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HIV Coreceptors: From Discovery and Designation to New Paradigms and Promise

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Abstract
Just over a decade ago, the specific chemokine receptors CXCR4 and CCR5 were identified as the essential coreceptors that function along with CD4 to enable human immunodeficiency virus (HIV) entry into target cells. The coreceptor discoveries immediately provided a molecular explanation for the distinct tropisms of different HIV-1 isolates for different CD4-positive target cell types, and revealed fundamentally new insights into host and viral factors influencing HIV transmission and disease. The sequential 2-step mechanism by which the HIV envelope glycoprotein (Env) interacts first with CD4, then with coreceptor, revealed a major mechanism by which conserved Env epitopes are protected from antibody-mediated neutralization. The Env-coreceptor interaction has become a major target for the development of novel antiviral strategies to treat and prevent HIV infection.

Key words: HIV, AIDS, coreceptor, CCR5, CXCR4, receptor, CD4, chemokine, GPCR, tropism, transmission, pathogenesis, fusion, entry, antibody, resistance, nomenclature, treatment, vaccine, microbicide, maraviroc

Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; Env, envelope glycoprotein; TCL, T cell line; M, macrophage; GPCR, G protein-coupled receptor

Introduction: The case for an HIV coreceptor(s)

The progressive depletion of T lymphocytes expressing the CD4 antigen was recognized from the outset as a defining feature of acquired immunodeficiency syndrome (AIDS) [1]. Remarkably soon after the discovery of a lentivirus designated human immunodeficiency virus (HIV) as the causative agent of AIDS [2], the CD4 molecule itself was implicated as a major receptor mediating entry of the virus into CD4-positive T lymphocytes [3, 4]. The major entry mechanism was shown to involve direct fusion between the membranes of the virion and target cell. The fusion process is mediated by the HIV envelope glycoprotein (Env), which is composed of two subunits derived from the gp160 precursor: the external gp120 subunit, which engages target cell receptors, and the gp41 subunit, which promotes the membrane fusion reaction [5, 6].

Despite the rapid identification of CD4 as the "primary receptor" for HIV, it soon became clear that the complexities of virus entry and tropism could not be explained by CD4 expression alone; several lines of evidence suggested that additional molecular components of the entry process were yet to be uncovered. For one, expression of recombinant human CD4 on otherwise CD4-negative human cell types rendered them permissive for HIV infection; however efficient human CD4 expression on murine cells failed to confer infection permissiveness, apparently due to a block at a very early step in the replication cycle [7]. This requirement for the CD4-expressing target cell to be of human origin was also manifested in cell fusion [8] and virus pseudotype assays [9] in which Env was the only HIV component, thereby indicating a critical difference in the ability of human versus murine cells to support the HIV fusion/entry step. Experiments with cell hybrids supported the involvement of an essential cofactor (perhaps a coreceptor?) specific to human cells, rather than the existence of a dominant negative inhibitory mechanism in murine cells [10-12]. A second line of evidence stemmed from the recognition that different strains of HIV-1, including isolates obtained from individuals at different stages of infection and disease progression, showed markedly different abilities to infect different CD4-positive human cell types [13-15]. Some isolates efficiently infected continuous T cell lines but not primary macrophages, whereas others showed strong preferential tropism for macrophages compared to T cell lines; these distinct phenotypes were referred to as T cell line-tropic (TCL-tropic, or T-tropic) versus macrophage-tropic (M-tropic), respectively. By and large, these phenotypes corresponded respectively to alternate phenotypic descriptions: syncytium-inducing versus non-syncytium-inducing, or rapid-high versus slow-low. Importantly, all HIV-1 isolates were found to replicate in activated primary CD4-positive T cells. A host of studies indicated that the Env gene was the major viral determinant governing these phenotypic distinctions (see citations in ref. [16]). In particular, a close correspondence was observed between target cell tropisms of different HIV-1 strains in infectivity assays and tar-

get cell specificities of the corresponding Envs in a cell fusion assay [17]. Additional cell hybrid experiments suggested that fusion specificities of different Envs reflected their preferential requirement for distinct "cofactors" (coreceptors?) expressed on T cell lines versus primary macrophages [18]. Identification of these fusion cofactors thus promised to provide critical insights into the mechanism(s) governing HIV tropism.

Identification of the First HIV Fusion/entry Cofactor (Coreceptor?) by Functional cDNA Cloning

The discovery of CD4 as the primary HIV receptor in the mid-1980s, coupled with the awareness that CD4 expression was insufficient to allow HIV Env-mediated fusion/entry, led to a decade-long search for the essential fusion/entry cofactor, a putative "coreceptor". A diversity of molecular candidates were proposed (reviewed in ref. [16]), but in no case did the experimental evidence meet the essential criteria. Success finally came in 1996 with the application of an unbiased functional cDNA library screening approach based on the ability of the cofactor-encoding cDNA to render murine cells expressing human CD4 capable of undergoing fusion with cells expressing HIV-1 Env [19]; for technical reasons, the initial focus was on the coreceptor for TCL-tropic strains. The protein identified by this approach, initially dubbed "fusin", was shown to render diverse CD4-expressing nonhuman cells permissive as targets in assays of both cell fusion and HIV-1 infection ("gain-of-function"); this result suggested that fusin was sufficient to confer HIV-1 fusion/entry susceptibility to nonhuman target cell types. Conversely, antibodies against a peptide derived from the fusin sequence blocked both cell fusion and HIV infection with human target cells that were inherently permissive when expressing CD4; thus fusin was necessary for CD4-dependent fusion/entry. A close correspondence was also observed between permissiveness of several human cell types expressing CD4 and their endogenous expression of fusin. Most importantly, both the gain- and loss-of-function criteria indicated that fusin acted selectively for Envs from TCL-tropic but not M-tropic strains in assays of cell fusion and HIV infection (Fig. 1) [19].

The coding sequence of the fusin cDNA suggested that the corresponding protein was a member of the superfamily of G protein-coupled receptors (GPCRs), which are characterized by 7 transmembrane segments with an extracellular amino terminus, an intracellular carboxyl terminus, plus three extracellular and three intracellular loops. The cDNA had been cloned previously by several independent groups, but no functional activity had been described, leading to its consideration as an "orphan" receptor. The closest homology was to a receptor for interleukin-8, a member of the family of small chemoattractant proteins called chemokines (CXC subfamily). This relationship suggested the possibility that fusin might be a chemokine receptor.

Identification of the Fusion/entry Cofactor (Coreceptor?) for M-tropic HIV-1 Strains

This notion was particularly intriguing in light of findings just a few months earlier on a seemingly unrelated problem, namely the identity of the secreted non-cytolytic HIV-inhibitory factor(s) released by CD8-positive T cells. This phenomenon, first described in the late 1980's [20], eluded biochemical definition despite intense experimental efforts. The first success came with a particular CD8 T cell system [21], in which all of the HIV-inhibitory activity was accounted for by three secreted proteins: RANTES, MIP-1α, and MIP-1β, all chemokines (CC subfamily). The block was shown to be at an early stage of the infection cycle. Interestingly, while potent inhibition was observed for several M-tropic HIV-1 isolates, minimal activity was seen against a TCL-tropic strain.

The identification of fusin, a possible chemokine receptor, as the major fusion/entry cofactor for TCL-tropic HIV-1, coupled with the inhibitory activity of specific chemokines against M-tropic strains, suggest-

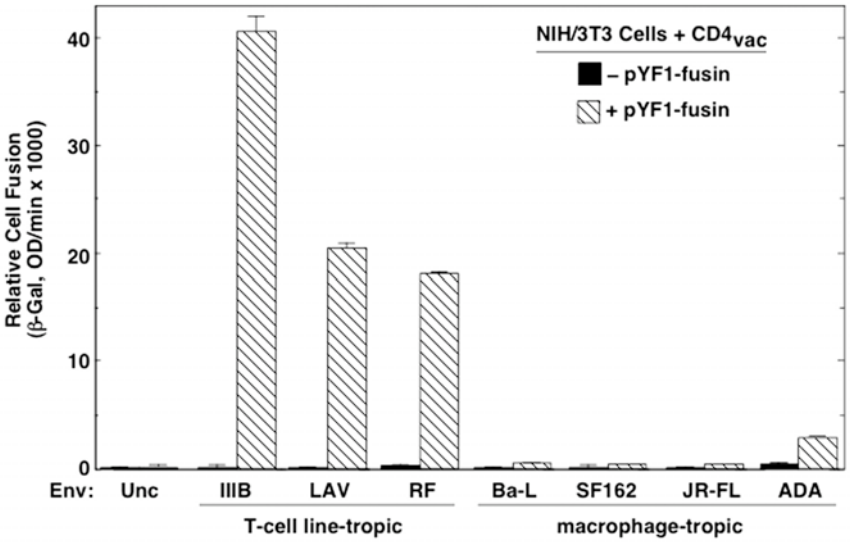

Fig. 1. Fusin functions preferentially for TCL-tropic Envs in cell fusion assay. Target cells were murine fibroblasts expressing vaccinia-encoded human CD4, without (filled bars) or with (cross-hatched bars) fusin. Cell fusion was performed with cells expressing the indicated Envs from either TCL-tropic or M-tropic HIV-1 strains, as measured by expression of the reporter gene β-galactosidase. From [19].

ed an obvious clue to the identity of the M-tropic cofactor: perhaps it is a chemokine receptor with specificity for the inhibitory chemokines (RANTES, MIP-1α, and MIP-1β). Fortuitously around the same time period, a chemokine receptor with precisely that specificity was isolated independently by two groups, and designated CC-CKR5 [22, 23]. In a flurry of activity, five near-simultaneous reports independently demonstrated that this molecule is an essential cofactor that functions along with CD4 to allow fusion/entry of M-tropic HIV-1 [24-28].

Rapid Expansion and Consolidation of a New Paradigm

The second half of 1996 witnessed dramatic "big bang" from the new nexus of HIV and chemokine receptors. The ligand for fusin was quickly identified as the CXC chemokine SDF-1 [29, 30], thereby demonstrating that the fusion/entry cofactor for TCL-tropic HIV-1 is indeed a chemokine receptor. According to the subsequent revision of chemokine receptor nomenclature [31], fusin became CXCR4 and CC-CKR5 became CCR5. Binding studies demonstrated direct CD4-dependent interactions of the gp120 Env subunit with CCR5 or CXCR4 [32-34]. These molecules could now justifiably be designated as "coreceptors" rather than as nebulous "cofactors".

The coreceptor discoveries provided a molecular explanation for the ability of different strains to enter and infect different types of CD4-positive human target cell lines (Fig. 2). M-tropic strains (generally corresponding to non-sycytium-inducing; slow-low) display this phenotype because their Envs function with CCR5 (highly expressed on primary human macrophages) but not CXCR4; by contrast, Envs from TCL-tropic strains (generally corresponding to syncytium-inducing; rapid-high) function with CXCR4 (highly expressed on human continuous T cell lines) but not CCR5. Envs from dual-tropic strains can function with either coreceptor. Activated human primary CD4-postive T cells express abundant levels of CCR5 and CXCR4, thus rendering them permissive for fusion/entry of all HIV-1 isolates. According to a newly devised nomenclature describing HIV tropism phenotype, M-tropic strains are now designated "R5", TCL-tropic strains are designated "X4", and dual-tropic strains are classified "R5X4" [35].

New Perspectives on HIV Biology Based on Coreceptors

In the ensuing decade since their initial identification, the HIV coreceptors have engendered entirely new paradigms for understanding basic mechanisms underlying the natural history of HIV, as well as for developing novel approaches to intervene in the HIV pandemic [36, 37].

Breadth of the coreceptor repertoire

The discoveries of CXCR4 and CCR5 as major HIV-1 coreceptors prompted the search for related molecules that might display coreceptor function. Indeed over the next several years, a number of additional chemokine receptors and related GPCRs were found to display such activities when co-expressed with CD4 in various in vitro assays [38, 39]. However there is only minimal evidence to date that any of these alternate molecules contribute to HIV-1 entry into natural CD4-expressing target cells, and evidence for physiological relevance in vivo has solidified only for the major coreceptors CXCR4 and CCR5; nevertheless the possibility that alternative molecules might provide this function under some circumstances remains open. CXCR4 and CCR5 are also the major coreceptors for HIV-2 and the related simian immunodeficiency virus (although CXCR4 usage is observed only infrequently in the latter case).

The HIV entry mechanism

With the knowledge that two distinct receptors (CD4 and coreceptor) are required for HIV fusion/entry, an obvious questions was: Does the virus randomly bind to whichever molecule it first encounters, or does the mechanism require a specific sequence of interactions? Biochemical experiments, including studies with purified soluble molecules, had clearly revealed a high affinity gp120/CD4 binding interaction long before

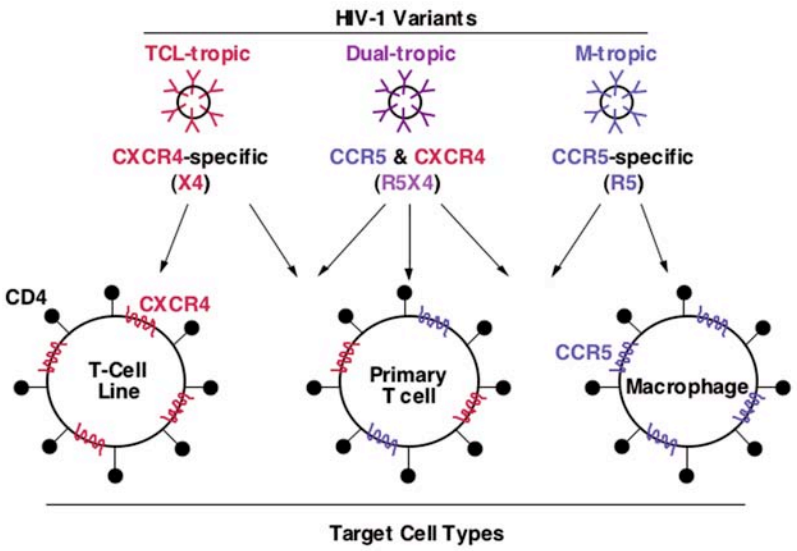

Fig. 2. Coreceptor usage and HIV-1 tropism. Adapted from [38].

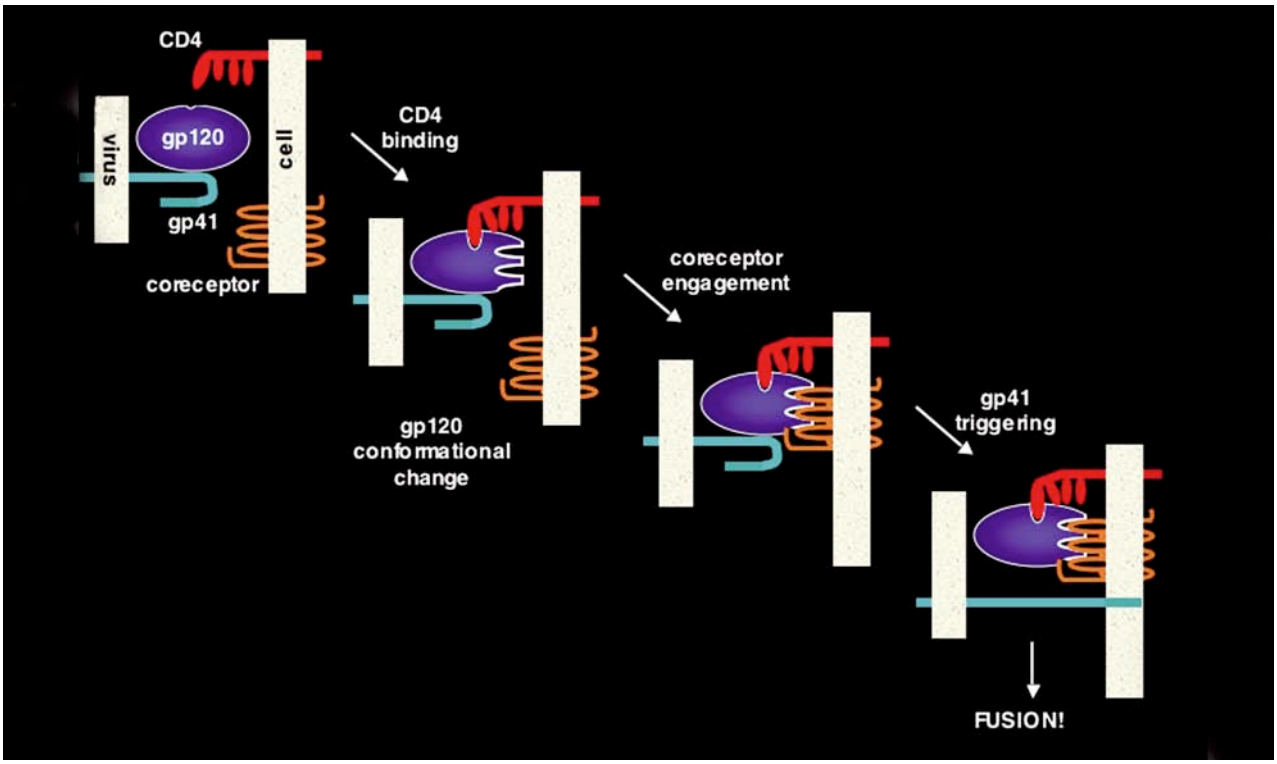

Fig. 3. Sequential 2-step receptor engagement model for HIV fusion/entry.

the coreceptor discoveries [3, 4]. Subsequent binding experiments [32, 33, 40] and functional assays [41] clearly demonstrated that CD4 binding induces conformational changes in gp120 that enable or enhance its interaction with coreceptor. In the generally accepted model [5, 6], gp120 binds to CD4 and is induced to undergo a major conformational change that either creates or facilitates exposure of the coreceptor binding site; gp120 interaction with coreceptor then triggers the gp41 subunit to promote the fusion reaction via another series of complex conformational changes (Fig. 3).

This model was brought to life by high resolution atomic structures of gp120 in both the CD4-bound [42] and unbound [43] states. These structural studies, combined with thermodynamic, biochemical, and immunochemical analyses, confirmed the phenomenon of "conformational masking" [44] by which highly conserved gp120 determinants involved in binding to receptors (CD4 and coreceptor) are created/exposed only transiently via the major conformational changes induced during the precisely choreographed sequence of gp120 interaction first with CD4, then with coreceptor. Particularly intriguing is the so-called "bridging sheet" formed from discontinuous regions of gp120 that create a contiguous surface in the CD4-bound conformation, but are physically separated in the unliganded structure. This CD4-induced surface plays a central role in binding to coreceptor [45]. The complex 2-step receptor interaction mechanism (CD4 followed by coreceptor) provides HIV with a major defensive strategy to protect its highly conserved epitopes against surveillance by the humoral immune system [46-48]. Also contributing to antibody evasion is the masking of potential epitopes within the oligomeric structure of Env on the surface of the virion and infected cell, as well as the continuously evolving pattern of Env glycosylation that acts as a "glycan" shield to protect conserved epitopes [49].

HIV tropism, transmission, and pathogenesis

The coreceptor discoveries enabled a molecular analysis of the mysterious relationships between HIV-1 tropism in vitro and the critical problems of HIV transmission and pathogenesis. Correlations between these phenomena had long been known [13-15]. Viruses isolated in the newly infected person invariably were found to be M-tropic, even though the "donor" might have harbored both M-tropic and TCL-tropic variants; this selectivity was observed independent of the mode of transmission (sexual, blood, or mother-to-child). The strictly M-tropic phenotype was found to persist during the asymptomatic phase of infection, a period often lasting several years. Not until the transition to the symptomatic phase were TCL-tropic variants first detected. Using assays to measure coreceptor usage, it was soon found that viruses isolated during the acute and asymptomatic phases invariably displayed the R5 phenotype; CXCR4-using viruses (R5X4, X4) were detected only in individuals at the symptomatic phase [38, 50] (Fig. 4)

A particularly remarkable discovery related to the selective transmission of R5 strains occurred within months after the identification of CCR5 as an HIV coreceptor. A CCR5 allele containing a 32 base-pair deletion within the open reading frame was detected, at particularly high frequencies in Caucasians; homozygosity for the newly identified CCR5 Δ32 allele,

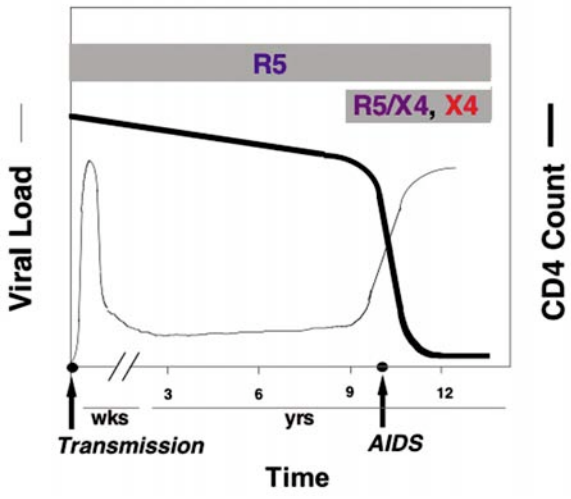

Fig. 4. Temporal evolution of HIV-1 in the infected person, from transmission, through the asymptomatic phase, and disease progression. Adapted from [38]

which encodes a truncated protein that is not expressed at the cell surface, was associated with nearly complete resistance to HIV-1 infection, and heterozygosity was correlated with slower rates of disease progression [51-54]. To date, CCR5 Δ32 homozygosity remains the only molecularly understood basis for resistance to HIV infection, although the protection is not absolute and additional unrelated genetic factors (often associated with innate or adaptive immunity) certainly influence susceptibility to HIV infection and disease progression [55-63]. A variety of genetic and regulatory factors related to the coreceptors and their chemokine ligands appear to have profound influences on HIV transmission and disease [64-67]. Recent findings suggest that the protective effects of the CCR5 Δ32 allele may reflect not only the absence of the functional coreceptor, but also on down-modulating activity of the mutant protein on CXCR4 surface expression [68]. While the absence of functional CCR5 was once thought to be innocuous, recent findings have indicated a protective role of the wild type allele in controlling the neuropathogenicity of West Nile Virus [69].

Novel anti-HIV Strategies Based on Coreceptors

The coreceptor discoveries, coupled with the resulting insights into the 2-step Env-receptor engagement mechanism, have engendered entirely new approaches to treat and/or prevent HIV infection by blocking the critical gp120/corecetor interactions required for HIV entry [70-77]. Such strategies can be based on directly targeting the coreceptors with coreceptor-binding agents (e.g. low molecular weight drugs, antibodies, chemokine derivatives) or coreceptor down-modulating treatments (e.g. gene therapy modalities including antisense or siRNA, "intrabodies", "intrakines", CCR5 Δ32); alternatively, the targets can be the corresponding binding sites on gp120. The former approach has the advantage of targeting a host gene product that will not mutate under selective pressure, and the near-normal physiology of individuals lacking a functional CCR5 gene suggests this molecule may be relatively dispensable for normal health; however several concerns arise, including the ability of escape mutants to use CCR5 in the presence of a small molecule CCR5 blocking agent [78-81], and the potential for such agents to select for CXCR4-using variants, perhaps pre-existing in the viral quasispecies [82]. The latter approach has the advantage of targeting highly conserved determinants on gp120, but suffers from the "conformational masking" phenomenon [44] noted above whereby these regions are inaccessible or unformed prior to CD4 binding; indeed antibodies against the conserved CD4-induced epitopes involved in coreceptor binding are generated at high frequencies in infected persons [83], but their minimal neutralizing activities provide little protection other than to force the virus quasispecies to maintain the "masked" conformation. A chimeric bifunctional protein containing soluble CD4 linked to a single chain fragment of an antibody against the conserved CD4-induced coreceptor-binding region of gp120 shows some promise as an antiviral strategy [84] (Fig. 5).

While antiviral strategies based on the HIV Env/coreceptor interactions have mostly focused on therapeutic agents to treat infected individuals, the coreceptors figure prominently in current perspectives on prevention of HIV infection. Vaccine approaches aimed at eliciting neutralizing antibodies must focus on the need to inhibit R5 variants [47, 48]. The rapidly emerging field of topical microbicides to prevent HIV-1 sexual transmission [85] has likewise focused on the critical importance of blocking R5 HIV-1, us-

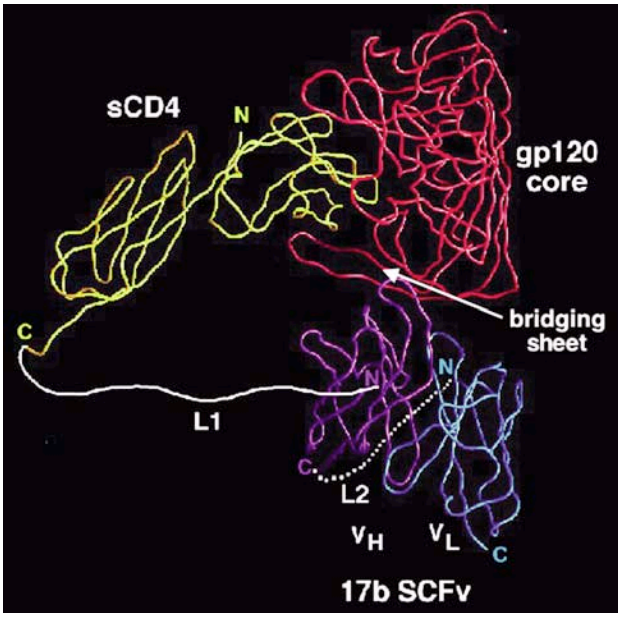

Fig. 5. sCD4-17b bifunctional recombinant protein, based on structure of Kwong et al. [42]. A soluble fragment of CD4 is attached via a flexible polypeptide linker to a single chain fragment of the 17b Mab against the CD4-induced bridging sheet of gp120. The protein displays potent neutralization of primary HIV-1 strains. Adapted from [84]

ing a variety of agents including small molecule CCR5 inhibitors [86] and chemokine derivatives [87].

HIV Coreceptors: A View to the Future

The past decade has witnessed an explosion of research activity on HIV interaction with coreceptors and their chemokine ligands. Fundamental new insights have been gained into the mechanism of HIV fusion/entry, factors affecting the natural history of HIV transmission and disease progression, and development of novel concepts for treating and preventing HIV infection. Yet profound gaps in our understanding remain, and it is likely that the coming years will provide answers to some of the most pressing questions.

Concerning the molecular mechanism of entry, the understanding of gp120 interaction with coreceptors still remains quite rudimentary at the molecular level. Biochemical, genetic, immunochemical, and structural analyses have implicated the critical importance of the sulfated N-terminus and the 2nd extracellular loop of CCR5 [88]; on the gp120 side, the bridging sheet and third variable (V3) loop are directly involved, with sequences in the latter domain determining the coreceptor usage phenotype (R5, X4, R5X5) [89]. The structures of CCR5 and CXCR4 await determination at the atomic level, a particularly challenging task in view of the fact that this has been achieved for only one GPCR: bovine rhodopsin. Some useful insights might be anticipated from crystal structures of gp120 in complex with coreceptor-based peptides, since several of these have been shown to interact specifically with gp120 (see Berger and Alkhatib, this volume).

The coreceptor discoveries have taken us a long way toward understanding the cellular tropism of different HIV isolates in vitro, and have also provided entirely new perspectives on the natural history of HIV infection in vivo. Yet the selection pressure(s) that govern the preferential R5 transmission (suppression of CXCR4-using variants?), the eventual appearance of X4 and R5X4 variants during disease progression in many cases, and the apparent greater pathogenicity of CXCR4-using HIV remain major mysteries. In all likelihood, a multitude of interrelated processes contribute to these phenomena [90, 91]. Recent studies have suggested that neutralizing antibodies might exert preferential suppressive action against CXCR4-using variants [92, 93].

In the coming years, we are likely to see the development of coreceptor-based antiviral strategies. Indeed, the orally bioavailable CCR5 blocking agent Maraviroc (Pfizer, trade name Selzentry) [94] is the first such therapeutic to be approved by the U.S. Food and Drug Administration, for use in combination with other antiretroviral drugs in adults harboring CCR5-using HIV-1 and showing elevated virus levels in blood [95]. Other compounds in this class are being actively developed, as are coreceptor targeted proteins such as antibodies and chemokine derivatives. Thus the HIV coreceptor story is likely to continue its rapid expansion in the years to come, hopefully with fundamental basic insights and major new applications waiting over the not-too-distant horizon.

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