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Review

HIV fusion and its inhibition

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1. Introduction

Although HIV-induced AIDS was first described almost two decades ago, it continues to be a disease of colossal proportions. As of the end of 2000, the World Health Organization estimates that over 36 million people are infected with HIV (WHO, 2000). This disease has claimed nearly 22 million lives, and an estimated 16,000 additional people become infected with HIV each day. Although death rates have declined in the United States and Western Europe, infection rates in developed nations remain high with an estimated 40,000 new infections annually in the United States. Additionally, the epidemic continues in Sub-Saharan Africa and Southeast Asia, and is a rising concern in Russia and Eastern Europe and Latin America. The search for a vaccine against HIV infection and/or disease progression is the focus of intense research and monetary investment, but in the interim, effective antiviral therapy has prolonged quality of life for many of those infected with this virus.

The predominant mode of HIV transmission world-wide is by sexual contact. Both free virus and virus-infected cells can cross the mucosal epithelium and infect cells expressing the viral receptors CD4 and a chemokine receptor (either CCR5 or CXCR4) in the sub-mucosal tissue (Bomsel, 1997; Ullrich et al., 1998). Although both CCR5-expressing cells (monocytes, macrophages and dendritic cells, and activated T cells) and CXCR4-expressing cells (resting T cells) are present in this tissue, viruses isolated from acutely infected individuals are predominantly CCR5-utilizing viruses (macrophage- or M-tropic) indicating that CCR5-tropic (R5) viruses are most often responsible for establishing infection in a new host (Berger et al., 1999; Moore et al., 1997). The reasons for this are not completely clear. It could be due to the relative abundance of macrophages vs. T cells as well as the relative

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activation state of the two cell types in mucosal tissue, since activated cells can be more easily infected than resting cells. Regardless of the mechanism, it appears that infected macrophages and/or dendritic cells leaving the sub-mucosal tissue are primarily responsible for dissemination of HIV through the body (Bomsel, 1997).

Acute HIV infection is associated with high levels of virus in the bloodstream and is often, but not always, accompanied by an acute viral syndrome (headache, fever, night-sweats, malaise, lymphadenopathy). Anti-HIV humoral and cellular immune responses can most often be detected within two to four weeks after infection occurs. Their appearance is usually associated with a decline in plasma viremia to a 'set point' that remains detectable in the majority of individuals unless antiretroviral therapy is instituted. Resolution of acute infection is typically followed by an asymptomatic phase that can last for months to years, during which time the infected individual remains clinically healthy but may display steadily declining CD4 + T cell numbers in the peripheral blood. During this time, there is ongoing and typically robust viral replication with 1–10 billion viral particles produced each day (Comar et al., 1996; Kaufmann et al., 1998; Perelson et al., 1996; Young and Kuritzkes, 1999). The level of plasma viremia correlates directly with the rate of progression to AIDS and typically increases as the CD4 cell number falls. When the number of CD4 + T cells falls below 200 per microliter, individuals most often present with AIDS-defining illnesses (particularly opportunistic infections and wasting), although other complications including Kaposi's sarcoma and B cell lymphomas can be seen in individuals with higher CD4 cell numbers. During the decline into AIDS, an increase in plasma viremia is also common.

While not always the case (de Roda Husman et al., 1999; Li et al., 1999), a switch in chemokine receptor utilization from CCR5 to CXCR4 may herald the transition from asymptomatic infection to AIDS (Abebe et al., 1999; Bjorndal et al., 1997; Connor et al., 1997; Peeters et al., 1999; Richman and Bozzette, 1994; Schuitemaker et al., 1992; Shankarappa et al., 1999; Tscherning et al., 1998). It is not known whether the coreceptor switch contributes to or results from disease progression. However, evolution of the virus quasispecies speaks to ongoing viral replication in the face of a host immune response that leads, in the absence of therapy, to progressive immune dysfunction (Pantaleo et al., 1993; Reimann et al., 1994).

State-of-the-art therapy for HIV infection, also known as highly active antiretroviral therapy (HAART), is successful in reducing plasma virus levels ('virus load') and extending the asymptomatic phase of infection and quality of life for many infected individuals (reviewed in Matsushita, 2000). HAART currently targets two different stages in the virus life cycle, consisting of two or three reverse transcriptase inhibitors combined with at least one inhibitor of the viral protease (see Fig. 1). Reverse transcriptase inhibitors in the form of nucleoside analogs and non-nucleoside compounds prevent the production of a DNA copy of the viral RNA genome, which is required for productive infection of the target cell and subsequent virus replication. The infectivity of nascent virus particles depends on the cleavage of the viral Gag polyprotein into its functional components by the virus-encoded protease. HIV protease inhibitors specifically antagonize the viral protease, preventing the maturation of nascent particles into infectious virions.

In many people, this combination antiretroviral therapy results in a dramatic reduction in viral load decreasing the rate of CD4 cell decline and the progression to AIDS. However, the mutability of HIV often leads to the emergence of drug resistant viruses, particularly in individuals who are unable to comply with the often complex treatment regimens. Additionally, current antiviral agents may produce prohibitive side effects in some individuals, which can further limit their utility. Recent development of novel classes of antiviral agents, targeting a different part of the viral life cycle, namely virus binding and fusion, have generated considerable enthusiasm that additional and possibly more tolerable drugs and drug combinations will be forthcoming.

A collaborative research seminar was convened by The Macrae Group, New York, NY, USA, December 8–10, 2000, in St. Lucia, West Indies, bringing together experts in the field of HIV

fusion and fusion inhibitors and scientists and clinicians interested in the current state of development of fusion inhibitors as therapeutic alternatives in the treatment of HIV infection. The meeting was chaired by Dani P. Bolognesi, Trimeris Inc., USA, Scott M. Hammer, Columbia University, USA, Martin S. Hirsch, Massachusetts General Hospital, USA, and John P. Moore, Weill Medical College of Cornell University, USA. In addition to the chairs, invited speakers included: Richard Wyatt, Dana Farber Cancer Institute, USA; Robert Gallo, Institute of Human Virology, USA; Min Lu, Cornell University, USA; Eric Hunter, University of Alabama at Birmingham, USA; Sam Hopkins and Thomas J. Matthews, Trimeris, Inc., USA; William Olson, Progenics Pharmaceuticals, USA; Erik De Clercq, Rega Instituut, Belgium; Martin Springer, Merck and Co., USA; Robert W. Doms, University of Pennsylvania, USA; Bahige Baroudy, Schering Plough Institute, USA; Shibo Jiang, New York Blood Center, USA; Don Wiley, Harvard University, USA; and Claude Nash, Viropharma, USA. 'Late breaker' presentations were selected from abstracts submitted by Robert Staudinger, New York University School of Medicine, USA; Dominique Schols, Rega Instituut, Belgium; Donald Mosier, The Scripps Research Institute, USA; Anna Gazumyan, Wyeth Ayerst Research, USA; and Girija Krishnamurthy, Wyeth Ayerst Research, USA.

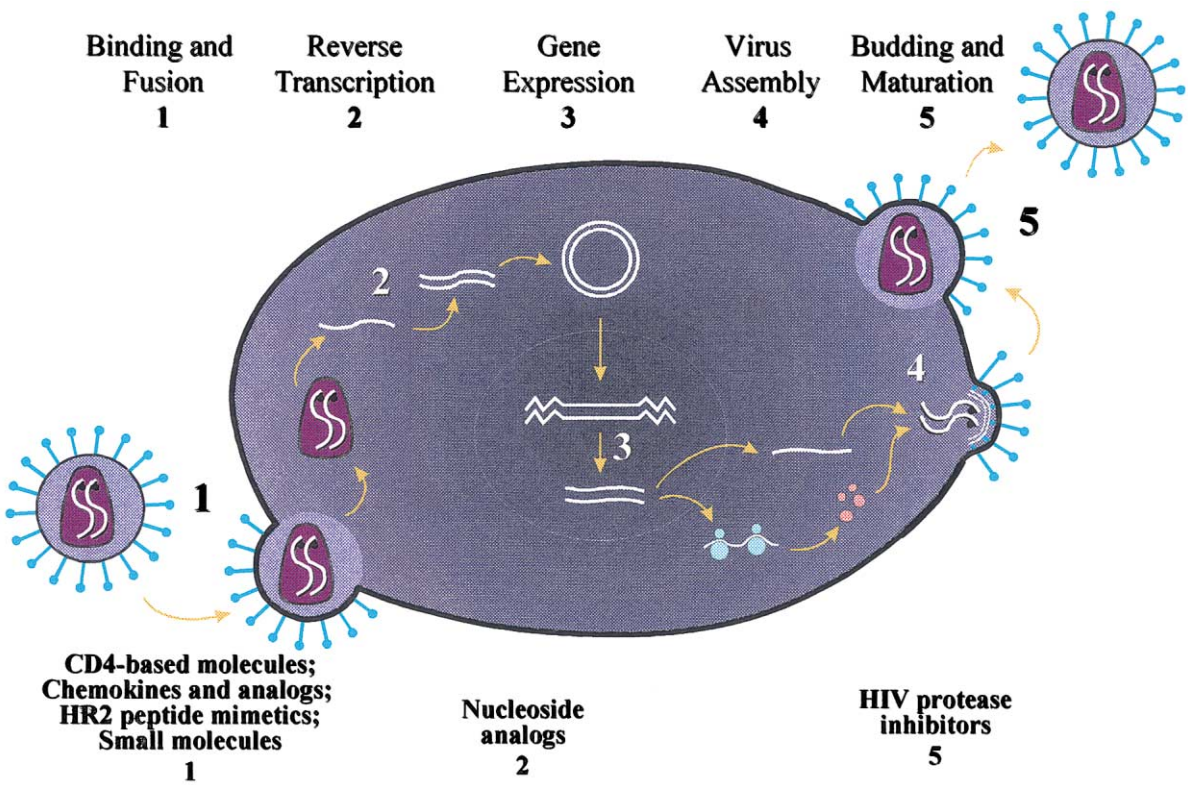

Fig. 1. Points of intervention in the HIV lifecycle. The lifecycle of HIV is depicted, from binding of the virion to CD4 and the chemokine receptor on the surface of the target cell, through reverse transcription, protein synthesis and processing, virus assembly, and budding and maturation of nascent virions. Current state-of-the-art therapy for HIV infection, termed highly active antiretroviral therapy or HAART, consists of a combination of drugs targeting reverse transcription (step 2 in the diagram) and virion maturation (step 5). These drugs act after HIV infection of target cells has occurred. The new class of therapeutic drugs discussed in this review target HIV binding and fusion with the target cell (step 1 in the diagram). Importantly, this point of intervention is before the virus infects the target cell.

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The intent of this article is to summarize what is known about the binding and fusion of HIV with the target cell, and provide an update on the modalities currently being explored to interrupt this process.

2. The fusion cascade

The HIV envelope glycoprotein (Env) is a type I membrane spanning protein that is synthesized and processed to its mature form by host cell machinery (for reviews, see McKeating and Willey, 1989; Wyatt and Sodroski, 1998). The polyprotein precursor is co-translationally transported into the endoplasmic reticulum where it is folded, oligomerized and glycosylated. En route to the cell surface, the carbohydrate moieties are modified and terminally sialylated in the Golgi apparatus, where the protein is also cleaved into its functional subunits, gp120 and gp41, by a furin-like protease. The 'native' Env expressed on the surface of cells and virions is an oligomeric protein, likely a trimer, composed of non-covalently associated heterodimers of gp120 and gp41. While the structure of the native Env 'spike' on the virion is poorly understood, the outer surface appears to be composed of variable domains and is heavily glycosylated (Kwong et al., 1998; Wyatt et al., 1998). These features contribute to the ability of this virus to evade detection and destruction by the immune system, as antibodies to carbohydrate structures are rare and antibodies made to the variable loops will select for 'escape variants' that will no longer be recognized by these antibodies (Wyatt et al., 1998). The gp120 and gp41 Env subunits each have specific roles in binding to and fusing with target cells. The binding sites for the receptors, CD4 and a chemokine receptor, are contained within gp120, and the gp41 protein appears to contain the elements required to mediate fusion. Thus, the fusion cascade involves a series of sequential, cooperative events mediated by gp120 and gp41 and the interactions between these two proteins.

While HIV can attach to the cell surface by interaction with a variety of molecules (Geijtenbeek et al., 2000; Mondor et al., 1998), binding of the HIV Env to CD4 is the initial step in the fusion cascade. Interestingly, the binding site for CD4 appears to be poorly exposed on the native Env of clinically relevant HIV isolates. This is suggested by the fact that while soluble gp120 molecules of primary isolates exhibit high CD4-binding affinity, primary isolate Envs in the native oligomeric conformation bind CD4 much less well (Brighty et al., 1991; Ivey-Hoyle et al., 1991). Binding of gp120 to CD4 induces conformational changes in Env, at least some of which are detectable by changes in antibody binding (Sattentau and Moore, 1993; Sullivan et al., 1998; Wyatt et al., 1995; Zhang et al., 1999). As mentioned previously, the native Env on virions is likely a trimeric molecule. Recent studies by Doms and colleagues indicate that at least two if not all three of the gp120 molecules in a trimer must be engaged by CD4 to produce the 'fusion-active' structure in this Env (reviewed in Doms, 2000). The conformational changes in gp120 include movement of variable loop regions (V1/V2 and V3) and result in the exposure and/or formation of a 'bridging sheet' between the inner and outer domains of gp120 (Kwong et al., 1998; Wyatt et al., 1998). A recent study has demonstrated that CD4 binding significantly reduces the flexibility of gp120, consistent with the formation of the ordered bridging sheet (Myszka et al., 2000). The bridging sheet contains highly conserved amino acids that constitute the conserved chemokine receptor binding site on the gp120 core (Kwong et al., 1998; Rizzuto and Sodroski, 2000; Rizzuto et al., 1998; Wyatt et al., 1998). Absence of this conserved structure on the native Env molecule may be another mechanism by which HIV avoids neutralization by the immune system, in that this receptor binding site may not even be formed until the virus is in close proximity to the cell membrane and bound to CD4.

Binding of gp120 to the chemokine receptor is an obligate step in the initiation of fusion and infection. Many different chemokine receptors have been shown to be permissive for fusion of virus and transfected target cells. However, only CCR5 and CXCR4 appear to be important for HIV infection of human lymphocytes (Edinger et al., 1999; Simmons et al., 1997; Zhang et al., 1998). Discovery that these seven-membrane-spanning-domain proteins were coreceptors for HIV infection seemed to answer the long-standing conundrum of HIV tropism. Now, with the isolation of primary HIV-2 and SIV strains that can fuse and infect CD4-negative/chemokine receptor-positive cells (Borsetti et al., 2000; Edinger et al., 1997; Gabuzda and Wang, 1999; Liu et al., 2000; Reeves et al., 1999), and the observation that FIV also uses CXCR4 (without CD4) as its receptor, it is hypothesized that chemokine receptors may be the primordial receptor for the lentiviruses (Poeschla and Looney, 1998; Willett et al., 1998). The ability of viruses to bind CD4 may be a more recent adaptation designed to protect the highly conserved chemokine receptor binding site from immune attack.

Interaction between gp120 and the chemokine receptor induces further conformational changes in Env, most importantly in gp41. The native structure of gp41 is also poorly understood, although several functional domains have been identified. The amino terminus in the ectodomain of gp41 consists of a stretch of hydrophobic amino acids resembling the fusion peptide of other fusogenic viruses (Bosch et al., 1989; Gallaher, 1987). The external portion of gp41 also contains two heptad repeat regions near the amino terminal (HR1) and carboxy-terminal end (HR2) of the gp41 ectodomain. In the most widely accepted model of the HIV Env-induced fusion cascade, the interaction of Env with its receptors exposes the fusion peptide, which then inserts into the target cell membrane making gp41 an integral protein in two different membranes. The three HR1 regions in the receptor-bound Env oligomer interact to form a coiled coil structure, and the three HR2 domains fold back like a hairpin into the hydrophobic grooves of the coiled coil to form a thermodynamically stable six-helix bundle. In the native Env structure, however, the structure of the gp41 ectodomain must be quite different. By analogy to the structure of influenza hemagglutinin, which has served as a model for fusion-inducing viral proteins (Skehel and Wiley, 1998), the hydrophobic fusion peptide may be sequestered in a hydrophobic environment (Skehel and Wiley, 2000). Data suggest that the HR1 coiled coil is not formed prior to receptor binding, as mutations in HR1 that abrogate coiled coil formation interfere with fusion but not with the production and oligomerization of Env (Wild et al., 1994). Likewise, formation of the six-helix bundle form of gp41 probably occurs subsequent to CD4 binding, as an HR2 peptide mimetic binds to CD4-triggered Env and prevents fusion (Furuta et al., 1998; Melikyan et al., 2000). Thus, prior to CD4 binding the HR1 and HR2 domains must be held apart in a configuration quite different than the six-helix bundle associated with the fusion process. Because the gp120–receptor interaction initiates the fusion cascade, it has been hypothesized that the gp120 protein provides a clamp to hold gp41 in the native Env conformation. Interaction of gp120 with the viral receptors alters the gp120–gp41 interaction, releasing gp41 to induce fusion (Doms and Moore, 2000).

The energy released during the formation of the six-helix bundle has been proposed to drive the thermodynamically unfavorable fusion between the viral lipid bilayer and the target cell membrane. In support of this hypothesis, a G652L mutation that results in increased fusogenicity (Cao et al., 1993) stabilizes the six-helix bundle structure by adding hydrophobic packing forces between HR2 and the trimeric coiled coil (Ji et al., 2000). Recent data suggest that CD4 binding alone may be sufficient to expose or induce the formation of the trimeric coiled coil of the gp41 molecule that signals the initiation of the fusion-active structure of the HIV Env (Furuta et al., 1998; Melikyan et al., 2000). However, while some laboratory-derived HIV-1 strains and primary HIV-2 and SIV strains can infect CD4-negative/chemokine receptor-positive cells, no HIV or SIV Env proteins identified thus far can induce fusion with target cells expressing only CD4. Thus, a function critical to the fusion cascade is mediated

by interaction of the chemokine receptor and gp120.

Virtually all the phases of the fusion cascade emphasize the important role of the interaction between gp120 and gp41 in this process. As described above, gp120 appears to hold gp41 in a metastable conformation in the pre-receptor-triggered Env. Gp120 then binds to the receptors, releasing gp41 to mediate fusion. Understanding the interaction between gp120 and gp41 has been hampered by the tenuous association of these two subunits in the Envs of laboratory-adapted HIV strains and the few primary isolates that have been studied. One tool that has been used to study the structure of the native Env glycoprotein is a soluble form of this molecule, produced by inserting a stop codon in the gp41 open reading frame just prior to the membrane spanning domain. Cells transfected with this truncated *env* secrete a 140 kD Env protein into the supernatant that contains gp120 and the ectodomain of gp41, some proportion of which is oligomeric (Earl et al., 1990; Edinger et al., 2000; Staropoli et al., 2000; Yang et al., 2000). However, the majority of the soluble gp140 in these studies is not cleaved and remains a continuous polyprotein rather than the non-covalently associated gp120 and gp41 as is found in the wild-type Env, due either to the high level of expression afforded by these transient expression systems or intentional removal of the proteolytic cleavage site (Earl et al., 1990; Edinger et al., 2000; Stamatatos and Cheng-Mayer, 1995; Stamatatos et al., 2000; Staropoli et al., 2000; Yang et al., 2000). Contact regions between gp120 and gp41 have been inferred by mutations that weakened or eliminated gp120 retention and Env function, or by differential antibody binding to monomeric gp120 and uncleaved, soluble gp140 (Binley et al., 2000; Cao et al., 1993; Kowalski et al., 1987; Sugiura et al., 1999; Wyatt et al., 1997). These methodologies have identified the extreme N- and C-termini of gp120 as associating with gp41, and portions of the N-terminus, the immunodominant regions near the cysteine mini-loop, and the middle of HR2 as contact regions in gp41. In the mutagenesis studies, however, disruption of the association could be due to indirect as well as direct effects. In the latter case, if uncleaved gp140 does not resemble native gp120, it is also likely that the gp120–gp41 interaction observed with this molecule may not precisely reflect that in the native Env (Binley et al., 2000). The interaction between gp120 and gp41 in the native Env is critical to Env function as it is most likely that this interaction sequesters the HR1 and HR2 regions from forming their highly stable, energetically favorable six-helix bundle.

3. Opportunities for intervention in the virus entry process

The process of the HIV envelope glycoprotein binding to and inducing fusion with a target cell offers many opportunities for intervention. As depicted in Fig. 2, modalities to inhibit virtually every step in this pathway are being explored (Chan and Kim, 1998; D'Souza et al., 2000; De Clercq, 2000; Doms and Moore, 2000; Moore and Stevenson, 2000). For the purpose of this discussion, we will define three categories of inhibitors according to their mode of action: (1) CD4 attachment inhibitors; (2) coreceptor interaction inhibitors; and (3) fusion inhibitors that target the viral gp41.

The initial interaction of gp120 on the virion with CD4 on the cell surface offers the highly conserved CD4 binding site on gp120 as a therapeutic target. Initial efforts to use a soluble form of CD4 to interrupt this process were thwarted by the rapid clearance of this molecule from the bloodstream and the lack of activity of soluble CD4 against primary isolates (Daar et al., 1990; Schacker et al., 1995). However, successful fusion of the gp120-binding portion of CD4 to the antigen-combining site of an immunoglobulin molecule has yielded a recombinant protein, PRO 542 from Progenics Pharmaceuticals, that combines the virus-binding and -neutralizing properties of the CD4 molecule with the long plasma half-life of the immunoglobulin molecule in a tetra-valent form (Allaway et al., 1995). PRO 542 appears to show anti-HIV activity in Phase I/II clinical trials (see below).

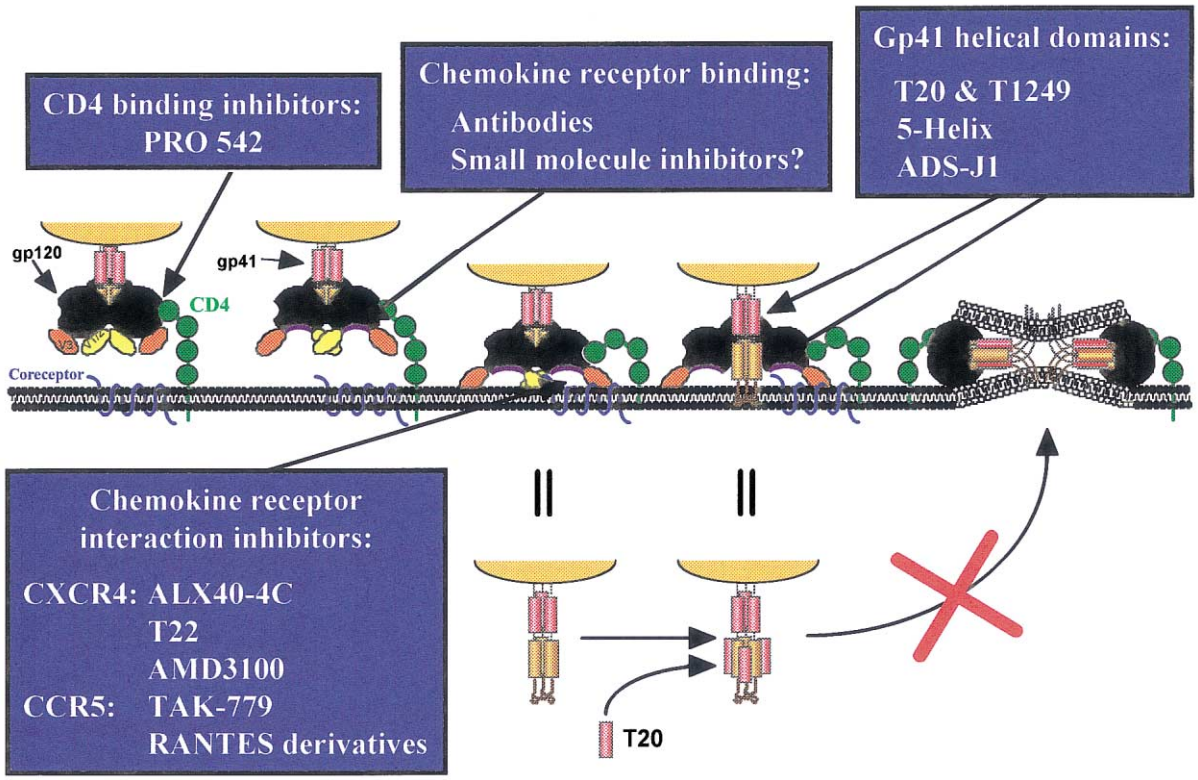

Fig. 2. Opportunities for intervention in the HIV fusion cascade. This schematic illustrates the current widely accepted model for the binding of the HIV envelope glycoprotein (Env) to its receptors, CD4 and a seven-membrane-spanning-domain coreceptor, and conformational changes that occur in Env leading to fusion with the target cell. As described in the text, the functional HIV Env exists as an oligomeric complex, likely a trimer of gp120–gp41 heterodimers, on the virion surface. While adhesion of HIV to the target cell may be facilitated by proteoglycan interactions (Mondor et al., 1998), the binding of the Env subunit gp120 to CD4 induces conformational changes in the Env that result in the exposure of previously masked regions of the Env protein. One such region is a highly conserved domain on the core of this protein believed to interact directly with a co-receptor, a member of the seven-membrane-spanning-domain G-protein coupled receptor family that serve as receptors for chemotactic factors. Interaction of Env with its receptors also induces conformational changes in the gp41 Env subunit, including exposure or formation of the trimeric coiled coil formed by the N-terminal heptad repeat region (HR1, depicted by the yellow cylinders) and insertion of the hydrophobic fusion peptide into the target cell membrane. The hydrophobic grooves formed by the HR1 coiled coil provide a tremendous attractive force for the C-terminal heptad repeat region (HR2, shown as pink cylinders) to form a six-helix bundle. The thermal stability of this six-helix bundle is such that its formation is believed to provide the force necessary to induce fusion of the viral and target cell membranes.

Several steps in the fusion cascade are suitable targets for pharmacologic intervention (listed in the boxes and detailed in the text). The CD4 and coreceptor binding sites on gp120 are highly conserved and are targets of neutralizing antibodies and perhaps other small molecule inhibitors. Both small molecule inhibitors and blocking antibodies directed at the coreceptors themselves have shown potent neutralizing activity. The gp41 fusion intermediate is also a target for inhibition by peptide mimetics (e.g. T20), small molecules and antibodies that bind this structure.

Shown below the fusion cascade schematic is a cartoon illustrating the mechanism of action of the current leading fusion inhibitor, T20. These figures show the predicted structure of the gp41 fusion intermediate (from which the gp120 molecules have been removed for the sake of clarity) with the formed HR1 coiled coil. Exogenous T20, which is a peptide mimetic of the HR2 domain, binds to the HR1 trimer preventing the formation of the six-helix bundle and thus preventing the fusion of the virus with the target cell. (Figure supplied by R.W. Doms, and previously published in Virology 276 (2000) 229–237.)

As described in the previous section, CD4 binding to Env induces the exposure or formation of the conserved chemokine receptor binding site on gp120, which is another target for intervention. A group of antibodies to CD4-induced epitopes bind to this chemokine receptor binding site (Rizzuto et al., 1998; Wyatt et al., 1995), and these antibodies are capable of neutralizing viruses whose Env proteins have been pre-bound to CD4 or which are naturally in the CD4-triggered conformation (Hoffman et al., 1999; Salzwedel et al., 2000). However, in the context of viral fusion, the Env binds CD4 on the cell and is tethered in close proximity to the chemokine receptors. Evidence suggests that antibodies to the chemokine receptor binding site on gp120 cannot readily gain access to this region to block the interaction with the chemokine receptor. It may be that small molecule inhibitors directed at this region of Env would be more effective in blocking the interaction between the Env and the chemokine receptor.

The chemokine receptors that serve as coreceptors for HIV infection belong to a family of G-protein coupled receptors (GPCR) with seven membrane spanning domains. These GPCR have traditionally been good targets for pharmaceutical intervention (Stott and Schild, 1996), and several agents have been described that inhibit HIV infection in vitro by binding to either CXCR4 or CCR5. CXCR4-directed inhibitors include ALX40-4C, T22, and AMD3100, all of which are cationic compounds suggesting that a charge-interaction is the crucial feature of binding of inhibitor, and possibly also of Env, to CXCR4. ALX40-4C is a small polypeptide, consisting of nine Arg residues stabilized by terminal protection and inclusion of D-amino acids (O'Brien et al., 1996). T22 ([$Tyr^{5,12}$, Lys^{7}-polyphemusin II) is a synthetic 18 amino acid derivative of polyphemusin, a horseshoe crab self-defense peptide (Tamamura et al., 1998). Both of these peptide inhibitors bind to the negatively charged surface of the first and second extracellular loops (ECL1 and 2) of CXCR4 (Doranz et al., 1997; Murakami et al., 1999) that are also involved in the interaction with HIV gp120 (Chabot et al., 1999). AMD3100 (Donzella et al., 1998; Schols et al., 1997), on the other hand, is a bicyclam compound that binds to a region spanning the fourth membrane spanning domain through the second extracellular loop (ECL2) to ECL3 of CXCR4. The interaction also appears to be electrostatic, as loss of negatively charged residues in this region, particularly Asp 171 and Asp 262, decreases binding and efficacy of the positively charged bicyclam (Labrosse et al., 1998; D. Schols, unpublished data). Of these three CXCR4-specific inhibitors, AMD3100 has gone through extensive pre-clinical development and is now in clinical trials (see below).

Inhibition via CCR5 is a particularly attractive option for two reasons. First, as mentioned above, the viruses that establish a new infection are generally CCR5-tropic. Thus, successful inhibition of infection via CCR5 might significantly decrease the probability of transmission. Second, individuals homozygous for a naturally occurring 32 base-pair deletion in the CCR5 gene and thus lacking this receptor are apparently immunologically normal. Of note, these individuals are also resistant to infection by R5 strains of HIV, and persons heterozygous for this CCR5 defect exhibit a slower progression to AIDS and death (Liu et al., 1996; Samson et al., 1996). The list of inhibitors targeting CCR5 is quite extensive, including small molecules, antibodies, chemokines and chemokine analogs. TAK-779 (Baba et al., 1999), a low molecular weight compound, inhibits productive interaction of the viral Env with CCR5 by inserting into a cavity formed between transmembrane helices 1, 2, 3, and 7 near the extracellular surface of the receptor (Dragic et al., 2000). At its effective concentration of 10 nM, this interaction does not induce signaling via CCR5 or down-modulation of the receptor, making TAK-779 an attractive candidate for pharmacologic inhibition of HIV infection (Baba et al., 1999). However, toxicity may limit the clinical utility of this compound. Several monoclonal antibodies to CCR5 inhibit infection of certain HIV isolates. One of these, PRO 140 (originally described as PA14; Olson et al., 1999), has inhibited infection of peripheral blood mononuclear cells by all R5 HIV isolates tested (Trkola et al., 2001). PRO 140 does not induce signaling or down-modulation of CCR5 at effective concentrations, nor does it

prevent CC-chemokine signaling. The epitope on CCR5 recognized by this antibody appears to span multiple extracellular loops, perhaps explaining its broad antiviral activity against diverse HIV-1 strains (Olson et al., 1999).

RANTES (regulated on activation, normal T cell expressed and secreted), one of the natural ligands for CCR5, is a broadly active inhibitor of macrophage infection by R5 HIV-1 isolates. Inhibition of HIV infection by RANTES and the other CC-chemokines correlates with down-modulation of their receptors, suggesting that this is their mechanism of action. RANTES is effective against HIV infection of T cells, but inhibits HIV infection of macrophages only weakly (Cocchi et al., 1995). However, chemokine derivatives, particularly amino-derivatives of RANTES, more potently inhibit infection of both T cells and macrophages (Mosier et al., 1999; Proudfoot et al., 1996; Simmons et al., 1997) and show promise as pharmacologic agents. In particular N-nonanoyl-RANTES (NNY-RANTES) exhibits potent inhibition of HIV infection in vitro and in a murine model (Mosier et al., 1999). A recent study showed that NNY-RANTES induced more rapid CCR5 internalization and slower reexpression than did AOP-RANTES (Sabbe et al., 2001), supporting this as the mechanism of increased efficacy of these N-terminal RANTES derivatives (Mack et al., 1998; Oppermann et al., 1999; Vila-Coro et al., 1999).

The plasticity of HIV and its ability to escape from the suppressive action of inhibitory agents raises concern about inhibiting HIV entry by blocking CCR5 utilization. As discussed above, viruses that establish infection in a new host are predominantly CCR5-tropic, and the appearance of variants that use CXCR4 as a coreceptor is a prognostic indicator for the progression to AIDS (Connor et al., 1997; Shankarappa et al., 1999; Xiao et al., 1998). Although the evolution from R5 to X4 tropic viruses is slow in infected humans, it is not unreasonable to suspect that suppression of HIV infection with a potent CCR5 blocking agent might accelerate the selection for X4 or dual-tropic (R5/X4) variant viruses (Este et al., 1999). Recent studies in the SCID-huPBL mouse model of HIV infection documented this occurrence with administration of AOP- and NNY-RANTES (Mosier et al., 1999). Plasma levels of the CC-chemokine analogs achieved in these experiments were unable to completely block infection (50–113 pM NNY-RANTES and 500–630 pM AOP-RANTES), and CXCR4-utilizing escape variants arose in the NNY-RANTES-treated animals within one week. While in vitro replication analysis suggested that the X4 variants were less fit than the parental R5 virus, these variants persisted in vivo for three weeks after removal of NNY-RANTES. However, in vitro studies with a non-agonist small molecule inhibitor of CCR5 utilization did not induce X4 escape variants (J. Moore, unpublished data), suggesting that the inhibitor and the infection system may influence the ability of the virus to escape neutralization and the mechanism used. These studies reinforce the plasticity of HIV in evading inhibitors, and the conclusion that inhibitors of virus binding and fusion should be used in combinations that would block replication at multiple points in the viral life cycle.

The gp41 molecule also provides an opportunity for interruption of the fusion process. Binding of Env to its receptors promotes conformational changes in gp41 including exposure and/or formation of the trimeric HR1 coiled coil, exposure and insertion of the fusion peptide into the target cell membrane, and formation of the six-helix HR1–HR2 bundle complex. As described previously, it appears that formation of the six-helix bundle is the driving force for the fusion process. In support of this hypothesis, molecules that target a fusion intermediate prior to formation of the six-helix bundle prevent fusion. Inhibitors active at this stage include peptide mimetics of the HR2 region (T20 and T1249) that bind the trimeric N-terminal coiled coil (Kilby et al., 1998; Wild et al., 1995), the recently described five-helix protein that targets the HR2 region (Root et al., 2001), and the ADS-J1 cyclic compound that binds to the hydrophobic pocket in the HR1 coiled coil (Chan et al., 1997; Ji et al., 1999). This group of inhibitors acts by preventing formation of the six-helix bundle. T20 and the second-generation T1249 peptide are progressing rapidly through clinical trial evaluations (see below).

4. Properties of an effective antiviral agent against HIV

As with any viral target, the properties of an effective antiviral agent against HIV include good pharmacokinetics, delayed drug resistance, and the ability to interfere with infection of all tissues that might be targets for the virus. In the case of HIV, it appears that the CCR5 and CXCR4 receptors may exist in different forms on different cell types. Immunoprecipitation studies detected monomers and oligomers of CXCR4 on macrophages, but only monomers on monocytes (Lapham et al., 1999). Additionally, differential reactivity of anti-CCR5 monoclonal antibodies indicated that there might be cell-type specific alterations in the CCR5 structure (Hill et al., 1998; Lee et al., 1999). Interestingly, altered CCR5 conformation might provide an explanation for the difference in the ability of RANTES to inhibit R5 infection of various cell types, reinforcing the need for broadly active inhibitors. Antiviral agents against HIV may also have special requirements that stem from the polyvalent nature of the Env. As mentioned previously, the HIV Env spike on virions and infected cells is most likely a trimer of gp120–gp41 heterodimers, with each heterodimer possessing the machinery necessary for receptor binding and fusion. The multimeric nature of this molecule invokes the possibility of cooperative interactions between the subunits of each Env oligomer. This indicates that an effective antiviral agent targeting utilization of chemokine receptors may also need to be multivalent or inhibit multivalent interactions despite interacting with only a single receptor molecule (dominant negative inhibition).

A case in point can be made from the experience of screening small molecules for their ability to inhibit HIV interaction with the chemokine receptor CCR5. During efforts to develop CCR5 antagonists it became evident that the ability to block the interaction of monomeric gp120 with CCR5 did not predict neutralization of HIV infection. Many compounds capable of blocking gp120–CCR5 interactions at nanomolar and subnanomolar concentrations had little or no antiviral activity, while similar inhibitors with equivalent or less gp120-blocking ability were potent antivirals. The discordance appeared to arise from the polyvalent nature of the CCR5–viral interaction. That the binding of HIV to CCR5 might be cooperative, and therefore difficult to inhibit, was supported by several lines of evidence. First, mutations in CCR5 that drastically reduced the affinity of the receptor for gp120 had little effect on the ability of the mutated receptors to support infectivity (Kuhmann et al., 1997). Second, immunogold electron microscopy revealed that both CD4 and CCR5 were organized in homogenous microclusters often separated by less than a viral diameter (M. Springer, in press). The latter observations implied not only the likelihood of cooperative binding to multiple receptors, but also that following initial interaction with a cluster of CD4 molecules the virus acted as a tethered ligand, greatly enhancing the kinetics of its productive interaction with CCR5. Inhibition of such a kinetically favored, cooperative interaction necessitates reducing free receptor levels to a minimum. An effective antagonist should: (1) block all states of the receptor to which gp120 can bind; (2) form complexes with the receptor that have very slow dissociation rates; and (3) limit viral access to receptor that has just arrived on the cell surface. Since a major source of 'new' receptor is recycling, a compound with antagonist properties (inhibiting recycling) would be more attractive than the one behaving as an agonist (stimulating receptor recycling). Additionally, the inhibitor–receptor complex should have sufficient stability to allow it to survive the recycling process, and be re-expressed on the cell surface intact.

Thus, an effective agent against HIV infection targeted at interactions between the oligomeric viral Env and the target cells (inhibiting CD4 binding or coreceptor interactions) would likely need to exhibit extremely stable binding to its ligand. This binding must not only be stable at neutral pH, but also at the low pH encountered in endosomes during the recycling process. In developing agents that block cell–cell fusion as well as virus infection, this principle applies not only to inhibitors directed against the receptors, as described in the example above, but also to inhibitors targeting the viral Env. Studies have

shown that the Env expressed on the surface of infected cells, which can mediate cell–cell fusion or be incorporated into budding virions, is also subject to endocytosis and recycling by virtue of trafficking signals in the cytoplasmic tail of gp41 (LaBranche et al., 1995; Rowell et al., 1995; Sauter et al., 1996). Thus, antiviral compounds whose mechanism of action involves binding to the viral Env glycoprotein should also be able to act on a polyvalent target and also withstand the low pH of the recycling endosome.

5. Attachment and fusion inhibitors in clinical trials

The previous sections have described the dynamics of binding and fusion employed by HIV in infecting target cells and the variety of opportunities within this fusion cascade for pharmacologic intervention. It is a testimony to the creative effort applied to these opportunities that several fusion inhibitors are already in clinical trials. The section below describes the current status of each of these clinical studies.

5.1. T20

T20 is a peptide mimetic of the HR2 region of gp41. Its dominant negative mechanism of action involves binding to the pre-hairpin fusion intermediate, preventing formation of the six-helix bundle that likely drives the fusion of the viral and target cell membranes. Pre-clinical in vitro data indicated broad activity against laboratory-adapted viruses and primary isolates (Rimsky et al., 1998), and antiviral activity was demonstrated in SCID-hu mice (D. Lambert and S. Barney, Trimeris, unpublished). While a range of sensitivity to this inhibitor was observed, innate differences in T20 potency did not appear to track with co-receptor utilization (CCR5 or CXCR4) by the virus, even when analyzing viruses obtained from infected individuals before and after the NSI → SI phenotypic switch. Rather, T20 resistant variants observed in vitro and in vivo have contained mutations in the HR1 region of the TM protein, with the most significant mutations located in the highly conserved GIVQQQ sequence near the amino terminus of the HR1 region (Kilby et al., 1998; Rimsky et al., 1998).

In both the proof-of-principle (TRI-001) and dose-escalation (TRI-003) clinical trials, treatment of HIV-infected individuals resulted in a decrease in viral load of up to 1.5 logs. The dose-escalation trial showed that twice-daily sub-cutaneous (SC) injections of 100 mg T20 was the most effective delivery/dose regimen. Pharmacokinetic analysis showed a half-life of 2.3–3.4 h following SC administration, and plasma levels suggested that 60–70% of the administered compound was bioavailable. The ability of T20 to penetrate the central nervous system (CNS) is unknown.

Participants in TRI-001 and TRI-003 were offered enrollment in a roll-over trial (T20-205), which evaluated a twice-daily 50 mg sub-cutaneous injection for safety and antiviral activity in combination with other antiretroviral drugs. Of the 71 participants enrolled, 41 remained for the entire 48 weeks of the trial. A post-trial survey revealed that the majority of participants felt that sub-cutaneous injections twice a day did not interfere with normal daily life. Non-compliance in this trial was not the result of adverse effects, although liver enzyme abnormalities, anemia, neutropenia and altered mental state were noted in some participants. As in TRI-001 and TRI-003, viral RNA decreased an average of 1.5 logs over the entire 48 weeks with a concomitant rise in CD4+ T cell count (mean = 85). Of those individuals completing the trial, 50% responded to T20 (33% of the initial 71 participants enrolled), with viral loads dropping below 50 RNA copies/ml in 22% of the final study population. Variant viruses resistant to T20 did develop during T20 monotherapy (TRI-003), but resistance data on the roll-over trial is not yet available.

A concern with peptide-based antiviral therapy is the potential for eliciting an antibody response to the antiviral agent, rendering the agent ineffective. Animal studies (cynomologus monkeys) showed that T20 was immunogenic, but that these T20-specific antibodies did not reduce the efficacy of the drug. The same result was observed in trial participants: antibodies generated by T20 administration did not adversely affect the antiviral

activity. Interestingly, in-patients with pre-existing antibodies reactive with T20, no increase in anti-T20 antibodies was detectable, and these antibodies did not reduce the plasma levels of T20 (TRI-003) or reduce antiviral activity (T20-205).

5.2. T1249

The second generation HR2 peptide mimetic combines the strengths of T20 with sequence changes that increase the potency and breadth of this compound. T1249 consists of 39 L-amino acids (T20 is 36) and begins two heptad repeats N-terminal to the start of T20. This incorporates the WMEW sequence that makes the deep hydrophobic pocket, thought to stabilize the interaction between HR2 and HR1 peptides. High affinity binding ($K_d = 1.1$ nM) to the HR1 region of gp41 is documented by CD spectroscopy and protease protection, as was done for T20.

In primate toxicology studies, antibodies to T1249 did arise and titers were maintained throughout administration but were not particularly high. A screen of 43 HIV+ sera revealed moderate titers of pre-existing anti-T1249 antibodies in four individuals and high titer antibodies in one individual. However, in vitro studies revealed no effect of these antibodies on the IC50 of this compound. Bioavailability of T1249 ranges from 62% (intramuscular) to 90% (sub-cutaneous), and sub-cutaneously injected doses ranging from 0.8 to 1.6 mg/kg results in plasma concentrations of >6 μg/ml (which is above the target concentration for antiviral activity) with a half-life of 4.8–5.6 h.

A phase I/II clinical trial has begun (T1249-101), having enrolled 72 participants with a plasma HIV RNA level >5000 copies/ml. Inclusion criteria for the study specify no antiretroviral therapy for at least two weeks prior to the start of the 14 day study. Endpoints for evaluation include assessments of plasma pharmacokinetics and the effect of treatment on plasma viral load following administration of doses ranging from 6.25 to 25 mg twice daily.

5.3. PRO542

The results of Phase I/II clinical trials on the tetrameric immunoglobulin-CD4 fusion protein (PRO542) have been published (Jacobson et al., 2000; Shearer et al., 2000). In the trial with adult subjects (3–6 per arm), IV doses up to 10 mg/ml were well tolerated and showed plasma half-lives of 3–4 days. The highest dose administered resulted in a sustained decrease in viral load in some subjects. A similar study in six children produced plasma RNA decreases of $> 0.7 \log_{10}$ in four individuals, and sustained virus suppression in three patients for the 14 day observation period. No antibodies to PRO542 were observed. Combination studies in vitro showed that PRO542 synergizes with T20 over a wide concentration range and ratio of compounds, which is reflective of the fact that these two antiviral agents act at different stages of the binding-fusion cascade.

5.4. AMD3100

The bicyclam AMD3100 is a neutral antagonist of CXCR4, inhibiting the binding of the natural ligand, SDF-1, and the anti-CXCR4 monoclonal antibody, 12G5, and completely blocking SDF-1-induced Ca flux in SupT1 cells at 100 ng/ml. In animal models, inhibition of infection of SCID-hu mice by an X4 virus required concentrations of AMD3100 in excess of 100 nM (Datema et al., 1996). However, no adverse effects of AMD3100 administration (BID) have been observed in doses as high as 8 mg/kg/day in dogs and 24 mg/kg/day in rats. The plasma half-life is similar in these two animal models (6–8 h), and AMD3100 can be found in a wide variety of tissues. Clearance is through the kidneys, and no mutagenic or clastrogenic effects of AMD3100 administration have been detected.

Phase I studies on the CXCR4 antagonist were completed in 1999, and Phase I/II dose escalation studies are ongoing. In Phase I study, AMD3100 was administered by a 15-min infusion, a SC injection or as an oral solution of 10–80 μg/kg. The study showed that the SC

injection was essentially completely absorbed, while there was no bioavailability by the oral route. While all routes were well tolerated, a transient increase in white blood cell (WBC) count to approximately three times baseline was observed approximately 6 h following drug administration. The increase spanned all white cell subsets, and may stem from CXCR4-blockade-induced demarginization.

In the Phase I/II open label, dose-escalation trial, HIV+ individuals with plasma RNA levels >5000 copies/ml were started with a continuous IV infusion of 2.5 μg/kg/h using an Alset infusion pump. Current enrollees are receiving 20 μg/kg/h, equivalent to 0.5 mg/kg/day, which is still below the toxic limit (resulting in a minor change in platelet count). Subjects are either receiving stable antiretroviral therapy (ART) or no ART, and are divided into two treatment arms: (1) irrespective of viral phenotype, (2) individuals with SI viruses as determined by the MT2 syncytium induction assay. Thus far, the results of this study show a 7–9 h plasma half-life, and a prolonged increase in WBC count.

6. Analysis of interaction between antiviral agents

Clinically relevant isolates of HIV can use either CCR5, CXCR4 or both chemokine receptors to mediate fusion and entry into the target cell. Virus isolated from infected individuals most often consists of a quasispecies rather than a single genotype, with continued replication and selective pressure amplifying the heterogeneity. In addition, studies in vitro and in small animals (Mosier et al., 1999) have demonstrated the ability of viruses to generate escape mutants in response to inhibitory agents. Thus, the most prudent antiviral strategy will employ drugs that target multiple steps in the viral lifecycle. Even within the confines of inhibitors of virus entry (step 1 of Fig. 1), there is the opportunity to affect receptor binding (e.g. PRO 542, TAK-779, AMD3100) and the fusion mechanism (T20, ADS-J1).

However, the various inhibitors use different mechanisms of action, which may be compatible or antagonistic. In the context of rapid evaluation of seemingly effective antivirals, different strategies exist for identifying synergistic and antagonistic effects of drug combinations. An attractive method is the Median Dose Effect (MDE) (Chou and Talalay, 1981) because this strategy requires fewer data points than the checkerboard method, it allows extrapolation outside the range of drug doses tested, and also allows evaluation of two or more drugs simultaneously. This method produces a 'Combination Index' (CI) that can identify synergy between drugs ($CI < 0.9$) and antagonism ($CI > 1.1$). The results of MDE analysis also correlate between in vitro and in vivo studies. For example, Zidovudine and stavudine exhibited antagonism in in vitro studies (Merrill et al., 1996), as well as in the ACTG 290 clinical trial (Havlir et al., 2000).

MDE studies of drugs targeting infection and fusion of HIV have produced interesting results (Tremblay et al., 2000). T20 showed synergism with either AMD3100 or TAK-779. AMD3100 was antagonized by met-SDF-1ß, particularly at high drug concentrations. Interestingly, TAK-779 and AOP-RANTES were synergistic against one primary R5 virus (RM), but exhibited dramatic antagonism against another primary R5 virus (JC). This result might be explained by the observation that some primary R5 viruses bind to a CCR5 epitope that is not blocked by AOP-RANTES (Simmons et al., 1997). Further study will be required to resolve this issue.

7. Attachment and fusion inhibitors in pre-clinical development

7.1. PRO 140

As previously mentioned, GPCR have traditionally been good targets for drug therapy. PRO 140 is a murine monoclonal antibody to CCR5 that broadly exhibits active blockade of entry into both T cells and macrophages by R5 viruses, but competes only weakly for RANTES binding. There are many potential benefits of antibody-based inhibitors including a larger recognition epitope on CCR5, multivalent binding potential, and a predicted long plasma half-life that allows an extended

dosing schedule. Additionally, while RANTES is a poor inhibitor of R5 infection of macrophages, PRO 140 is equally potent on both macrophages and T cells. Attempts to generate resistant variant viruses have been unsuccessful over 31 weeks in culture, making PRO 140 a very interesting therapeutic candidate. Attempts to humanize this antibody are currently underway in an effort to improve the clinical utility of this agent.

7.2. ADS-J1

Like T20 and T1249, ADS-J1 is compound designed to inhibit the formation of the fusion-driving six-helix bundle. However, the mechanism of action of this molecule is quite different from the HR2 peptide mimetics. This cyclic compound was discovered by a sandwich ELISA-based screen designed to detect the formation of the six-helix bundle. Briefly, a rabbit antiserum generated against the six-helix bundle, but which could still detect HR1 and HR2 in isolation, was used to coat ELISA plates. HR1 was added to the plate, followed by test compounds, and finally HR2. Formation of the six-helix bundle was detected using a monoclonal antibody specific for this structure, NC-1 (Jiang et al., 1999). In this ELISA, the negative result (inability to form the six-helix bundle) indicated that ADS-J1 was able to prevent the formation of this fusion-active structure. Interestingly, ADS-J1 was one of 16 compounds predicted by computer modeled docking to fit into the hydrophobic pocket of the HR1 peptide coiled coil identified by X-ray crystallography (Chan et al., 1997; Debnath et al., 1999). However, functional testing revealed that compounds able to block the binding of HR2 to the HR1 coiled coil required interaction with Lys at position 574 in HR1, forming a salt bridge similar to that formed between this residue in HR1 and Asp 632 in HR2 in the intact gp41 protein (Jiang and Debnath, 2000). At this early stage in the study of ADS-J1, no preclinical pharmacokinetic or toxicity data were available.

7.3. NNY-RANTES

Amino-terminal derivatives of RANTES are more potent inhibitors of HIV infection than the natural chemokine because they greatly decrease the rate of receptor recycling, effectively sequestering CCR5 inside the cell. Notably, studies in SCID-huPBL mice showed that 60% of mice maintaining plasma levels of NNY-RANTES between 50 and 113 pM were protected from infection by HIV-1. To achieve these levels, however, NNY-RANTES was administered in both a bolus dose in addition to continuous infusion, and preliminary data suggest that either dosage scheme alone was ineffective (Mosier et al., 1999). Viruses resistant to NNY-RANTES were recovered from the four mice that became infected while undergoing treatment. Two of the resistant isolates still used only CCR5 and mutations associated with resistance mapped outside the V3 loop (Mosier et al., 1999). The other two treatment failures had adapted to use CXCR4 in addition to CCR5 due to mutations in the V3 loop. Interestingly, the R5X4 resistant variants were only maintained in the presence of drug, and showed rapid reversion following withdrawal of NNY-RANTES. More recent experiments have shown that a combination of NNY-RANTES and AMD3100 at 1.2–3.6 μM/ml prevented R5 HIV-1 infection as well as the emergence of resistant isolates. Further development is ongoing to improve the pharmacokinetics of this class of antiviral compounds. Preliminary data indicate that newer RANTES derivatives are more potent and can sequester CCR5 for >48 h.

8. Guidelines for effective anti-HIV therapy

In current HIV therapy, 30–50% of initial treatment regimens fail for a variety of reasons including resistance and poor adherence. Within each class of currently used HIV inhibitors, cross-resistance is relatively common, shrinking the options for alternative therapy from the seemingly large number of 15 approved drugs. Only 30–80% of cases respond to salvage therapy regimens, with the percentage decreasing after a second failure and further decreasing after multiple treatment failures.

Building on the experience of the last 15 years, several lessons are obvious. Waiting to change a drug regimen until viral loads reach high levels only allows resistant viruses to disseminate and

establish new reservoirs of infection. Treatment with a combination of drugs is most effective when a larger number of drugs is used and includes inhibitors directed at more than one step in the viral replication cycle. In vitro drug-resistance testing of patient virus should be utilized to guide the choice of therapeutic agents employed in each case. It is also prudent to change drug combinations at the earliest reliable indication of treatment failure. Thus, having new classes of drugs available should increase the success rate of salvage therapy.

9. Other viruses

The rapid progress in understanding and inhibiting HIV fusion owes much to the many years of research on influenza virus. Although the trigger for fusion mediated by influenza hemagglutinin (HA) is low pH rather than receptor binding, the fusion cascade in this virus also involves the spring-like formation of a trimeric coiled coil that results in the projection of the fusion peptide toward the target cell membrane (White, 1995) followed by folding of the protein into a 'hairpin' conformation that juxtaposes the fusion peptide and the membrane-spanning domain of the molecule (Bullough et al., 1994). It now appears that other viral and cellular proteins utilize similar mechanisms to induce membrane fusion (Chan and Kim, 1998; Skehel and Wiley, 1998).

Coiled coil motifs have also been described in the fusion proteins of Ebola virus, Moloney murine leukemia virus, simian virus 5, respiratory syncytial virus (RSV), human parainfluenza virus type 3, and measles virus. Of these viruses, fusion inhibitors are actively being developed for RSV, which causes lower respiratory infections and pneumonia in immunocompromised, pediatric and geriatric populations (McIntosh and Chanock, 1990). Like T20 in the HIV system, peptide mimetics of the HR2 region of RSV F_1 potently inhibit infection by this virus in vitro. A small molecule, RFI-641, has also demonstrated in vitro antiviral activity against both A and B subgroups of RSV. This dendramer-like compound appears to act after virus attachment by binding a highly conserved target in the F protein. Pre-clinical experiments showed efficacy in animal models of RSV infection. Phase I studies of this compound have demonstrated safety in vivo, and Phase II clinical trials are in progress.

10. Summary and conclusions

HIV infection remains a significant issue in global health. Intensive research is focused on vaccine strategies and targets, but novel therapeutic drugs are urgently needed to ameliorate the disease course in currently infected individuals. HAART is extending disease-free life for many infected individuals. However, drug resistance and toxicity limit the utility of HAART for increasing numbers of people. Several novel antiviral drugs are being developed that target the HIV entry process. The compounds described herein fall into three categories: inhibitors of CD4 binding, coreceptor interaction, and fusion. A few of the entry/fusion inhibitors are currently in clinical trials, including the peptide-based fusion inhibitors T20 and T1249, the tetrameric immunoglobulin-CD4 fusion protein PRO 542, and the CXCR4 antagonist AMD3100. Other compounds are being evaluated in pre-clinical studies. With the addition of the entry/fusion inhibitors, the arsenal of anti-HIV drugs has been increased from three classes of compounds to six. Studies have shown synergistic antiviral effects among the newer compounds as well as between the new and existing drugs. These new agents provide hope for salvage therapy for individuals failing HAART, as well as alternatives for initial therapy for newly infected individuals. The success of fusion/entry inhibitors in HIV therapeutics has also sparked development of analogous antiviral agents targeting binding and fusion of other fusogenic viruses.

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