HIV-1 entry – an expanding portal for drug discovery

Wade S. Blair, Pin-Fang Lin, Nicholas A. Meanwell and Owen B. Wallace

The advent of highly active antiretroviral therapy (HAART) – combinations of protease and reverse transcriptase inhibitors – provided a potent and clinically effective method of suppressing viral load in HIV-1-infected individuals. However, although initially successful, a broader clinical experience has revealed limitations in this therapeutic regimen, with up to 40% of treated individuals ultimately failing to sustain control over viral replication. Significant advances in understanding the process by which HIV-1 enters host cells have brought into clear focus a target for drug discovery not represented in the current clinical armamentarium. In this article, the mechanism of HIV-1 entry is reviewed in the context of representative antiviral agents that interfere with key steps in this process.

Clinical control of HIV-1 infection has evolved to the current triple drug regimens that comprise combinations of inhibitors of the two viral enzymes reverse transcriptase and protease. Although initially dramatically successful at reducing viremia and prompting suggestions that long-term therapy might lead to a cure[1], recent developments have provided a more sobering picture of the nature and challenge of HIV-1 infection[2]. Consistent and long-term clinical benefit from triple therapy remains elusive owing to intolerance, non-compliance with drug dosing schedules and, most ominously, the development and transmission of resistant viruses[3]. Eradication of the infection appears unlikely because of the persistence of infection in reservoirs with low turnover rates[4]. As a consequence, the search for anti-HIV agents continues unabated, with renewed emphasis placed on novel targets in an attempt to increase the repertoire and efficacy of clinically available drugs.

Although the HIV-1 entry process was one of the earliest mechanisms examined as a target for therapeutic intervention, progress was difficult and success elusive. However, the elucidation of coreceptors that facilitate virus entry by triggering fusion, and the recently solved X-ray crystal structure of elements of the HIV-1 envelope glycoprotein gp120, have provided crucial new insights into the mechanism of HIV-1 entry and afforded significant new opportunities for HIV-1 drug discovery. In addition, HIV-1 entry has been validated as a clinically relevant target, with successful proof-of-principle studies conducted with T20 (Trimeris, Durham, NC, USA), a peptide based on elements of gp41, which interferes with the membrane fusion process at the later stages of HIV-1 entry[5]. In this article, we summarize the HIV-1 entry process, highlighting potential new opportunities for drug discovery and discussing representative inhibitors, with emphasis accorded to those compounds that have been advanced into clinical trials (Table 1).

The process of HIV-1 entry can be conveniently discussed in the context of three sequentially distinct steps:

- Attachment of the virus to host cells
- Interaction of the virus with coreceptors
- Fusion of the virus and the host cell membranes (Fig. 1).

However, it needs to be emphasized that this is an oversim-

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 1359-6446/00/$ – see front matter PII: S1359-6446(00)01484-7

Table 1. HIV-entry inhibitors under development

Compound	Type	Target	Mode of drug administration	Clinical phase	Company
PRO542	Protein	Gp120	Intravenous	I/II	Progenics (Tarrytown, NY, USA)
FP21399	A bis-azo dye derivative	Gp120	Intravenous	II	Lexigen (Lexington, MA, USA) (the US subsidiary of Merck KGaA, Darmstadt, Germany)
Zintevir	Oligonucleotide	Gp120	Intravenous	II	Aronex (The Woodlands, TX, USA)
PRO2000	Polymeric sulfonate derivative	CD4	Topical gel	I/II	Procept (Cambridge, MA, USA)
SPC3	Peptide	GalCer or post-CD4 binding	Intravenous	II	Columbia Labs (Miami, FL, USA)
Dextrin 2-sulfate	Polymeric poly-sulfonate derivative	Accessory receptor	Intraperitoneal Topical	II/III I/II	ML Laboratories (Warrington, UK)
TAK779	Quaternary ammonium derivative	CCR5	Subcutaneous	Preclinical	Takeda (Osaka, Japan)
AMD3100	Bicyclam derivative	CXCR4	Intravenous	I/II	AnorMED (Langley, British Columbia, Canada)
T20 (pentafuside)	Peptide	Gp41	Intravenous, subcutaneous	II	Trimeris (Durham, NC, USA)

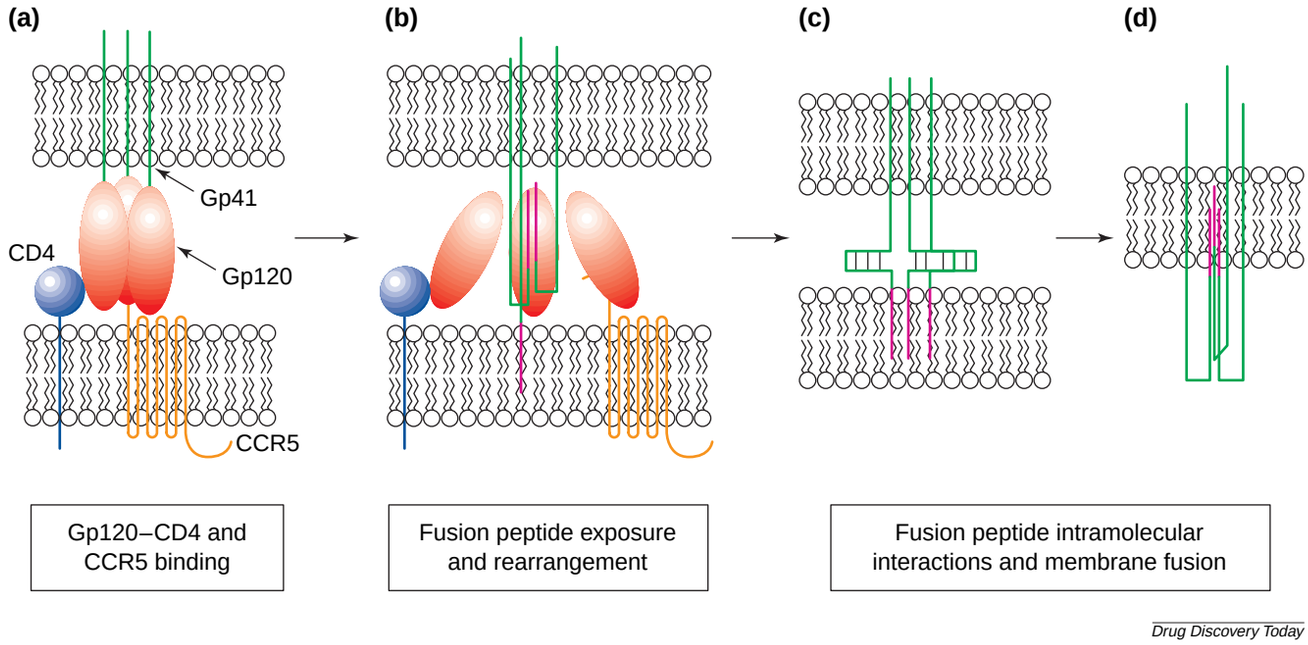

Figure 1. *A model for HIV-1-induced cell fusion. The HIV envelope on the surface of virions (native) is composed of a trimeric gp120 (shown in green)–gp41 complex. In such a complex, the gp41 fusion peptide (not shown) is buried. The binding of the glycoprotein gp120 to CD4 results in conformational changes that induce exposure of the gp120 V3 (third hypervariable region) loop that subsequently interacts with structural elements of appropriate coreceptors, depicted in this example as the chemokine receptor CCR5 (a). This interaction triggers conformational changes in gp41, unmasking the fusion peptide and facilitating its insertion into the host cell lipid bilayer (b). Intramolecular interactions between the C- and N-peptide regions of gp41 result in a hairpin configuration that leads to juxtaposition of the host cell and viral membranes (c) before fusion. The events mediating the final steps of membrane fusion are unresolved, but the final outcome (d) is such that the fusion peptide and the transmembrane domain of gp41 are present in the same lipid bilayer.*

plification because each of these steps represents the aggregate of a more complex series of biochemical events. Virion attachment is mediated by the specific binding of gp120 to the cellular receptor CD4, although it is also facilitated by non-specific interactions between envelope proteins and other cell-surface molecules such as heparan sulfate proteoglycan, galactosyl ceramide, mannose receptors and adhesion molecules[6]. The interaction of gp120 with CD4 results in the exposure of domains of gp120 that subsequently interact with one of several cell type-specific coreceptors, leading to destabilization of the gp120–gp41 protein complex. As a consequence, the gp41 subunit undergoes a conformational rearrangement, exposing the hydrophobic fusion peptide that inserts into the host cell membrane and initiates the fusion process (Fig. 1)[7–9].

Attachment: inhibitors of the gp120–CD4 interaction

Crystallographic studies of gp120–CD4

HIV-1 particles are enveloped in a host cell-derived lipid bilayer, which contains the virus-encoded envelope glycoprotein complex gp120–gp41 that is required for the attachment of virions to target cells and the subsequent fusion of viral and cellular membranes. The gp120–gp41 complex also promotes the fusion of infected cells with uninfected neighboring cells, resulting in syncytium formation, and is the principal determinant of viral tropism, antigenicity, pathogenicity and HIV-induced immunosuppression and neurotoxicity. Although expressed on the membrane as a complex of two proteins, gp120–gp41 is initially synthesized in infected cells as a 160 kDa protein that is cleaved by a cellular protease to generate the mature and functional exterior envelope glycoprotein complex. Proteolysis generates the fusion peptide at the N-terminus of gp41, which also contains a membrane-spanning sequence and a long cytoplasmic tail to anchor it into the membrane. Gp120 remains attached to gp41 through a network of non-covalent interactions located in both the N- and C-termini.

The gp120 protein comprises five variable regions, designated V1 through V5, which are exposed on the protein surface and are interspersed with five conserved regions. The gp120–gp41 complex is trimeric in nature and it has been estimated that there are ~72 of these complexes on the membrane of a single HIV-1 virion[7]. Gp120 binds with high affinity (K_D in the low namolar range) to the host cell-surface glycoprotein CD4, the principal receptor for HIV-1, which is expressed on the surface of human helper T cells and monocytes/macrophages, the primary target cells for HIV-1 infection *in vivo*. A detailed understanding of this interaction has been afforded by the recently solved crystal structure of a trimolecular complex consisting of an HIV-1 gp120 core fragment, the V1/V2 domains of CD4 and the Fab fragment of a neutralizing monoclonal antibody (mAb) designated 17b (Ref. 10). These crystallographic studies have revealed that the gp120 core is composed of an inner and an outer domain joined by a β-sheet, referred to as the bridging sheet. The inner domain faces the envelope trimer axis and, presumably, gp41, whereas the outer domain is mostly exposed on the surface of the trimer. Importantly, the X-ray crystal structure shows that CD4 binds in a recessed pocket on gp120, which is formed by elements from both major domains and the bridging sheet. The deep cavity of this pocket, which is occupied by the aromatic side chain of Phe_{43} of CD4, is crucial for gp120 binding, and confirms earlier studies that identified this interaction as a potential target for inhibitors of HIV-1 virion attachment[10–12].

The development of antiviral agents that target gp120 presents a major challenge because of the genetic diversity inherent in this HIV-1 envelope protein, a factor that might be responsible for the poor potency often demonstrated by gp120 inhibitors against diverse clinical isolates. Because HIV-1 appears to retain infectivity over a wide range of CD4–gp120 affinities, the success of this approach will depend on developing agents with very high affinities for gp120, to ensure activity against a broad spectrum of clinical HIV-1 strains. However, the fact that the CD4-binding pocket in gp120 is composed of highly conserved amino acids suggests that compounds that target this site might be clinically effective against a wide spectrum of HIV-1 strains.

Soluble CD4 fragments and derivatives as HIV inhibitors

A logical attempt to develop clinically effective inhibitors of gp120-mediated HIV-1 infection evaluated soluble CD4 (sCD4) fragments generated from the extracellular domain of this receptor. The recombinant protein exhibited potent inhibitory activity against laboratory strains of HIV-1 without affecting human major histocompatibility complex (HLA) class II-specific T-cell interactions[13]. However, in Phase I/II clinical trials, sCD4 failed to demonstrate efficacy, despite achieving significant drug concentrations in serum[14]. It was subsequently shown that primary HIV-1 isolates required 10- to 1000-fold higher concentrations of sCD4 to block virus replication *in vitro* when compared with laboratory HIV-1 strains[14]. This has been attributed to a reduced affinity of sCD4 for the gp120 present on primary isolates, combined with an increased retention of gp120 on the virion surface when compared with laboratory HIV-1 strains[15].

A refinement of this approach has resulted in the identification of PRO542 (CD4–IgG2), which is a recombinant heterotetrameric fusion protein in which the V1 and V2

domains of human CD4 are fused with the constant region of human IgG2 (Ref. 16). Although PRO542 contains four gp120-binding sites and, consequently, has potent HIV-1 neutralizing activity, the protein exhibits weak activity against some primary HIV-1 strains. Nevertheless, PRO542 possesses several advantages over sCD4 that might make it a more successful clinical candidate. More specifically, the CD4–IgG2 protein is less likely to potentiate antibody-dependent enhancement of HIV-1 infection when compared with CD4–IgG1 and, importantly, the pharmacokinetic profile of PRO542 is superior, with a terminal half-life in rabbits up to 100-fold longer than sCD4. Progenics (Tarrytown, NY, USA) is currently developing PRO542 as a prophylactic agent for occupational or prenatal exposure to HIV-1 (Ref. 17). In Phase I/II clinical trials, single intravenous infusions of 0.2 to 10 mg kg^{-1} PRO542 were well tolerated and non-immunogenic, with no adverse events reported (J. Jacobson *et al. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*, 26–29 September 1999, San Francisco, CA, USA, abstract 323). As a consequence of a preliminary indication of antiviral efficacy in this study, multi-dose Phase I/II clinical trials are currently underway that will determine the efficacy and ultimate potential of this protein.

Small-molecule inhibitors of the gp120–CD4 interaction

The bis-azo dye FP21399 (Lexigen Pharmaceuticals Corp, Lexigen, MA, USA; Fig. 2), identified from a chemical library using a cell-based fusion assay, is active against a broad spectrum of HIV-1 strains including clinical isolates, with EC_{50} values of 0.03–1.8 μM (Ref. 18). Mechanism of action studies showed that FP21399 weakly inhibits CD4–gp120 binding (IC_{50} ≈5 μg ml^{-1}) and competes with the binding of a monoclonal antibody directed against the V3 loop of gp120, suggesting viral entry as a target[19]. Phase I clinical trials showed that intravenous administration of FP21399 was well tolerated by patients during the four-week treatment period and that 4 of the 13 treated patients displayed a decrease in viral load (B.J. Dezube *et al. 5th International Conference on Retroviruses and Opportunistic Infections*, 1–5 February 1998, Chicago, IL, USA, abstract 650). This promising result has led to the initiation of Phase II clinical trials with FP21399 designed to establish the viability of this compound as a clinically useful HIV inhibitor.

The 17-base G-quartet phosphothiorate nucleotide, zintevir (AR177, T30177; Aronex, The Woodlands, TX, USA) (Fig. 2) inhibits laboratory and clinical strains of HIV-1 infectivity in cell culture, with EC_{50} values of 0.12–3 μM (Ref. 20). Although it was proposed that inhibition of integrase might contribute to the antiviral activity of zintevir, more recent studies implicate the HIV-1 envelope protein as the primary target of zintevir in cell culture[21]. Zintevir has progressed successfully through a Phase I clinical study, where the necessary serum levels were achieved by intravenous infusion, and Phase II studies have now been initiated to establish antiviral efficacy[22].

Inhibition of HIV by agents that bind to CD4

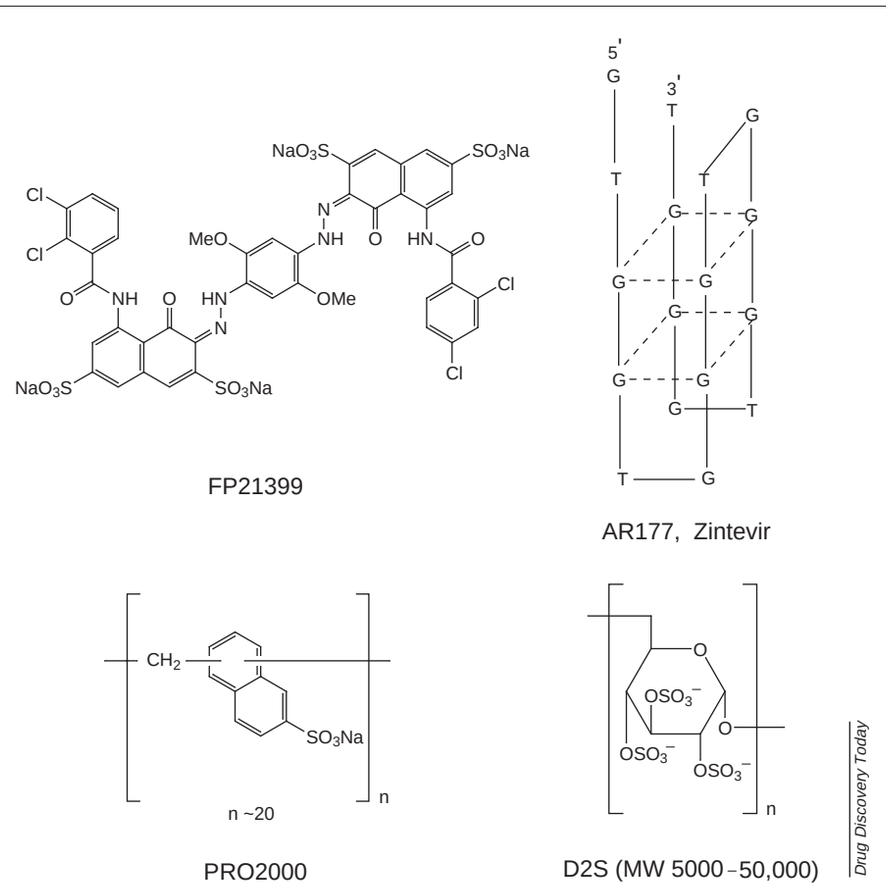

Figure 2. *The structure of several inhibitors of glycoprotein gp120–CD4 interactions.*

Agents that specifically target the host cell protein CD4 represent a plausible strategy to control HIV infection. However, because of the significant inherent risk of this approach, including possible deleterious effects on immune function, it has been neither aggressively nor broadly pursued. Nevertheless, Procept (Cambridge, MA, USA) is examining this concept in a clinical setting with PRO2000 (Fig. 2), a 5 kDa naphthalene sulfonate polymer that binds to CD4 and interferes with gp120–CD4 binding (IC_{50} ≈0.4 μg ml^{-1}). PRO2000 inhibits HIV-1 infection in a wide range of cell types (EC_{50} ≈13 μg ml^{-1})[23], but lacks specificity because it also exhibits activity against herpes viruses and *Chlamydia trachomatis*[24]. PRO2000 is currently in development as a topical preventative of HIV-1 infection, administered as a 4% gel, which was well tolerated in initial clinical studies, and the compound is expected to advance into Phase II trials (Procept, Press Release, 11 March 1999).

Interference with the function of attachment factors

A variety of anionic polymers that bind to proteins that facilitate attachment of HIV-1 to target cell membranes (e.g. galactosyl ceramide or heparan sulfate) have demonstrated antiviral activity[25]. However, it is likely that such inhibitors are more suited for topical rather than oral prophylactic use, given the lack of target specificity and the pharmacokinetic issues associated with compounds of this polar nature. Representative is dextrin 2-sulfate (D2S) (Fig. 2), a synthetic derivative of an enzymatic hydrolysis product of starch that consists predominantly of (1,4)-linked anhydro-D-glucopyranosyl units. Those derivatives possessing a sulfate moiety on the 2-position of the glucan ring exhibit the best combination of anti-HIV-1 activity (IC_{90} ≈69 μg ml^{-1} against primary isolates) and low anticoagulant activity[25]. ML Laboratories (Warrington, UK) is currently evaluating D2S in Phase II/III trials as Viraldon, an intraperitoneally administered agent for HIV patients in the later stages of the disease, and in Phase I/II clinical studies as Emmelle, an intravaginal gel formulation for the prevention of heterosexual HIV transmission[26].

Another agent that interferes with attachment factor function is SPC3, a synthetic, multi-branched peptide derived from a conserved sequence of the gp120 V3 loop. SPC3 displays eight GPGRAF motifs, radially branched on an uncharged polylysine core matrix, a structural feature that enhances ligand avidity. This peptide potently inhibits HIV-1 and HIV-2 infectivity in CD4-positive T cells (EC_{50} ≈50 nM) and HIV-1 infection of CD4-negative human colon epithelial cells (EC_{50} ≈1.8 μM). Mode-of-action studies have established that inhibition of HIV-1 entry into CD4-positive lymphocytes occurs at a step subsequent to virion binding, while infection of CD4-negative cells is inhibited at the step of galactosylceramide-mediated HIV-1 attachment[27]. Currently, SPC3 is being evaluated in Phase II clinical studies sponsored by Columbia Laboratories (Miami, FL, USA)[28].

Inhibition of gp120 and coreceptor interactions

Although the interaction of gp120 with CD4 is an obligatory step for efficient infection of cells by HIV-1, it became clear shortly after the discovery of CD4 that an additional receptor(s) or coreceptor(s) was required for HIV-1 entry and infection. The identity of these coreceptors was elucidated only recently, when it was demonstrated that several members of the seven transmembrane (7TM)-spanning, G-protein-coupled chemokine receptor family could function in this capacity[29]. In fact, differential expression of chemokine receptors on the surface of CD4-positive cells determines HIV-1 tropism in almost all cases. For example, CXCR4 was the first HIV-1 coreceptor identified and is utilized by syncytium-inducing (SI) primary HIV-1 isolates and laboratory-adapted HIV-1 strains. Such viruses replicate efficiently in primary T cells and immortalized T-cell lines and are referred to as T-tropic or X4 viruses. By contrast, most non-syncytium-inducing (NSI) HIV-1 primary isolates utilize the chemokine CCR5 receptor and replicate in macrophages and primary T cells, but not in most immortalized T-cell lines. CCR5-utilizing viruses are referred to as macrophage (M)-tropic or R5 viruses. Following the discovery of CXCR4 and CCR5, many additional chemokine receptors have been shown to mediate HIV-1 entry in cell culture but the relevance of these coreceptors *in vivo* remains controversial.

Coreceptor recognition

Current models predict that HIV-1 entry is initiated by the interaction of gp120 with CD4, which results in exposure of gp120 domains, sequestered in the native form, that recognize the appropriate coreceptor proteins. In support of this hypothesis, the binding of gp120 to coreceptor molecules can be significantly enhanced by the presence of CD4 (Refs 30–32), and several HIV-2 strains can be induced to infect CD4-negative cells by pretreatment with soluble CD4 (Ref. 33). In addition, the affinity of mAbs that recognize epitopes in the putative coreceptor-binding region of gp120 is significantly enhanced by CD4 binding[30–32].

Early studies using chimeric HIV-1 envelopes demonstrated that coreceptor specificity was determined primarily by sequences in the V3 region of gp120 (Refs 34,35). However, recent mutagenesis and antibody neutralization studies combined with structural analysis of the gp120–CD4–mAb ternary complex revealed that other regions in gp120 are also involved[10,12]. HIV-1 domains pre-

dicted to interact with coreceptors include the highly conserved stem of the V1/V2 element, proximal to the base of V3, and sequences on the surface of the bridging sheet. The selection in tissue culture of CD4-independent HIV-1 and HIV-2 variants[33,36] demonstrates that productive infection can be established in the absence of CD4 binding. These HIV variants contain alterations in their respective envelopes that presumably result in the stabilization of an exposed coreceptor-binding domain.

HIV-1 coreceptors as antiviral targets

Based on the current level of understanding, it is anticipated that antiviral therapies that target the CCR5 or CXCR4 receptors would be effective at different stages of disease in an infected individual. NSI/R5 HIV-1 isolates are the most common viruses transmitted and predominate during the asymptomatic phase of the disease, whereas SI/X4 HIV-1 isolates are more prevalent in the later stages of the disease. The emergence of CXCR4-utilizing HIV-1 variants in infected individuals corresponds to the switch in viral phenotype from NSI to SI, and is associated with disease progression[37]. Consequently, antivirals targeting CCR5 would be expected to be most effective for early intervention therapies, whereas antivirals targeting CXCR4 should provide more effective therapy for patients in the later stages of AIDS.

HIV-1 coreceptors represent attractive and potentially tractable targets for therapeutic intervention in view of the number of marketed drugs that modulate 7TM, G-protein-coupled receptors. However, as a strategy for antiviral drug discovery, several potential issues have emerged that advocate some caution. One prominent concern is the potential for HIV-1 to utilize multiple coreceptors for infection, providing a mechanism for switching between different HIV-1 strains that might allow rapid escape from therapies targeting one or two coreceptors. Although a legitimate concern, currently available data suggest that this might not be a serious issue. Because individuals homozygous for a defect in CCR5 expression are refractory to HIV-1 infection[38], alternative coreceptor utilization by HIV-1 does not appear to be a significant factor in the establishment of an infection and, therefore, might not be a significant factor in escape from antiviral therapies directed against CCR5 (Ref. 39). Consistent with this, infection of primary T cells by HIV-1 isolates capable of utilizing CCR5 or other coreceptors (Bonzo) was completely inhibited by AOP-RANTES (aminooxypentane-regulated on activation, normal T-expressed and secreted), a CCR5 antagonist, or inhibitors of CCR5 and CXCR4 used in combination[39]. Consequently, small-molecule antagonists of CCR5 or CXCR4 might indeed prove to be effective components of HIV-1 combination therapies. However, a related concern is the possible selection for more virulent X4 viruses in the presence of CCR5 inhibitors, thus resulting in accelerated disease progression[40]. Such a phenomenon might dictate the administration of CCR5 and CXCR4 inhibitors in combination.

An additional issue relating to the targeting of cellular receptors for antiviral therapy is the potential for deleterious effects resulting from perturbation of the natural function of the cellular target. This appears to be less of a problem for CCR5 (Ref. 38), but antiviral compounds that interfere with CXCR4 function might produce some serious problems because deletion of CXCR4 or stromal cell-derived factor 1α (SDF-1α) expression in mice resulted in an embryonic lethal phenotype, suggesting that CXCR4 function is essential in mammals[41,42]. Obviously, resolution of these issues awaits the clinical assessment of potent and selective chemokine inhibitors.

Chemokine-derived inhibitors of HIV-1 infection

The β-chemokines macrophage inflammatory protein 1α (MIP-1α), MIP-1β and RANTES, which are natural ligands for CCR5, are potent inhibitors of HIV-1 replication in tissue culture, an observation that set the stage for the discovery of CCR5 as a functional HIV-1 coreceptor[29]. The mechanism of chemokine-mediated inhibition of HIV-1 replication appears to be bimodal, with inhibition of virus entry resulting from direct competition for receptor binding, as well as chemokine-mediated downregulation of HIV-1 co-receptors[29]. Several modified β-chemokines have been identified that are potent inhibitors of HIV-1 replication *in vitro*, and which have been useful in elucidating fundamental aspects of the biochemical pharmacology of coreceptor function. However, there are several significant obstacles to overcome in terms of developing chemokines or chemokine-derived molecules into anti-HIV therapeutics. These include problems associated with drug delivery of polypeptide drugs, issues associated with the production of deleterious side effects owing to ligand–chemokine receptor promiscuity and the potential for interference with the inflammatory response, and the reported potential of chemokines to enhance viral replication under certain circumstances[43]. Consistent with some of these concerns, BB10010 (British Biotech, Oxford, UK), a MIP-1α derivative, failed to affect HIV-1 viral load or CD4 counts in Phase I clinical trials owing to inadequate plasma concentrations[44].

Peptide inhibitors of coreceptor binding

Several peptide-derived inhibitors of CXCR4-mediated HIV-

1 infection have been described, of which T22 (Fig. 3), an 18 amino acid analog of polyphemusin II derived from horseshoe crab red blood cells, is the most prominent[45]. Both T22 and a derivative, T134 (Seikagaku Corp, Tokyo, Japan) can specifically inhibit infection by X4 HIV-1 strains and prevent SDF-1α–CXCR4 binding and the binding of a mAb (12G5) to the CXCR4 receptor[45–47]. Another peptide-derived inhibitor of CXCR4-mediated HIV-1 infection is ALX404C (NPS Allelix Corp, Toronto, Ontario, Canada), an oligocationic peptide consisting of nine D-arginine residues, which functions by competing with gp120 for CXCR4 binding, probably by interacting with the first and second extracellular loops of CXCR4 (Ref. 48). Although ALX404C was advanced to Phase I/II clinical trials, further development of this drug was not pursued.

Small-molecule inhibitors of HIV-1 coreceptors

Small-molecule inhibitors of chemokine receptors are beginning to emerge in both the patent and primary scientific literature as the role of these receptors in human physiology and pathophysiology is being explored and elucidated. NSC651016 (Fig. 3; Science Applications International Corp, Frederick, MD, USA and The National Cancer Institute-Frederick, Frederick, MD, USA), an analog of distamycin, was recently shown to interact with CCR5, CCR3, CCR1, CXCR4, but not CCR2 or CCR2b receptors[49]. NSC651016 exhibited antiviral activity against X4 HIV-1 laboratory strains (RF and IIIB), several clinical isolates, including AZT (3′-azido-3′-deoxythymidine, zidovudine)-resistant virus, HIV-2 and simian immunodeficiency virus (SIV). In addition, inhibition of HIV-1 infection mediated by CXCR4 or CCR5 was directly demonstrated[49]. However, the potency of NSC651016 in HIV-1 antiviral assays is not impressive (EC_{50} = 1.5–7 μM) and its chemokine receptor promiscuity will probably be a serious issue *in vivo*.

A more promising small-molecule inhibitor of CXCR4-mediated HIV-1 infection is the bicyclam AMD3100 (AnorMED, Langley, British Columbia, Canada) (Fig. 3), which is currently in Phase II clinical trials. AMD3100, a bis-tetraazamacrocycle, is a potent inhibitor of HIV entry (IC_{50} = 1–10 ng ml^{-1}) that specifically inhibits infection of X4 HIV-1 and HIV-2 strains but not R5 HIV-1 isolates[50,51]. This compound competes with the mAb 12G5 and SDF-1α for CXCR4 binding and inhibits SDF-1α-mediated signal transduction, although AMD3100 alone has no effect on signaling[51,52]. AMD3100 did not interfere with the binding of mAbs to CCR5, nor did it affect CCR5-mediated cell signaling, indicating selectivity for the CXCR4 receptor[51,52]. It is not yet clear whether AMD3100 competes directly for the gp120-binding site on CXCR4 or whether the compound prevents gp120 binding by inducing conformational changes in CXCR4 via an allosteric mechanism. Confirmation of CXCR4 as the molecular target of AMD3100 was provided by resistance studies showing that mutations in CXCR4 confer partial resistance[53], and that mutations in the HIV-1 envelope, including V3 loop sequences, are associated with a more complete resistance to this drug[54].

In Phase I clinical trials, AMD3100 was well tolerated at several doses, showing that CXCR4 antagonism might not necessarily be acutely toxic in humans (C. Hendrix *et al. 6th International Conference on Retroviruses and Opportunistic Infections*, 31 January–4 February 1999, Chicago, IL,

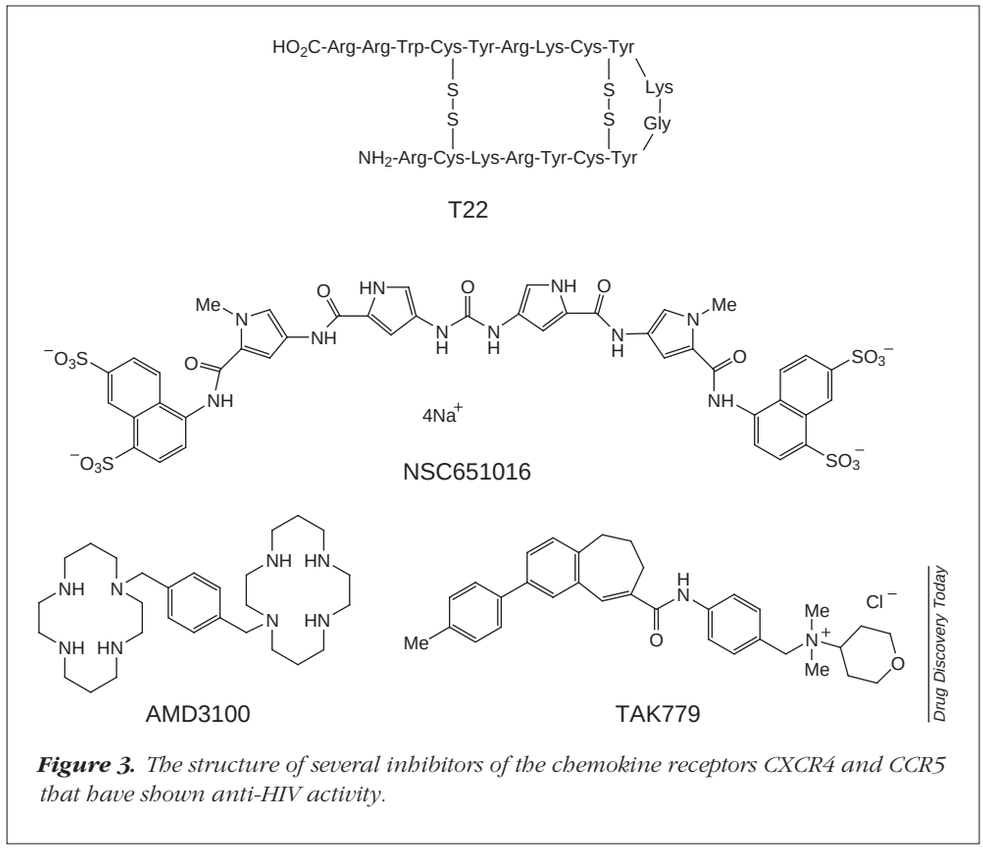

Figure 3. *The structure of several inhibitors of the chemokine receptors CXCR4 and CCR5 that have shown anti-HIV activity.*

USA, abstract 610). These data are encouraging, given that studies of CXCR4 knockout mice predicted the potential for significant problems. A major issue relating to the clinical utility of AMD3100 is its low oral bioavailability, necessitating that the compound be administered by injection, thus severely limiting its potential for long-term use. In addition, because AMD3100 is only effective against X4 but not R5 HIV-1 isolates, its antiviral efficacy is predicted to be limited to patients in the later stages of the disease. Nevertheless, AMD3100 is in the unique position of potentially establishing proof-of-principle in a clinical setting for the efficacy of HIV-1 coreceptor inhibitors.

Although there have been many patent applications filed claiming small-molecule CCR5 antagonists, to date, little has been published that confirms these structures as anti-HIV agents. This aspect is of particular importance because ligand recognition by the CCR5 receptor involves, in part, regions of the extracellular N-terminus and exposed loops between the membrane spanning domains. By contrast, receptor activation is thought to occur in a classic fashion by interaction of elements of the ligand with residues in the transmembrane domain, a mechanism analogous to that by which the C5a receptor is activated. Because the interaction of gp120 with the CCR5 protein is thought to be restricted to extracellular domains primarily responsible for ligand recognition, rather than signal transduction, CCR5 antagonists or agonists that bind only to the membrane-spanning region might not inhibit HIV-1 infectivity. However, compounds that will enable the investigation of these concepts are beginning to emerge, and the first small-molecule inhibitor of CCR5-mediated HIV-1 infection, TAK779 (Takeda Chemical Industries, Osaka, Japan; Fig. 3), was described recently[55].

TAK779 potently blocked the interaction of MIP-1α, MIP-1β and RANTES with CCR5 (IC_{50} ≈1 nM), inhibited CCR5-mediated Ca^{2+} signaling, but had no significant effect on RANTES–CCR1, eotaxin–CCR3, or thymus-and-activation-regulated chemokine (TARC)–CCR4 interactions. However, TAK779 is not completely selective for CCR5 because it inhibits the binding of monocyte chemoattractant protein (MCP-1) to CCR2b, although the 20-fold higher IC_{50} for CCR2 ligand-binding provides a reasonable margin of selectivity. While biochemical studies that would more precisely define the mechanism of inhibition have not been described, preliminary data indicate that TAK779 influences accessibility of specific residues located within the second extracellular loop of the CCR5 receptor (Y. Izawa *et al. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*, 26–29 September 1999, San Francisco, CA, USA, abstract 912).

Consistent with its receptor-binding profile, TAK779 inhibited the replication of several R5 HIV-1 strains, including clinical isolates (EC_{50} = 1–6 nM; EC_{90} = 7–27 nM), but not the replication of X4 HIV-1 strains (EC_{50} >20 μM) or HIV-2 (Ref. 55). The antiviral activity of TAK779 *in vitro* is reduced by less than ten times in the presence of 50% human serum and the compound exhibits a half-life of 8.7 h after intravenous administration to rats. Excellent lymph node penetration was also seen in rats with a substantially longer half-life of almost 23 h in this tissue (M. Baba *et al. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*, 26–29 September 1999, San Francisco, CA, USA, abstract 914). Animal toxicology studies with TAK779 are reportedly in progress as a prelude to clinical studies. However, the permanently charged nature of this molecule restricts drug delivery to parenteral routes of administration, and clinical evaluation will be by subcutaneous dosing.

Inhibitors of gp41 function

Functional aspects of gp41

The conformational changes induced in gp120 upon binding to CD4 and a coreceptor set the stage for gp41 to mediate the final stage of HIV-1 entry, membrane fusion. Although the precise sequence of biochemical events involved in the activation of gp41 and the complex process of fusion are not well understood, some insight can be drawn from influenza, which represents a paradigm for virus–host cell fusion mechanisms. The 12 residues at the N-terminus of gp41 comprise the fusion peptide, a sequence of hydrophobic residues essential for membrane fusion, which has significant sequence homology with the fusion peptides of other viruses, including influenza. In its native state, gp41 exists in a non-fusogenic conformation, in which the fusion peptide is buried within the envelope complex. By analogy with influenza, this might represent a metastable conformation, maintained in the presence of gp120 by a network of non-covalent interactions. The dual interaction of gp120 with cell-surface CD4 and a chemokine receptor results in the release of gp41 from these constraints. This enables the protein to adopt a more stable conformation in which the fusion peptide is projected away from the viral lipid envelope and into the host cell membrane, thus initiating the fusion process[8]. At this stage of the process, there appears to be little interaction between remote sequences of gp41 but, as fusion proceeds, specific helical regions of gp41 interact with each other, creating a hairpin-like structure that juxtaposes virus and host membranes. The crystal structure of a fragment of the final complex of the gp41 core[56–58] has been solved, and predicts that

the fusion-active hairpin exists as a trimer of helical bundles, in which three C-helices pack against a central N-peptide coiled coil. The structure reveals prominent contacts between the C-helices and the N-peptide coiled coil, and deep cavities within hydrophobic grooves formed by the N-terminal elements, which are potentially useful targets for inhibiting HIV-induced fusion[8,56].

Inhibitors of gp41-mediated membrane fusion

This step of the HIV-1 fusion process emerged as a potential drug target when it was discovered that a 38-amino acid peptide corresponding to residues 558–595 of gp160 (42–79 of gp41; Fig. 4), designated DP107 (T21), prevented infectivity in cell culture[59,60]. This finding has led to a greater understanding of the virus–host cell fusion process and the development of a new class of HIV-1 inhibitors[60]. A second peptide, DP178 (T20, pentafuside; Fig. 5), comprising a sequence of 36 amino acids from a domain (residues 643–678 of gp160; residues 126–161 of gp41) of gp41 proximal to the TM-spanning region and predicted to be helical, was subsequently found to be a considerably more potent antiviral agent, with an EC_{50} of ≈1 ng ml^{-1} in cell culture[60]. A series of biochemical studies[61,62] suggested that the two gp41 domains defined by T21 and T20 interacted during the fusion process, a hypothesis confirmed recently by crystallographic studies[56,57]. Available evidence is consistent with the hypothesis that T21 and T20 function by associating with their cognate sequences on gp41 after its conformational rearrangement, intercepting a transient intermediate and interfering with the intramolecular interactions that appear to be essential for completion of the fusion process[56,57,59–62]. T20-resistant HIV-1 variants selected in culture contain mutations that localize to the N-peptide region of gp41, thus confirming gp41 as the target[63].

A Phase I clinical trial conducted with T20 in a cohort of 17 HIV-1-infected individuals, of which seven were naive to antiretroviral therapy, has provided proof-of-concept for this mechanistic approach to HIV-1 inhibition in a clinical setting[5]. T20 was administered at doses of 3, 10, 30 or 100 mg kg^{-1} by intravenous infusion over a 20 min period every 12 h for 14 consecutive days without significant drug-related side effects. The two higher doses produced a significant reduction in plasma viral RNA by the end of the study period, with the 100 mg kg^{-1} twice-a-day dosing regimen being the more efficacious. A median 1.96-log fall in plasma viral RNA was measured by reverse transcription PCR (RT–PCR) methodology at the 100 mg kg^{-1} dose but increases in CD4 T-cell counts were variable and not statistically significant. In an extension of this study, T-20 was administered by subcutaneous injection at a dose of 50 mg kg^{-1} twice-a-day to 55 HIV-1-positive individuals who exhibited resistance to all three classes of HIV inhibitors as the result of prior exposure. This dosing regimen was combined with oral antiretroviral agents and, after 16 weeks, a reduction of at least 1-log in viral load was measured in 60% of the patients. T20 was well tolerated in this study and the results establish this new antiviral chemotype to be clinically beneficial in a difficult-to-treat patient population. However, although subcutaneous injection is a simpler dosing paradigm than intravenous infusion, convenient drug delivery remains a key challenge to the development of T20.

More recently, Trimeris (Durham, NC, USA) has identified a second peptidic HIV fusion inhibitor, T1249, that is claimed to be highly potent against a broad range of clinical isolates. Moreover, T1249 is reported to exhibit efficacy against T20-resistant virus and, following an investigational new drug (IND) application filing in April 1999, entered Phase I trials later in the year (*Reuters Medical News*, 14 May 1999). Trimeris has recently entered into an agreement with Roche (Basel, Switzerland) to develop HIV fusion inhibitors.

Attempts have already been made to identify molecules substantially smaller than T20 that function in a similar fash-

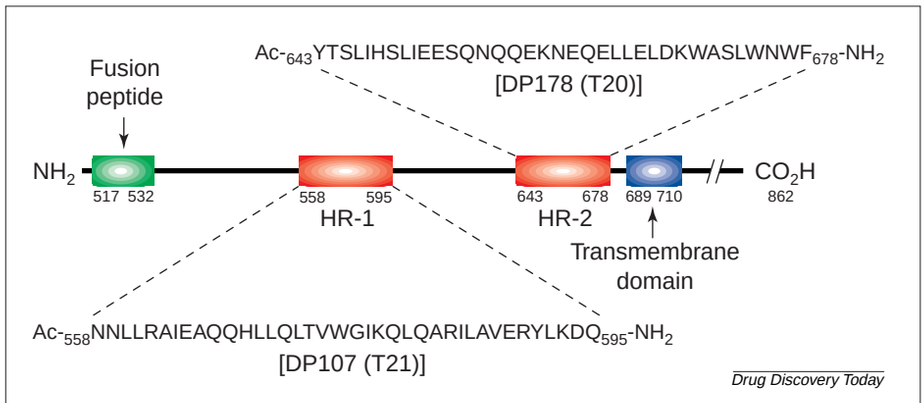

Figure 4. *A schematic depiction of gp41 that identifies the location of the fusion peptide, the membrane-spanning domain and the cytosolic region. The two heptad repeats from which peptide-based fusion inhibitors have been identified are designated as HR-1 (N-terminus) and HR-2 (C-terminus).*

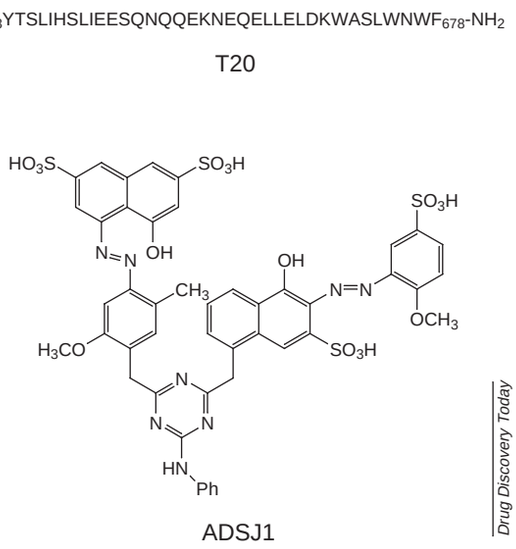

Figure 5. *The structure of ADSJ1, an inhibitor of gp41 function.*

ion[64–66]. Using the published crystal structures of the C- and N-termini of gp41, computer-based docking algorithms were used to select compounds from commercial sources that would bind to the deep hydrophobic cavities created by the association of the trimeric N-terminal helices that interact with the C-terminal sequences[64]. From 16 compounds predicted to bind, two inhibited HIV-1-mediated cell fusion and showed antiviral activity in cell culture, with ADSJ1 (New York Blood Center, NY, USA; Fig. 5) being the most potent. Preliminary mechanistic studies are consistent with ADSJ1 interfering with the interaction of the C- and N-terminal sequences of gp41 during the fusion process. Although ADSJ1 is the first non-peptidic inhibitor of this function of gp41, compounds with lower MW and reduced acidity will be required if clinically convenient and useful drugs are to be developed.

Conclusions

Inhibition of HIV-1 entry has re-emerged as an attractive opportunity for drug discovery based on several recent developments that have provided crucial new insights into the mechanism of viral entry. The identification of chemokine receptors as obligate cofactors for infection of host cells has very quickly resulted in the characterization of the first potent antiviral agents that will enable an evaluation of the potential utility of this target in a clinical setting. Given the high level of interest in this area, it is very likely that additional chemokine receptor ligands that demonstrate potent antiviral activity and improved pharmacokinetic properties will be disclosed in the near future. The description of the crystal structure of a gp120–CD4 complex has revealed highly conserved domains that represent potential opportunities for the design of drug candidates targeting a viral protein renowned for its inherent sequence diversity. Of particular significance has been the successful Phase I/II clinical evaluation of Trimeris' pentafuside (T20), establishing it as the prototype of a new class of HIV-1 fusion inhibitors. Although T20 suffers from drawbacks often associated with peptide-based drugs, attempts have already been made to identify small molecules that function in a similar fashion, and it might only be a matter of time before more drug-like candidates are discovered.

Advances in the understanding of virus entry in the context of HIV-1 replication have occurred at an opportune time, coinciding with a heightened appreciation of the challenge of HIV-1 infection as the limitations of current therapy are recognized and defined. Clinically effective HIV-1 inhibitors with novel modes of action and convenient dosing schedules will very quickly find an important place in the pharmacopoeia. The complexity of the virus entry process presents several opportunities for pharmacological intervention, offering considerable promise that an exciting new class of drugs, targeting unique elements of the virus life cycle, will emerge in the near future.

Acknowledgements

We thank H. Wang and C. Deminie for critical reading of the manuscript, and D. Morse for preparation of the manuscript.

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Collaboration…

Pharmacopeia (Princeton, NJ, USA) has entered into a research collaboration agreement with **Organon** (Oss, The Netherlands) to examine small-molecule antagonists of interleukin 8 (IL -8), one of the chemokines responsible for the recruitment of white blood cells to sites of injury leading to inflammation. Several active IL -8 antagonists have already been identified by Pharmacopeia by the screening of its small-molecule library against the IL -8 receptor. Under the terms of the agreement, Organon will provide funding for Pharmacopeia to chemically optimize the lead compounds they have already produced. Both companies will then work together to identify a clinical candidate for development and commercialization by Organon.

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