapeutic strategies that target these receptors in an attempt to block HIV entry. In this review, Scott Cairns and Patricia D'Souza discuss these potentially powerful approaches and how they complement existing antiretroviral drug therapy.

Chemokines and HIV-1 second receptors: The therapeutic connection

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In 1996, the discovery that HIV uses invariant host proteins as co-receptors for entry, coupled with the observation that one of these proteins, CCR5, is dispensable for health, galvanized the search for ways to stop HIV entry. The rapid pace at which researchers are exploring the HIV-chemokine connection is evident in the large number of publications on this topic as well as the rapid translation of laboratory findings into possible therapeutic applications. During the past year, significant advances have been made in two general areas: First, the identification and characterization of additional chemokine receptors that serve as co-receptors for entry of HIV-1 and related lentiviruses into the cell; and secondly, the translation of basic research observations into an array of therapeutic options designed to block, sequester or prevent the use of chemokine receptors by HIV.

Here, we highlight those studies that have formed the basis for the development of antiviral intervention strategies that target this early step in the virus life cycle (Fig.1). By focusing on viral entry, these novel strategies may strengthen the armamentarium against HIV infection and AIDS because they complement existing antiretroviral drugs that target viral enzymes involved in post-entry steps.

Chemokine receptors and HIV entry

HIV infection is initiated by interaction of the virion envelope glycoproteins (gp120/41) with at least two cellular receptors: the CD4 molecule and a seven-transmembrane domain G-protein coupled chemokine receptor[1]. Macrophage-tropic (M-tropic) strains of HIV-1 replicate in macrophages and CD4$^+$ T cells and use the CC chemokine receptor CCR5[2–6]. These HIV-1 viruses are newly classified as R5 based on their co-receptor usage[7]. The CCR5 co-receptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype, and by the related lentiviruses HIV-2 (ref. 8) and simian immunodeficiency virus[9] (SIV) . T-tropic isolates of HIV-1 replicate in primary CD4$^+$ T cells, established CD4+ T cell lines, as well as macrophages. All of these viruses use the CXC chemokine receptor CXCR4, and many of them also use CCR5 (refs. 10–12). Those viruses that only use CXCR4 are referred to as X4, whereas viruses that use both receptors with comparable efficiency are referred to as R5X4 (ref. 7). Although CCR5 and CXCR4 are believed to be the primary receptors for entry of HIV-1, nine additional chemokine receptors, including one encoded by cytomegalovirus have been shown by *in vitro* assays to serve as co-receptors for HIV and SIV (Table 1). The biological role of any of these receptors in HIV infection or pathogenesis awaits clarification. Within the human genome, there exist approximately fifty additional open reading frames with sequence similarity to chemokine receptors; some of these may ultimately contribute to the growing list of chemokine receptors with HIV co-receptor activity (W. Haseletine, unpublished).

Variation in expression of chemokines and their receptors

The R5 viruses are the strains most commonly transmitted between people and are present early in the course of disease. The importance of CCR5 in transmission of HIV was underscored by the finding that individuals who have a homozygous 32 base-pair (bp) deletion in the CCR5 gene are highly resistant to infection with HIV-1 (refs. 13–15). This deletion, which is carried in homozygous form by approximately one percent of Caucasians of European descent, results in a prematurely truncated protein that remains intracellular.

Approximately 10–15 percent of Caucasians are heterozygous for the Δ32 allele and levels of CCR5 expression on T cells in these individuals are lower than in individuals who are homozygous for the wild-type allele. Although there is little evidence that heterozygosity affects transmission, infected heterozygotes progress to disease more slowly, exhibit lower viral loads and slower rates of CD4$^+$ T cell decline, and have a higher likelihood of being long-term non-progressors than homozygous wild-type individuals[15–17]. Other genetic polymorphisms in CCR5 and its

Table 1 Summary of HIV and SIV coreceptors

Chemokine Receptor	Ligand Type	Ligand	Virus
CXCR4[10]	CXC	SDF-1	X4, R5X4
CCR2[5,6]	CC	MCP-1, MCP-2, MCP-3	R5X4
CCR3[5,6]	CC	Eotaxin, RANTES, MIP-1α, MCP-3, MCP-4	R5X4
CCR5[2–6]	CC	MIP-1α, RANTES, MIP-1β	R5, R5X4,HIV-2, SIV
CCR8[46,68]	CC	I-309[46]	R5X4, HIV-2, SIV
STRL33/BONZO[69–71]	?	?	R5X4, SIV
BOB/GPR-15[70,72]	?	?	R5X4, SIV
GPR-1[72]	?	?	SIV
V28/CX_3CR[73,74]	CX_3C	Fractalkine[76]	R5X4, HIV-2, SIV
APJ*	?	?	R5X4, SIV
US28 (cytomegalovirus)[75]	CC	RANTES, MIP-1α, MCP-1	R5X4

All strains of HIV and SIV bind to CD4. Eleven coreceptors are listed that bind different HIV and SIV strains. ?, not yet tested or unknown. R5 refers to CCR5-using virus, X4 refers to CXCR4-using virus. R5X4 refers to dual tropic CCR5- or CXCR4-using virus. In CC or β- chemokines, the cysteine pairs are adjacent. In CXC or α- chemokines, each of cysteines is separated by an intervening amino acid. In CX_3C chemokines, each pair of cysteines is separated by three intervening amino acids. References are provided only for those ligands discovered in 1997. *, Edinger, A.L. *et al.*, manuscript submitted.

Table 2 Status of anti-HIV therapeutic strategies involving chemokine receptors

Strategy	Therapy	Target	Status
Immune restoration	Down-regulation of CCR5 on CD4+ T cells	CCR5	phase I human trials
	Use of -/-CCR5 cells	CCR5	concept
Gene therapy	Ribozymes	CCR5, CXCR4	Preclinical
	Intrakines	CCR5, CXCR4	Preclinical
	Single chain mAbs	CCR5, CXCR4	Concept
	Anti-sense	CCR5, CXCR4	Concept
Immunotherapy	mAbs	CCR5, CXCR4	Preclinical
Suicide vectors	Modified cytopathic viruses or vectors	HIV-infected cells	Preclinical
Chemokines/altered	MIP 1-α	CCR5	Phase I human trials
Chemokines/peptides	met-RANTES	CCR5	Preclinical
	AOP-RANTES	CCR5	Preclinical
	T22	CXCR4	Preclinical
	ALX40-4C	CXCR4	Preclinical
Small molecule antagonists	AMD3100	CXCR4	Preclinical

regulatory regions are known to occur[18] and may contribute to the considerable variation in CCR5 expression levels (20-fold), and course of disease between individuals who express two wild-type CCR5 alleles[19,20].

Recently it has been found that individuals homozygous for a polymorphism in a noncoding region of the gene encoding stromal derived factor-1 (SDF-1), the ligand for CXCR4, experience delayed disease progression[21]. Although much remains to be learned about genetic factors that may influence resistance to infection or disease progression, current understanding of naturally occurring variation in chemokines and their receptors offers hope that strategies designed to affect levels of these molecules may have therapeutic application.

Ex vivo manipulation of CCR5 protein expression

Novel strategies are being implemented to examine mechanisms to sequester or prevent the expression of chemokine receptors in order to make cells resistant to infection with HIV (Table 2). An intriguing observation is that activation of CD4+ T cells with immobilized monoclonal antibodies (mAbs) to the cell surface molecules CD3 and CD28 results in a population of CD4+T memory cells that have down modulated transcription of CCR5 and produce factors that inhibit R5 as well as X4 virus replication. These CD4+ T cells resist infection with R5 viruses, but are still susceptible to infection with X4 viruses[22,23]. Resistance of the stimulated cells to HIV-1 infection is short-lived *in vitro*, with re-acquisition of HIV infectability occurring within one week after the stimuli are removed.

These findings illustrate that CCR5 levels can be experimentally manipulated and support studies in HIV-infected individuals to examine the safety and efficacy of administering CD4+ T cells in which CCR5 expression is diminished. Two approaches that capitalize on these observations are at different stages of development. In the first, a clinical trial has been initiated in which HIV-positive subjects have been infused with three doses of their own CD4+ cells, which had been previously stimulated in culture with anti-CD3 and anti-CD28 mAbs. Two out of three participants in this trial experienced sustained increases in CD4+ cells for more than four months post-infusion (B. Levine, unpublished). Although the protocol is unlikely to have wide clinical application because of the expense and cumbersome nature of the treatment strategy, these preliminary data suggest that manipulation of the CD28 signal transduction pathway as a means of decreasing expression of CCR5 may have therapeutic potential.

A second approach is to supply HIV-infected individuals with hematopoietic stem cells that are genetically resistant to HIV infection. These cells are capable of giving rise to renewable populations of the lymphoid and myeloid lineages. To ensure successful engraftment of the infused hematopoietic stem cells, the recipient would need to undergo relatively harsh treatments: depletion of existing lymphoid cells to prevent graft rejection and prophylactic measures to prevent graft-versus-host disease. The limited availability of resistant stem cells would also make this treatment impractical for large-scale use. Nevertheless, in those relatively rare instances such as AIDS-associated lymphoma, where cytoablative regimens constitute the normal standard of care, reconstitution with genetically resistant stem cells may ultimately play a role in treatment.

Gene therapy to prevent chemokine receptor expression

Gene therapy is based on the premise that insertion of anti-HIV genes into target cells will render them resistant to HIV infection and/or replication. In current gene therapy strategies, cells are taken from a donor and transduced with a vector capable of expressing the protective gene as well as a marker gene that allows selection of the transduced cells. Many technological difficulties exist in adapting this strategy to large-scale clinical testing: inefficient gene transfer using current vectors; inability to target viral reservoirs; restricted range of cell types susceptible to current retroviral vectors; and expensive, labor intensive *ex vivo* manipulations of target cells. Some of these obstacles may be resolved with the development of improved gene-based delivery systems and the use of pluripotent stem cells with self-renewal capacity which will permit the entry of genetic therapy into the mainstream of therapeutic options available to infected individuals. Several gene therapy approaches, all still at the stage of *in vitro* experimentation, highlight the potential impact of this technology on the treatment of HIV disease.

Intrakines. In this approach to block surface expression of CCR5 and CXCR4, vectors have been engineered to express modified forms of SDF-1 or the CCR5 ligand, RANTES, which have been altered to express retention signals on their carboxy termini that prevent secretion. The modified intrakines, so called because they remain inside the cell, bind to their cognate receptors, trapping them in the endoplasmic reticulum (ER) where they are rapidly degraded. SDF-1 and RANTES intrakine-expressing lymphocytes resist infection with X4 (ref. 24) and R5 (ref. 25) viruses, respectively. Importantly, at least by *in vitro* measures, the modified lymphocytes appear functionally uncompromised despite the absence of the chemokine receptors. Intrakine-modified cells may offer at least two advantages over strategies that in-

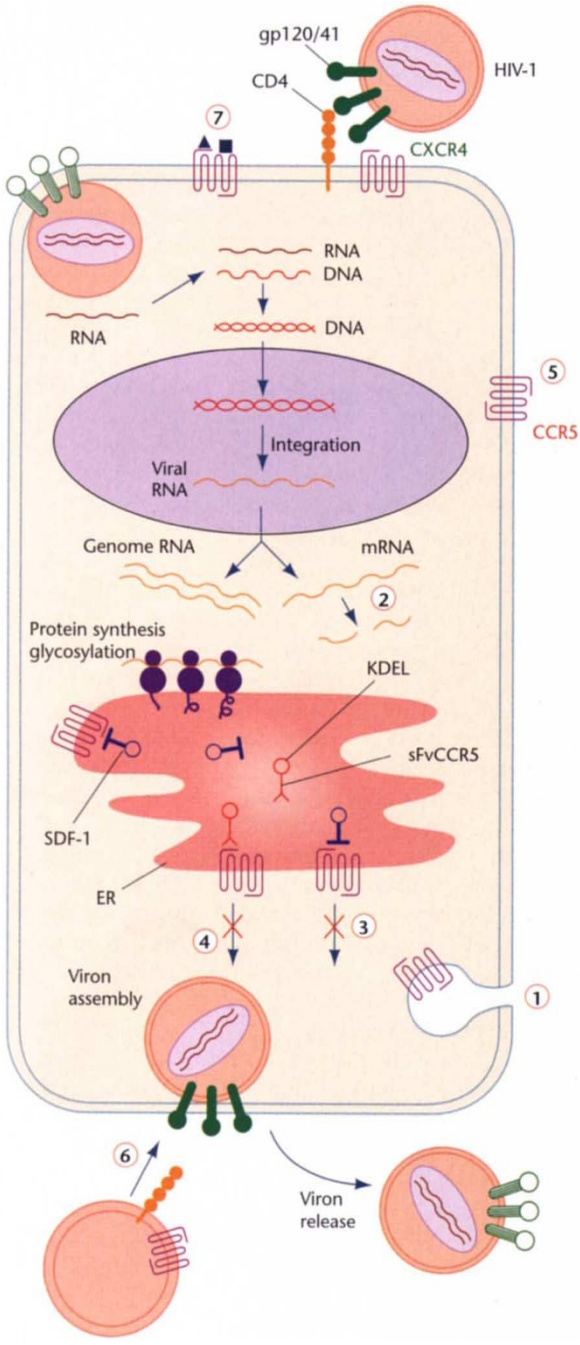

Fig. 1 Chemokines, coreceptors and the HIV life cycle. HIV binds to susceptible cells via CD4 and a chemokine receptor. This is followed by viral entry, reverse transcription, integration, viral RNA synthesis and processing, viral protein synthesis, virion assembly and budding. Steps at which HIV/chemokine receptor interactions can be disrupted, include: (1) Downregulation of chemokine receptors with anti-CD3 and CD28 mAbs; (2) site for ribozyme action; (3) and (4) newly synthesized CXCR4 and CCR5 form complexes with intrakines or intrabodies and remain trapped in the ER; (5) extracellular mAbs to CCR5 block interaction with gp120/41; (6) suicide viruses target gp120/41 expressing cells; and (7) chemokines, peptides, small molecules bind to their cognate receptor and prevent interaction with gp120/41.

volve direct administration of bioactive chemokines: They circumvent problems associated with the putatively short half-life of chemokines in circulation (<10 min)[26]; and they minimize potential inflammatory effects that could result from the systemic administration of bioactive chemokines.

Intrabodies are intracellular antibodies that bind to and prevent expression and function of their target molecules. Previous studies have demonstrated the feasibility of genetically engineering human cells to produce intrabodies that bind to HIV-1-encoded proteins intracellularly and prevent their function and/or incorporation into virions[27–29]. This strategy is now being applied to trap chemokine receptors using specific mAbs, although data on the effectiveness of this approach are not yet available.

Ribozymes are enzymatic RNA molecules that can be designed to specifically recognize and cleave other RNAs. By cleaving mRNA sequences, ribozymes can prevent or diminish translation of proteins encoded by the targeted sequences. Ribozyme targeting sequences can be modified to recognize many accessible sites within target RNA sequences. In the first such studies to target HIV, ribozymes were targeted to HIV *gag* sequences[30] and leader sequences at the 5′ end of the HIV genome[31]. In current studies, CCR5-specific ribozymes have been developed that appear to cleave their targets *in vitro*, and can be expressed to appreciable levels in target cell lines (J. Rossi, unpublished).

Other less well developed gene therapy strategies include the use of antisense methods in which the therapeutic gene would prevent the translation of the chemokine receptor by binding to, and blocking translation of the targeted mRNA. Further exploratory gene therapy strategies may include vectors that express secreted chemokines as well as other co-receptor ligands as potential competitors of gp120 interaction with chemokine receptor.

Strategies to prevent chemokine receptor expression have several advantages. First, they aim to block viral entry rather than limit virus production by infected cells. Second, they complement existing antiretroviral drug strategies that interfere with highly polymorphic virally encoded determinants involved in replication and viral particle maturation[32,33]. Third, they are unlikely to be immunogenic making them good candidates as therapies that require prolonged maintenance of the therapeutic gene. Finally, these approaches could be modified to trap or inactivate more than one chemokine receptor by using bi-cistronic expression vectors, perhaps making cells resistant to both R5 and X4 viruses.

Monoclonal antibodies

Another strategy to inhibit the interaction of HIV with its co-receptor is by administering mAbs to the chemokine receptors. Passive immunotherapy with a mAb to CCR5 may have at least two clinical applications: prevention of maternal-fetal transmission of HIV-1, and post-exposure prophylaxis. Whereas the efficacy of mAbs in the prevention and treatment of infectious disease is not well-studied, the concept of using neutralizing antibodies in chronic HIV infection has been tested, albeit with suboptimal reagents. In most cases, treatment has been well tolerated and modest reductions in viral burden were observed in some patients[34,35].

A variety of mAbs have been developed to target chemokine receptors. The first such reagent was 12G5, a murine mAb against CXCR4 (ref. 36). Of the mAbs to CCR5, one particular murine mAb designated 2D7, completely blocked the binding and chemotaxis mediated by RANTES, MIP-1α and MIP-1β. The mAb also efficiently blocked the infectivity of several R5 and R5X4 viruses. Mapping studies have revealed that 2D7 maps to

the second extracellular loop of CCR5, an important domain for both gp120 and chemokine binding[37].

The development of mAbs for therapeutic use is still subject to several obstacles including the high costs of production of these reagents, accessibility of the targeted cellular population, the necessity for injection and possible immunogenicity of the mAb. The latter issue may be diminished to a large extent with the use of humanized mAbs. However, the possible effects of these reagents on the targeted cell population beyond simple receptor blockade, such as receptor down regulation or clearance of receptor-expressing cells, must be taken into account. Resolution of these issues will undoubtedly dictate their future utility as potential therapies or transmission blockers. Importantly, these natural products offer the benefit of inducing fewer of the toxic side effects that are sometimes associated with small molecule therapeutics.

Suicide vectors targeting HIV-infected cells

With knowledge about the necessary components for viral entry, new strategies that specifically target HIV-infected cells have been described that advance the field of targeted gene delivery and may have a potential impact on drug delivery. Two recent reports demonstrate that when the rhabdoviruses rabies virus[38] and vesicular stomatitis virus[39] (VSV) were engineered to express CD4 and CXCR4 instead of their normal envelope proteins, these viruses could infect HIV-infected cells, presumably by interacting with surface-expressed gp120/41 glycoproteins. In fact, the engineered VSV virus was capable not only of infecting HIV envelope-expressing cells, but of killing the cells as well[39]. Similar experiments have subsequently been reported with an HIV-based vector[40].

It is still too early to predict the clinical applicability of these 'suicide vectors.' Potential concerns include: accessibility of the vector to various sites of infection *in vivo;* their ability to target only productively infected cells that express gp120; the transmissability of the vectors from person to person or mother to infant; and the possible adverse effects of administering a live replicating vector to immunosuppressed recipients. Nevertheless, the findings may have broad pharmacological applications and could offer a convenient way to target and deliver drug to infected or cancerous cells[41]. Hypothetically, the incorporation of drugs into liposomes that express CD4 and one or more of the chemokine receptors, or perhaps a single chimeric receptor capable of interacting with the envelopes of M- and T-tropic isolates, could constitute an ideal drug delivery vehicle in which only those cells expressing gp120 would be targeted by the drug. Uninfected or 'innocent bystander' cells would then avoid the unwanted side effects associated with drug delivery.

Natural ligand and peptide based strategies

Because chemokines are able to compete with HIV-1 envelope glycoprotein for binding to the chemokine receptors and can also down regulate their cognate receptor[42], they are obvious therapeutic candidates. Chemokines secured a central role in the HIV field as a result of the observation that MIP-1α, MIP-1β and RANTES inhibit the replication of R5 viruses[43]. Similarly, SDF-1 has been shown to competitively block viral entry of X4 viruses[44,45]. Other recently described CC chemokines with anti viral properties include I-309 the ligand for CCR8[46] and macrophage-derived chemokine (MDC), which exhibits a broad range of suppressive activity against diverse primate lentiviruses[47]. However, the initial MDC observations were made on material purified from the supernatant of immortalized $CD8^+$ T cells and require verification with recombinant protein.

Several studies are planned or at the proof-of-concept stage using biologically active or inactive variants of these molecules. One such agent that has already been tested in the clinic is a variant of the CCR5-binding chemokine MIP-1α, called BB-10010. Although MIP-1α is not the most specific or effective ligand for CCR5, it was selected based on existing safety data as a stem cell protectant during cytotoxic cancer chemotherapy. In a phase I study of BB-10010, no consistent changes were noted in viral load, CD4 counts, or HIV isolate co-receptor usage (L. G. Czaplewski, unpublished). This is the predicted result considering the median plasma concentration of BB-10010 was only 3.5 ng/ml after six days of treatment, much lower that the 90–900 ng/ml range required to see antiviral effects of chemokines *in vitro*. In the absence of innovative dosing strategies to improve the efficacy of BB-10010, this ligand is unlikely to succeed in the clinic.

Apart from the problems of low oral bioavailability of peptides or proteins like MIP-1α, therapeutic interventions based on administration or over expression of these bioactive compounds are also compromised because of the key role these molecules play in inflammation. Other *in vitro* observations also complicate the therapeutic use of chemokines. A first concern is the observation that in certain circumstances, the β-chemokines can actually enhance the replication of X4 isolates[48] (A. Kinter, unpublished). Conversely, SDF-1, has been found to stimulate certain R5 isolates[48]. In both of these instances, the stimulatory effect seems to depend upon the ability of the chemokine to transmit intracellular signals to the target cell following interaction with its receptor on the target cell membrane. A second concern is that under certain circumstances, MIP-1α, MIP-1β and RANTES can enhance[49] CCR5-mediated fusion, entry and replication of R5 strains in macrophages, in contrast to their inhibitory properties in T cells. Because macrophages are likely to be among the first cells exposed to HIV and constitute a reservoir for the virus, these observations engender caution in the systemic use of chemokines.

Modified β-chemokines that block HIV infection without inflammatory side effects or the HIV-1-stimulatory effects of the parent molecules are second generation compounds with therapeutic promise. Two β-chemokine derivatives that bind CCR5 and lack cellular signaling capabilities are under investigation: RANTES(9-68), a truncated form of RANTES[50]; and aminooxypentane (AOP)-RANTES, a version that is chemically modified at the amino terminus[51]. Both of these compounds exhibit increased potency when compared to RANTES and inhibit the infection of primary lymphocytes by R5 viruses in tissue culture experiments without stimulating X4 replication. AOP-RANTES was also able to inhibit replication of R5 strains in primary human macrophages. Although, the unmodified β-chemokines block HIV infection of dendritic cells[52], the inhibitory properties of these modified chemokines on dendritic cells and other sites of viral entry remain unknown.

Two peptides that specifically block the CXCR4–HIV interaction are T22 (ref. 53) and ALX40-4C (ref. 54). T22 is an 18-amino acid peptide derived from the hemocyte debris of the horseshoe crab. It specifically blocks membrane fusion and infection by X4 viruses as well as chemotaxis in response to SDF-

1. ALX40-4C is a highly cationic peptide containing nine arginines. This compound also blocks HIV envelope interactions and SDF-1 interaction with CXCR4.

Although the initial laboratory data on the ability of these peptides to block the interaction of HIV envelope with chemokine receptors is encouraging, these agents are relatively large, expensive to manufacture and, because they are peptides, are expected to have limited oral bioavailability. Moreover, the optimum concentration of these compounds required to block HIV entry and chemokine-mediated biological effects *in vivo* is unknown. Therefore, before these compounds enter the therapeutic pipeline, careful comparative titrations will be essential to identify concentrations of these agents at which their anti-HIV effects are dissociable from their chemokine-inhibitory activity. The latter parameter is of particular relevance because interruption of the chemokine receptor–ligand interaction may have an impact on normal lymphocyte trafficking and immune responses.

Small molecule inhibitors

Small molecules offer several potential advantages over protein-based approaches because they can be easily synthesized and rationally designed to exhibit improved oral bioavailability. Design and screening of these types of inhibitors are active areas of investigation and offer exciting alternative strategies to inhibit HIV entry. The biopharmaceutical industry has already had substantial experience in successfully targeting members of the seven-transmembrane receptor family such as those involved in autoimmune and inflammatory disorders. This previous experience is likely to hasten the discovery and development of molecules relevant to HIV infection. Two recent reports[55,56] describe the feasibility of using a small molecule to block HIV entry. Both describe the anti-HIV activity of AMD3100, a member of the heterocyclic family of compounds called bicyclams. AMD3100 was first reported in 1992 to inhibit replication of T-tropic HIV isolates, but its mechanism of action was unknown[57,58]. The more recent studies demonstrate that AMD3100 binds to CXCR4 and inhibits the interaction between X4 envelope and CXCR4. The drug also inhibits the interaction between CXCR4 and its natural ligand, SDF-1, preventing its function as both an HIV-1 co-receptor and a CXC chemokine receptor. Previous *in vivo* studies of AMD3100 in the SCID-human mouse model have shown that it is nontoxic, and that efficacious steady state levels of drug (100 ng/ml plasma) can be maintained by subcutaneous injection or implantable minipump administration[59]. Although these preclinical and animal studies raise hope about its therapeutic use, its poor oral bioavailability dampens enthusiasm that AMD3100 will reach the clinic.

In drug discovery, early studies to determine bioavailability and toxicity in animal models will be important predictors of clinical efficacy. Although the effect of down regulating CXCR4 in adult humans is unknown, the observation that ablation of SDF-1 expression in transgenic mice resulted in an embryonic lethal phenotype emphasizes a careful and deliberate interpretation of the data along the pathway of drug development. Still, the identification of small molecules like AMD3100 that are capable of interrupting the interaction of HIV-1 with CXCR4 establishes a process for the discovery and development of additional HIV-1 antagonists, as well as for other chemokine receptor functions. If such inhibitors of membrane fusion can be developed that are easy to ingest and exhibit limited toxicity, they may ultimately prove to be valuable contributors to therapeutic drug cocktails, which are currently comprised only of drugs that inhibit HIV-1 reverse transcriptase and protease enzymes.

The road ahead: Laboratory to clinic

Almost two years after the discovery of chemokine receptors as HIV co-receptors, the pace of scientific progress in this area remains relentless and the challenges formidable. Although CCR5 and CXCR4 are the two primary HIV-1 co-receptors, the growing number of co-receptors accessible to the diverse HIV strains circulating in the human population poses a concern. Chemokine receptors constitute a relatively invariant host target; however, the inherent plasticity of the virus and its capacity to use alternative co-receptors complicate the design of therapeutic strategies. This challenge is reminiscent of that seen in the development of current antiretroviral agents. When used as monotherapies, current antiretrovirals are inevitably associated with the selection of viral variants that resist the antiviral agent. It is now clear that only the use of multiple antiretroviral agents targeting two critical, but distinct, enzymes in the viral life cycle can have a lasting impact on viral load. An insight from these observations is that strategies targeting chemokine receptors are likely to succeed only as potential adjuncts to conventional antiretroviral therapy, rather than stand-alone therapies.

Strategies to inhibit viral spread by blocking entry of R5 viruses are likely to have their greatest impact when they are employed soon after infection, during a period of high viral homogeneity[60]. In chronically infected individuals, approaches that prevent viral use of CCR5 may impose sufficient selective pressure to drive HIV toward the more virulent R5X4 and X4 phenotypes. The latter viruses emerge in approximately 50 percent of patients late in the course of infection and are often associated with a rapid disease course[61]. Although the biologic role and specific contributions of the individual co-receptors to the disease process remain unknown, the ability of HIV to mutate and to escape from co-receptor antagonists targeting CCR5/HIV interactions implies that it is prudent to consider strategies to block multiple co-receptors for a significant anti-viral effect *in vivo*.

To optimize inhibitor design, additional basic knowledge of the molecular interactions between envelope and chemokine receptors as well as the interactions of the chemokine receptors with their natural ligands will be crucial. It will also be essential to quantitate the breadth of reactivity of potential anti-chemokine receptor reagents against a diverse panel of primary viral isolates[48]. Presently, the breadth and potency of potential therapeutic agents against a minimally passaged panel of primary viral isolates is the best laboratory surrogate test for predicting clinical efficacy.

The war on AIDS is far from over. An effective and cheap preventive vaccine is essential to prevent the expanding global spread of HIV/AIDS. The new information on HIV co-receptors and entry has several implications for vaccine design. These were recently reviewed[62] and will only be briefly summarized. The evidence that transmission of HIV is mainly restricted to R5 strains during person-to-person transmission, coupled with the observation that individuals homozygous for a deletion of CCR5 are highly resistant to infection with R5 viruses, argues effectively that candidate envelope-containing vaccines should focus on the R5 phenotype. Moreover, the finding that all M-tropic strains of HIV-1 irrespective of their geographic clade use

CCR5 as their predominant co-receptor during viral entry[12,63,64] offers hope that the presence of multiple genetic subtypes may not be a formidable hurdle to vaccine development.

If the sequence of events in viral entry is conserved across HIV-1 genetic subtypes, then envelopes from all HIV-1 strains are likely to share conserved features for CD4 and CCR5 or CXCR4 binding. Therefore, studies to identify and stabilize these conserved structural features may have a significant impact on HIV vaccine design. Of relevance to this latter point is the observation that some envelope glycoproteins of HIV-1 (ref. 65), HIV-2 (ref. 36) and SIV (ref. 66), as well as all the published FIV isolates[67] can use chemokine receptors independent of CD4 for viral entry suggesting that these proteins may already constitutively express a post-CD4 binding motif. Whether these structures can be incorporated into an effective vaccine, and whether they will be accessible to immune recognition are issues for future investigation.

In an era when antiviral treatments challenge patients to adhere to complicated drug regimens and an HIV vaccine is still not on the immediate horizon, the development of novel strategies or vaccines that focus on HIV entry and transmission will come none too soon. By providing new targets for drug development and vaccine design, these findings may contribute to the development of the first generation of HIV therapeutic and preventive measures that focus on the interaction of HIV with host proteins.

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