[Frontiers in Bioscience 3, d44-58, January 1, 1998]

CHEMOKINE RECEPTORS AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Received 12/15/97 Accepted 12/19/97

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1. ABSTRACT

Primate lentiviruses infect target cells by interacting with the cell surface protein, CD4 and additional molecules, termed coreceptors. Recently, HIV-1 coreceptors have been identified as seven transmembrane spanning, G-protein coupled receptors of the chemokine receptor family. Thus, expression of CD4 and an appropriate coreceptor is both necessary and sufficient to render target cell permissive for fusion with virions or infected cells. The spectrum of tissue tropisms exhibited by primate lentiviruses can be largely explained by differential utilization and distribution of coreceptors. This article reviews what is currently known about the selective utilization of particular coreceptors by primate lentiviruses and the nature of the envelope/coreceptor interaction, with particular reference to two important HIV-1 coreceptors, CCR-5 and CXCR-4. It has become clear that these interactions are somewhat 'plastic': Variability is evident, both in the selection of coreceptor and the way in which different viral strains interact with their cognate coreceptors. The implications of these findings both for attempts to block HIV infection with coreceptor targeted agents and for understanding HIV replication *in vivo* is discussed.

2. INTRODUCTION

In common with all other retroviruses, the initial event in the life cycle of primate lentiviruses is association with specific cell surface receptors that mediate virus entry. Since the identification of CD4 as an essential component of the receptor for HIV and SIV more than a decade ago (1), it has been widely appreciated that additional cellular molecules are required for the entry of HIV/SIV into target cells (2-4; figure 1). It was shown at an early stage that the expression of CD4 on the surface of human cells could render them susceptible to infection by HIV-1 virions or HIV-1 enveloped pseudotypes, and also permitted envelope induced cell fusion (1). Conversely, expression of human CD4 on the surface of murine cells did not confer susceptibility to infection, although CD4-positive murine cells are fully competent for binding the viral envelope protein gp120. Later, it became clear that this observation also held true for HIV-2 and SIV and was not specific to murine cells (2-4). In fact, almost all non-human cells remain refractory to HIV-1 infection even when engineered to express human CD4, (although HIV-2 and SIV are somewhat less species restricted). In addition, it was shown that the resistance of CD4-positive murine fibroblasts to HIV-1 could be reversed by fusion with CD4-negative human cells, indicating that there is no dominant block to virus entry (5). Taken together, these observations strongly implied the existence of additional human cell specific cell surface molecules that participate in virus entry. Until recently, the identity of these `coreceptors' had remained elusive. However the identification of several chemokine receptors as molecules that, in conjunction with CD4, can mediate the entry of HIV and/or SIV into target cells has led to an explosion in interest in this field, and a very rapid advance in our understanding of how primate lentiviruses infect target cells.

Table 1. Coreceptor utilization by primate lentiviruses

VIRUS STRAIN		CORECEPTORS UTILIZED[a]						
		CCR-2b	**CCR-3**	**CCR-5**	**CXCR-4**	**GPR-15** (BOB)	**STRL-33** (Bonzo)	**GPR-1**
HIV-1	Primary non SI or M-Tropic	1/18	6[b]/22	46/46	1/46	4/13	4[c]/14	0/3
	Primary SI	1/10	2/10	13/25	24/24	1/3	1/3	0/1
	T-cell line adapted	0/2	0/2	2/4	4/4	0/1	1[d]/1	
HIV-2		0/4	5/10	10/13	6/17	5/6	4/6	
SIV		0/4	0/4	9/9	0/9	3/3	3/3	2/2

Notes
[a] Number which utilize the coreceptor / number tested, (data from references 11, 12, 20, 24-37, 49, 56, 62, 66, 79, 91).
[b] includes 1 strain, JRFL for which contradictory data have been reported
[c] includes 3 strains for which contradictory data are reported
[d] IIIB strain, contradictory data reported.

3. HIV AND SIV TROPISM

Additional evidence for the existence of primate lentivirus coreceptors derived from the observation that HIV/SIVs have a range of overlapping but readily distinguishable tropisms. For example, while many immortalized human T-cell lines can be infected by laboratory adapted HIV-1, HIV-2 and SIV strains, other CD4 positive cell lines are sometimes selectively permissive only for specific virus types (4). Heterogeneity in tropism is also evident within a given virus type. In the case of HIV-1, virtually all strains replicate efficiently in CD4+ peripheral blood lymphocytes (PBL). However many freshly isolated or primary strains are also able to propagate efficiently in primary macrophages but not in immortalized T-cell lines and are thus termed M-tropic. Conversely, other strains, particularly those which have been adapted to growth in immortalized T-cell lines (T-tropic) generally do not efficiently infect primary macrophages (6-9). A proportion of isolates (termed dual-tropic) are able to replicate well in both T-cell lines and primary macrophages in addition to PBL (10-12). Dual-tropic and T-tropic strains induce cell fusion in primary and immortalized T-cells and are also referred to as syncytium inducing (SI).

In almost all cases, the determinants of HIV/SIV tropism can be mapped to the viral envelope gene (13-19), thus indicating that tropism is restricted at the level of virus entry. A significant finding was that a major determinant of HIV-1 T-verses M-tropism is the third hypervariable (V3) region of the surface glycoprotein, gp120 (13-15). While other regions of the viral envelope (notably V1) have also been also shown in to influence HIV-1 tropism (17-19) it remains true that the tropism of a particular strain is very often determined by the V3 region of the viral envelope.

An important prediction arising from these data was that T-tropic and M-tropic HIV-1 strains, as well as HIV-2 and SIV, would make use of distinct coreceptors for virus entry. As we will discuss, this prediction proved at least partially accurate, and it appears that the role of the envelope in determining viral tropism is manifested at the level of coreceptor selection. In addition, there is variation in the 'promiscuity' of viral envelopes in that some are restricted to the use of a single coreceptor, whereas others are able to interact with several. It is becoming clear that (with a few exceptions) the spectrum of tropisms associated with primate lentiviruses can be largely explained by coreceptor utilization and distribution

4. CHEMOKINE RECEPTORS: ENTRY COFACTORS FOR HIV AND SIV

A large body of recent work has established that several chemokine receptors are capable of mediating cell fusion and infection by HIV and SIV strains. The crucial advance was made by Feng *et al*. (20) who identified Lestr/fusin, a member of the 7 transmembrane (7TM) spanning, G-protein coupled receptor family, as a fusion cofactor for T-tropic HIV-1 strains. Thus, expression of CD4 and fusin on the surface of murine cells was sufficient to render them susceptible infection by T-tropic HIV-1 strains. Fusin is expressed on a wide range of human cell lines, those which do not express fusin are not infected by T- tropic HIV-1. Importantly, cells expressing both CD4 and fusin remained refractory to M-tropic HIV envelope mediated fusion, consistent with the earlier prediction that fusion cofactors for T- and M-tropic HIV-1 strains would be distinct moieties. Furthermore, a CXC chemokine (SDF-1), has subsequently been identified as a ligand which both binds fusin and specifically blocks infection by T-tropic but not M-tropic HIV-1 strains (21,22). Based on the identification of its chemokine ligand, fusin was renamed CXCR-4, the term used for the remainder of this article.

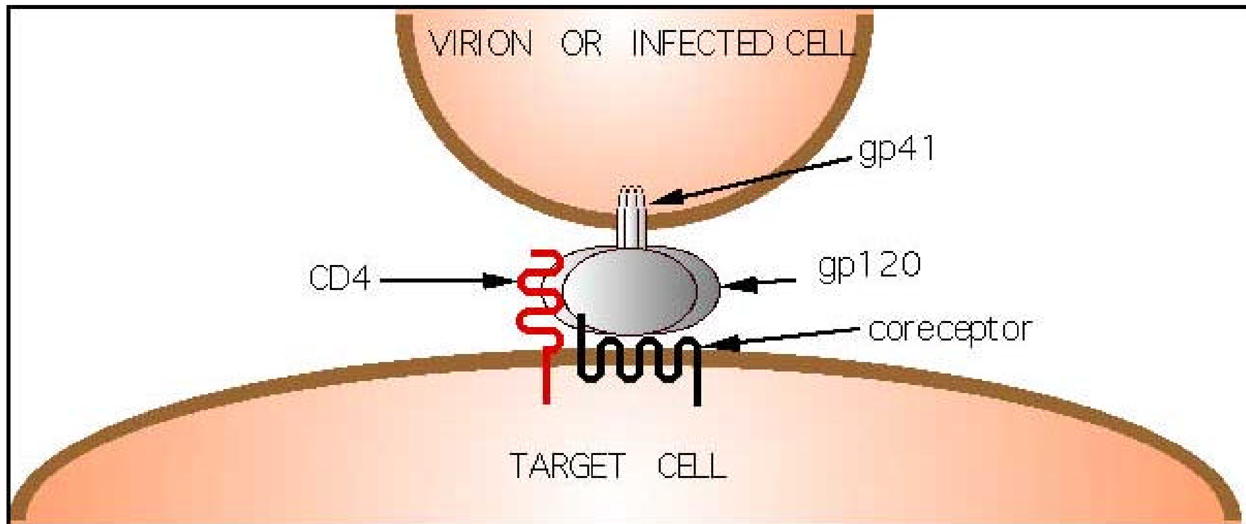

Figure 1. HIV envelope induced membrane fusion requires both CD4 a coreceptor. Expression of CD4 on the surface of a target cell is fully sufficient to permit envelope binding, but additional cell surface molecules are required for completion of the fusion reaction. Evidence suggests that the initial interaction of the viral envelope gp120 protein with CD4 triggers a conformational change enabling further gp120 interactions with the coreceptor. The identity of particular virus coreceptors and sequences which participate in the envelope interaction is isolate dependent. Following coreceptor binding, addtional conformational changes are thought to occur, resulting in the fusion of virus and target cell membranes.

4.1 Additional Coreceptors for HIV-1, HIV-2 and SIV strains

The discovery that CXCR-4, whose closest known homolog is the IL-8 receptor (CXCR-2), constitutes a functional coreceptor for T-tropic HIV-1 strains gave added significance to the earlier finding that the C-C chemokines MIP-1-alpha, MIP-1-beta and RANTES are able to block infection of CD4+ human T-cells by M-tropic but not T-tropic HIV-1 (23). Thus, since certain chemokines are known to bind several 7TM receptors it became likely that discovery of a chemokine receptor that is recognised by all three of these chemokines would also result in the identification of an M-tropic HIV-1 specific coreceptor.

Thus, very soon after the identification of CCR-5 as a receptor for MIP-1-alpha, MIP-1-beta and RANTES a number of groups independently showed that CCR-5 served as a coreceptor for a wide range of M-tropic strains (24-28). In fact, to date all M-tropic HIV-1 strains (which includes isolates of very diverse genotypes (12, 24, 29)) that have been analysed for coreceptor usage have been shown to be capable of utilising CCR-5 (table 1 and refs therein). Expression of this coreceptor is largely confined to primary T-cells and macrophages, consistent with its role as a coreceptor for M-tropic HIV-1 strains. Nevertheless, it soon became clear that a more restricted subset of M-tropic HIV-1 strains could also recognise a related chemokine receptor, CCR-3 (25), and one particularly promiscuous strain (89.6) could make use of no less than 4 distinct coreceptors; CCR-5, CXCR-4, CCR-3 and CCR-2b (27). In addition, other primate lentiviruses can exploit these and other cell surface molecules during virus entry (30-37, table 1). HIV-2 strains are capable of using a number of of the same coreceptors as HIV-1 including CCR5, CCR-3 and CXCR-4 as well as other recently identified coreceptors (33, 37, table 2). A number of SIV strains are capable of utilising CCR-5, but usually not CXCR-4, as a coreceptor (30-35). However, since the same SIV strains are fully capable of replication in a number of human T-cell lines which do not express significant levels of CCR-5, it was immediately apparent that additional SIV specific coreceptors should exist (32). The recent identification of three additional coreceptors, Bonzo/STRL33, BOB/GPR15 and GPR1 which support infection by SIV strains was thus predictable (32-35). The susceptibility of CCR-5 negative cell lines to SIV infection can, in at least some instances, be explained by the presence of Bonzo/STRL33, BOB/GPR15 and/or GPR1. Interestingly, Bonzo/STRL33 and BOB/GPR15 are also functional for some HIV-1 and HIV-2 isolates (33, 35, 36). While both are expressed on primary T-cells, and Bonzo/STRL33 is expressed on monocytes, what contribution these coreceptors make to the establishment and maintenance of HIV-1, HIV-2 or SIV infection *in vivo* is not yet known. The resistance of CCR-5 negative primary T-cells and macrophages cells to M-tropic HIV-1 infection (see below) suggests that these receptors play a relatively minor, if any role in HIV-1 infection *in vivo* (38, 39). However the ability of some dual-tropic HIV-1 strains to infect macrophages when they are apparantly incapable of infecting transfected cells where CCR-5 is the only expressed coreceptor (11), potentially indicates a role for Bonzo/STRL33, or another as yet unidentified coreceptor.

4.2 CCR-5 is the major coreceptor utilized by HIV-1 *in vivo*

A long observed, but poorly understood phenomenon associated with the HIV-1 epidemic is the existence of individuals who have been repeatedly exposed to virus but remain uninfected. In some of these cases, a substantial *in vitro* infection resistance (specifically to CCR-5 utilizing, M-tropic strains) of PBL and macrophages is evident (38, 39). In addition, it is well known that there is large variation in the rate at which disease progresses in individuals who do become infected. Analysis of CCR-5 genes has revealed the existence of a defective CCR-5 allele which may contribute to each of these observations (40-42). Specifically, approximately 10% of the CCR-5 alleles in Caucasian populations contain a 32 base pair deletion which results in a frame shift and premature truncation of the receptor. These truncated receptors are not expressed on the cell surface and therefore do not function as HIV coreceptors. About 20% and 1% of Caucasians are heterozygous and homozygous, respectively, for the CCR-5 delta32 allele and, importantly, homozygousity for the CCR-5 delta32 allele is associated with a significant degree of infection resistance (41, 42). In fact only four individuals (out of several thousand examined) have been shown to be both HIV infected and homozygous for CCR-5 delta32 (43-46), whereas the expected frequency of homozygotes would be approximately 10 per 1000 HIV-1 infected individuals, given the frequency of this genotype in the general population. Individuals who are heterozygous for the two major CCR-5 alleles do not manifest a high degree of infection resistance (41, 42). However, once infected, the progression of disease in heterozygotes appears to be somewhat retarded. This phenotype is associated with a measurably reduced virus load post seroconversion, and a decrease in frequency of symptomatic primary infection (47). Taken together, these observations strongly imply that the major route of HIV transmission both between individuals and between cells within an individual (at least during the early stages of infection) is mediated by the CCR-5 coreceptor.

CCR-3 is only expressed in a restricted sub-population of T-cells (48) and is, therefore, unlikely to play a major role in HIV infection of these cells. However, it is relatively abundant on microglial cells, the major targets of HIV-1 in the brain (49). Eotaxin, the physiological ligand for CCR-3, and a CCR-3 reactive monoclonal antibody have both been reported to posses infection inhibiting properties in primary brain cultures. M-tropic strains that are unable to use CCR-3 can also infect brain cultures, suggesting that both CCR-3 and CCR-5 play a role in infection of these cells.

An interesting, but as yet unexplained, observation is association of a CCR-2b polymorphism with retarded disease progression (50). This is unexpected given that the great majority of HIV-1 strains are not able to use CCR2b as a coreceptor. The mutant CCR-2b allele, which encodes a receptor with a single valine to isoleucine change, is invariably associated with an intact CCR-5 allele. Since the two genes are very closely linked, it is quite likely that the mutant CCR-2b gene is associated with some, as yet unidentified, defect in CCR-5 expression, although this has not yet been thoroughly investigated. Wide variation of CCR-5 expression levels among individuals homozygous for intact reading frames has been documented, as has a measurably lower expression level in CCR-5 delta32 heterozygotes (51). It might well be that coreceptor expression levels contribute to the very large variation in rates of disease progression, particularly since the amount of CCR-5 expressed on PBL from different donors correlates with their ability to support the replication of M-tropic strains *in vitro* (51).

5. MECHANISM OF ACTION OF CORECEPTORS

Accumulating evidence indicates that the SU envelope glycoproteins of primate lentiviruses directly interact with their cognate coreceptors (52-56). Soluble gp120 from T-tropic HIV-1 has been reported to form a precipitable complex with CXCR-4 (52) and specifically bind to cells expressing CXCR-4 with an affinity in the nanomolar range (53). In addition, a few groups have demonstrated that HIV-1 and SIV envelope glycoproteins are able to compete with beta chemokines for CCR-5 binding (54-56). Significantly, this competition is much more efficient in the presence of CD4, either coexpressed with CCR-5 on the cell surface, or in soluble form as a pre-formed complex with gp120. The inference from these data is that the interaction of HIV or SIV with a target cell is initially with CD4, thereby inducing a conformational change in gp120 which facilitates subsequent binding of envelope to the coreceptor. This scenario is supported by the previous observation that a number of HIV-2 strains can be induced to infect CD4 negative cells by pre-treatment with sCD4 (57). In this case a virion/sCD4 interaction presumably induces a stable envelope conformation that enables interaction with a coreceptor in the absence of cell surface CD4. Why this phenomenon should be restricted to HIV-2 remains obscure, but perhaps sCD4 complexes with the gp120 of other primate lentiviruses adopt a coreceptor-binding conformation that is less stable than that obtained with HIV-2. The ability of HIV-2 to be adapted to efficiently infect CD4 negative cells, using either CXCR-4, CCR-3 or the orphan receptor V28 (57-59), probably represents an extension of this phenomenon. In this case, the viral envelope presumably adopts a stable CXCR-4 binding conformation in which the coreceptor binding site is revealed without the need for prior 'activation' by CD4.

5.1 Envelope binding sites on virus coreceptors

All of the identified HIV/SIV coreceptors are members of the seven transmembrane spanning G-protein coupled receptor family. As such, they contain an N-terminal extracellular domain and three extracellular loops (ECL-1-3). A number of studies have attempted to determine which of these extracellular domains are determinants of coreceptor function, and by implication, what are the amino acid residues that interact with the virus envelope (60-67). In most cases, the functional coreceptor phenotype cannot be mapped to a single linear sequence. It is therefore likely that the virus binding site or sites on the coreceptor involve

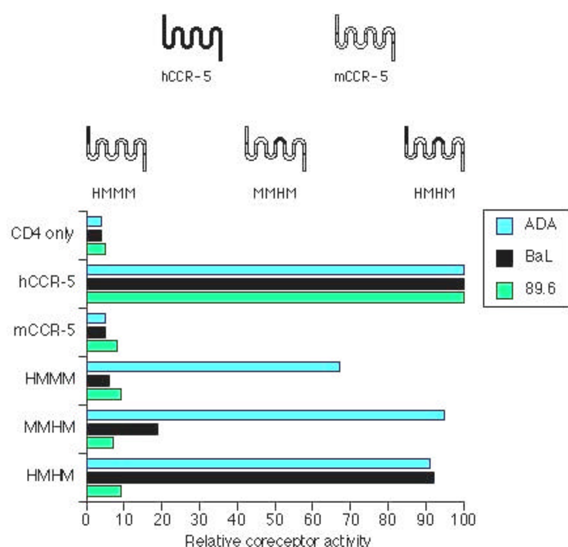

Figure 2. Examples of differential utilisation of human/murine CCR-5 chimeras by M-tropic and dual-tropic HIV-1 strains. The relative ability of human/murine CCR-5 chimeras to support fusion induced by the envelopes of two M-tropic (ADA and Ba-L) and one dual-tropic (89.6) HIV-1 strain is indicated. While ADA is efficiently able to utilize chimeras containing a single human extracellular domain, BaL requires the presence of two, and 89.6 requires an intact human CCR-5 molecule.

multiple extracellular domains, and that within each of these domains multiple amino acids contribute to coreceptor function. While this complexity has largely frustrated attempts to define precise virus binding sites, studies of chimeric and mutant coreceptors have, to some extent, illuminated our understanding of how HIV/SIV interact with coreceptors and suggested that several, functionally redundant, interactions between coreceptor and virus envelope are likely to occur. As we will discuss, this has implications both for the feasibility of using coreceptor targeted inhibitors of virus entry as therapies and for understanding the evolution of coreceptor usage *in vivo*.

5.2 Determinants of CCR-5 coreceptor function

Historically, the generation of chimeric molecules consisting of a functional retrovirus receptor and a closely related, but non-functional homologue has proven to be a successful strategy for rapidly mapping the determinants of virus receptor function. Several studies have attempted to map the extracellular determinants of CCR-5 coreceptor function using such an approach (60-66). Fortuitously, a murine homologue of CCR-5 had been previously identified that is 82% identical to the human receptor but non-functional as a coreceptor for a all HIV-1 strains tested so far. At least four groups have used human/murine CCR-5 chimeras to map functional domains (60-63). There is broad agreement in the general conclusions among these three studies, some discrepancies are probably the result of methodological difference, details in the derivation of the chimeric coreceptors and polymorphisms in murine CCR-5 alleles. Of the four extracellular CCR-5 domains, human and murine proteins differ in sequence in three. Remarkably, each of these extracellular human CCR-5 domains is independently capable of conferring some degree of coreceptor function on an otherwise inactive murine receptor (60-62). Conversely there is no single extracellular domain of human CCR-5 that cannot be functionally substituted by its murine counterpart. (at least in the case of most murine CCR-5 alleles) Importantly, these chimeric coreceptors are often functional for a significantly more restricted range of HIV-1 strains; the differential abilities of virus strains to utilize various chimeric receptors strongly implies that different virus strains interact with CCR-5 in different ways (61, 62). Examples of the strain dependent differences in the ability of HIV-1 isolates to utilize human/mouse chimeric CCR-5 receptors are presented in figure 2.

Some strains, in particular those which are dual-tropic (i.e. are also capable of using CXCR-4 as a coreceptor) appear to be particularly sensitive to perturbations in the human CCR-5 extracellular domains. In fact, none of the three human CCR-5 extracellular domains that differ in sequence to the murine homologue can be replaced by murine sequences without (at least partially) compromising dual-tropic coreceptor function (61, 62). This is a suprising observation, since human and mouse CCR-5 receptors are much more similar in sequence than are CCR-5 and CXCR-4, a fully functional coreceptor for dual tropic HIV-1 strains. In contrast the M-tropic strains ADA and SF162 and are able to quite efficiently utilize chimeric coreceptors where any one of the 3 divergent extracellular domains is of human origin (61, 62, figure 2). Other M-tropic strains such as Ba-L, M23 and E80 have intermediate properties, in that a larger number of chimeras are functional than for dua-tropic strains, but some chimeras which are functional ADA or SF162 coreceptors do not support fusion mediated by these additional envelopes. For example, while no single human extracellular domain is sufficient to confer high level Ba-L coreceptor function on murine CCR-5, (figure 2) inter-domain synergy can be observed; chimeras containing two extracellular domains of human CCR-5 origin exhibit near wild type Ba-L coreceptor function, while each individually has little or no activity in the context of an otherwise murine receptor.

Interestingly the results obtained using a CCR-5 allele from an NIH Swiss mouse for chimera construction differ significantly from those obtained using other murine CCR-5 alleles (63). It is apparent that murine CCR-5 polymorphisms are largely responsible for this discrepancy, and implicate specific amino acids in the envelope/CCR-5 interaction (see below). It is noteworthy, therefore that given the complexity of envelope coreceptor interactions, in particular the participation of multiple coreceptor domains, non-functional CCR-5 homologues are unlikely to provide a neutral background for analysis using chimeras or site directed mutant receptors. Thus, which extracellular domains in CCR-5 that are 'flagged' as being important for coreceptor function is dependent not only on the virus strain, but also on the non-functional partner selected for the generation of chimeras.

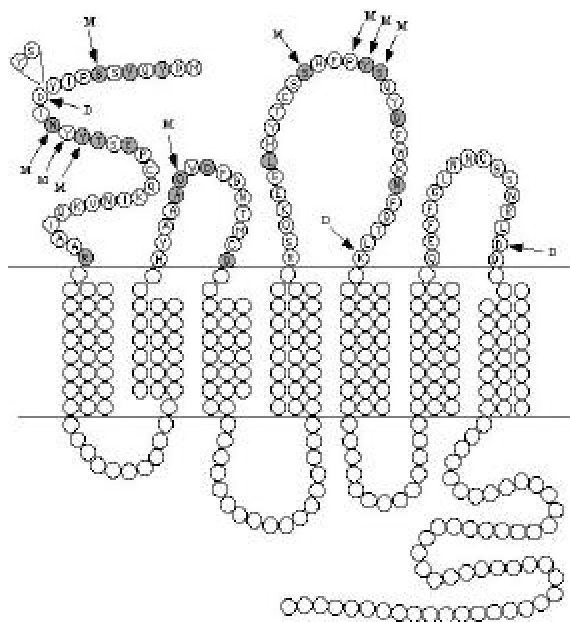

Figure 3. Summary of mutational analyses of CCR-5. Amino acid residues that differ between human and murine (BALB/c) CCR-5 are indicated by shading. Arrows indicate ammino acids that when mutated either individually or in combination compromise coreceptor function for M-tropic (M) or dua-tropic (D) HIV strains. Note that some mutations are performed in the context of human/mouse CCR-5 chimeras, where coreceptor activity is dependent on the presence of a single human extraceellular domain. (data from refs 63, 66, 67)).

This premise is largely born out when the above studies are viewed in conjunction with others which have employed CCR-5/CCR-1, CCR-5/CCR-2b, CCR-5/CXCR-2 and CCR-5/CXCR-4 chimeras to map determinants of coreceptor function for both HIV-1 and SIV strains (64-67). However, it is clear that the N-terminal extracellular domain of CCR-5 is capable of conferring coreceptor function for some HIV-1 M-tropic and dual-tropic strains on multiple, otherwise inactive, 7TM receptors (65). Loss of function as a result of partial truncations in the CCR-5 N-terminus, in the context of a CCR-5/CCR-2b chimera, support the role of this domain in coreceptor function, although the particular amino acids involved are clearly strain dependent (64). As with human/murine CCR-5 chimeras however, the N-terminus is dispensable in an otherwise intact human CCR-5 molecule, since it can be truncated or replaced with those of CCR-2b, CCR-1, CXCR-2 and CXCR-4 without drastically compromising M-tropic coreceptor function (64, 65). Dual-tropic strains are again more fastidious than M-tropic strains in their ability to interact with N-terminally substituted CCR-5 chimeras; of the aforementioned 7TM receptors, only the CCR-1 N-terminus can functionally replace that of CCR-5, and then only for one of the two dua-tropic strains analysed (65). These data have been interpreted as suggesting that dual tropism evolves from M-tropic viruses that retain the ability to interact specifically with the N-terminus of CCR-5 while acquiring the ability to interact with the ECLs of CXCR-4 at the expense of interaction with CCR-5 ECLs. We would argue that the interaction of dua-tropic envelopes with CCR-5 also remains critically dependent on CCR-5 ECLs, since replacement of these with corresponding murine CCR-5 sequences results in a dramatic loss of coreceptor function (61, 62, figure 2).

Mutational analyses of CCR-5 extracellular domains in the context of an otherwise intact molecule has not proven to be particularly informative for M-tropic HIV-1, since many point mutants retain substantial coreceptor function (66). Nevertheless, partial or total loss of dua-tropic coreceptor function (dependent on the particular strain) as a result of an aspartate to alanine mutation at position 11 in the N-terminus has been documented (66). The partial effect of this mutation for the 89.6 strain is markedly enhanced when combined with alanine substitution of lysine 197 (ECL2) and/or aspartate 276 (ECL-3). Again this, indicates that the CCR-5 N-terminus is a critical, but not the sole, determinant of dua-tropic coreceptor function.

In the case of M-tropic HIV-1 strains, studies in our laboratory have exploited human/murine CCR-5 chimeric coreceptors whose activity is dependent on the presence of a single human CCR-5 extracellular domain (68). Residues of human origin were substituted, either individually or in combination, with corresponding murine sequences. These experiments indicate an important role for residues serine 7, asparagine 13, and tyrosine 15 in the N-terminus and serine 180 in ECL-2. In addition, individual amino acids were identified (for example tyrosine 184 and serine 185) that had little or no effect on coreceptor function when mutated individually, but dramatic losses in coreceptor result when both are substituted. Thus, it is evident that not only do multiple CCR-5 domains contribute to coreceptor function, but within these domains multiple amino acids play an important role.

In addition, the identification of non functional CCR-5 alleles from African green monkey and NIH Swiss mouse has indicated an especially critical role for tyrosine 14 in the N-terminus, glutamine 93 in ECL-1 and proline 183 in ECL-2 (63). Indeed, mutation of tyrosine 14 or proline 183 in an otherwise intact CCR-5 molecule renders it non-functional as a coreceptor for several M-tropic HIV strains.

A summary of the mutational analyses performed to date is depicted in figure 3. Although residues which are important for function are somewhat scattered throughout the extracellular domains, several important residues are closely clustered within the N-terminus and in ECL-2. As yet, we cannot determine whether these sequence motifs constitute spacially distinct envelope recognition domains, or form an single envelope binding site in the context of a folded protein. Alternatively some of these amino acids could be responsible for influlencing the overall structure of the CCR-5 extracellular domains and modulate coreceptor function without directly contacting the viral envelope.

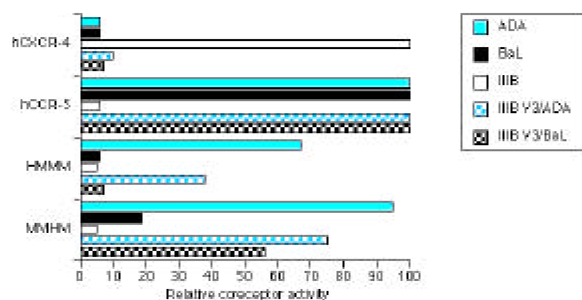

Figure 4. The role of the V3 loop in determining coreceptor selection and in modulating envelope CCR-5 interactions. The ability of the T-tropic strain IIIB to use CXCR-4 but not CCR-5 is reversed by the substitution of V3 sequences with those of M-tropic strains ADA or BaL. IIIB strains containing V3 loops of either Ba-L or ADA are differentially able to utilise human/murine CCR-5 chimeras. A single amino acid change in the V3 loop influences the viruses ability to use a chimera containing a human N-terminal domain, but has little effect on utilization of a chimera containing human ECL-2 in an otherwise murine CCR-5 context.

The CCR-5 sequences involved in recognition by other primate lentiviruses are less well characterized. However, in the context of CCR-5/CCR-2b chimeras, ECL-2 is required for recognition by several SIV strains (67). Like HIV-1 strains, different SIV strains use CCR-5 in different ways: For other SIV strains, ECL-2 is dispensable provided that an intact CCR-5 N-terminus is present.

5.3 Determinants of CXCR-4 coreceptor function

With the aim of adopting similar approaches to analyse structure function relationships in CXCR-4, we and others have cloned the murine homolog of this receptor (69-71). Unfortunately, murine CXCR-4 was found to constitute a functional coreceptor for at least some T-tropic and dua-tropic HIV-1 strains (71,72), precluding the construction of informative chimeras. In addition, both rat and feline CXCR-4 receptors were also found to support infection by at least some T- tropic HIV-1 strains (73, 74). These observations were suprising given the large body of literature indicating that almost all non-human cells remain refractory to HIV-1 infection when engineered to express human CD4, whereas most human cell lines are highly susceptible to T-tropic HIV-1 infection when CD4 is expressed (1-4). This apparent anomaly can only partially be explained by species specific differences in expression patterns. While we obtained no evidence for differential expression of CXCR-4 mRNA in vivo (in mouse versus human tissues) it does appear that CXCR-4 expression is somewhat more restricted in immortalized murine cell lines as compared to human counterparts (71). However, there are examples of both murine and feline cells that contain abundant CXCR-4 mRNA but after CD4 expression, remain resistant to T-tropic HIV-1(74, 75). Species specific post-translational processes are also unable to account for this anomaly, since transfection of CXCR-4 negative fibroblasts with CD4 plus either human or murine CXCR-4 renders them permissive for HIV-1 fusion or infection (71, 72). It remains unclear why some cells remain resistant to infection when they apparently express a full complement of receptors that are capable of supporting fusion/infection in the context of a different cell line of the same species. Indeed, human macrophages express CXCR-4 but are not susceptible to T-tropic HIV-1 infection. It may be that the apparent need for relatively high levels of CD4 expression by CXCR-4 utilizing HIV-1 strains is a limiting factor for infection in some cases (76). Alternatively, it is possible that there are cell type specific, post-fusion blocks to virus infection (77, 78).

Although a rat CXCR-4 homolog was found to be a functional coreceptor for the T-tropic isolate LAI, it is non-permissive for the T-tropic HIV-1 isolate NDK and the HIV-2 isolate ROD. Thus, human/rat CXCR-4 chimeras have been used to map functional determinants for these strains (79). Suprisingly, the sequence requirements for ROD and NDK are similar, in that both require the presence of a human CXCR-4 derived ECL-2, These two strains and LAI appear rather insensitive to deletions in the N-terminus, almost total removal of this region is required before complete loss of function is observed, although partial effects on NDK and ROD are evident with smaller deletions. A variant of ROD (ROD/B) which is capable of infecting cells expressing CXCR-4 in a CD4 independent manner shows a similar partial dependence on an intact N-terminal CXCR-4 domain (80).

CXCR-4 chimeras with more distantly related 7TM receptors such as CXCR-2 and CCR-5 indicate an absolute requirement for ECL-2 in this context (65). However, chimeras that, in addition, contain the CXCR-4 N-terminus and/or ECL-1 are more active, and are recognised by a wider range of strains. Thus, it appears that envelope-CXCR-4 interactions are as complex and strain dependent as is the case with CCR-5.

5.4 Viral determinants of coreceptor recognition

In many cases, transplantation of the V3 loop of HIV-1 can, in a absolute manner, change the tropism of a T-tropic to that of an M-tropic virus and vice versa (13-16). In fact, this phenotypic switch can be accomplished by minor changes in the V3 sequence, and correlates closely with a switch between CXCR-4 and CCR-5 coreceptor utilization (25, 61, 81). Furthermore, dua-tropic viruses that use both CXCR-4 and CCR-5 can be derived simply by manipulation of V3 loop sequences (81). Thus, the V3 loop is an excellent candidate for being a component of a coreceptor binding site. The additional observation that minor changes in V3 loop sequences influence the ability of envelopes to interact with chimeric coreceptors supports this hypothesis (61). For instance, the V3 loop sequences of ADA and Ba-L differ by a single amino acid, and while both are able to confer CCR-5 utilization on the T-tropic IIIB envelope, only the ADA V3 loop permits utililisation of an human/murine CCR-5 chimera containing the human CCR-5 N-terminus in an otherwise murine background. Similarly, two amino acid changes in the V3 loop of SIV mac236, influence the ability of this strain to recognise a panel of CCR5/CCR-2b chimeras. An example of the role of V3

sequences in determining coreceptor selection and influencing specificity for chimeric coreceptors is given in figure 4.

Nevertheless, the V3 loop is not the sole determinant of coreceptor recognition. In some cases, alternative tropisms can be mapped to non-V3 sequences (17-19), and while the V3 loop sequences of ADA and JRFL are identical, only the former envelope protein is capable of efficiently utilising BOB/GPR15 (33). In addition, the M-tropic Ba-L envelope does not efficiently utilise a human/murine CCR-5 chimera where only ECL-2 is of human origin. However, a chimeric virus containing a Ba-L V3 sequence in the context of the T-tropic IIIB envelope is able to use this chimera more efficiently (figure 4). This suggests, somewhat paradoxically, that the T-tropic IIIB envelope contains non-V3 sequences that facilitate interaction with the CCR-5 ECL-2.

While these observations provide genetic evidence for the interaction of multiple envelope domains with multiple coreceptor domains, it remains true that binding sites on coreceptors and envelopes cannot yet be unambiguously identified. In the context of proteins that are capable of induced conformational change and for which little or no structural information is available, the effect of introducing sequence changes on the conformation of distal regions of envelope and coreceptor is not easily predicted. It is also important to recognize that there is considerable scope for the introduction of laboratory artifacts in such experiments: The very act of culturing virus in the laboratory imposes different selective pressures on viral populations that are likely to impact on the nature of their interaction with receptors. In addition there are examples of very minor envelope sequence changes drastically influencing coreceptor recognition (60, 67, 81), thus different results are likely to be obtained with cloned versus uncloned forms of the same virus isolate. Furthermore, the relative expression levels of coreceptors on transfected cells, as compared to the natural targets of virus infection, remain largely unexplored and clearly affect the efficiency of infection. Indeed, over-expression of some chemokine receptors render cells permissive for viral envelope induced cell fusion, while cells expressing lower levels of the same coreceptor remain resistant to infection by cell-free virus (82). Therefore, a degree of caution is warranted in interpreting the results of the aforementioned studies. Despite these caveats, it is abundantly clear that there is considerable plasticity in coreceptor usage, both in terms of the viruses ability to use one or more of a number of 'optional' coreceptors and the ability of strains to use a given coreceptor in different ways.

6. PLASTICITY OF HIV CORECEPTOR USAGE - IMPLICATIONS FOR PATHOGENESIS AND TREATMENT

6.1 Coreceptor targeted inhibitors of HIV entry

The identification of coreceptors that mediate primate lentivirus entry might well have major significance for attempts to treat HIV infection. The chemokines themselves are inhibitors of HIV entry (21-23), and modified forms of RANTES, which bind CCR-5 yet fail to induce signal transduction, have similar properties (83, 84). Recently, beta chemokine homologues encoded by Kaposi's sarcoma associated herpesvirus have also been shown to block HIV-1 infection (85, 86). The lack of an overt phenotype associated with CCR-5 delta32, even among homozygotes (40, 42), suggests that antagonists targeted specifically to this coreceptor should be relatively free of undesirable side effects. However, this is less likely to be the case for CXCR-4 targeted antagonists; mice in which the SDF-1 has been ablated have a lethal defect in B cell lymphopoesis (87). Nevertheless, it might be possible to develop small molecule inhibitors that block viral interactions with CXCR-4 without compromising SDF-1 signal transduction, Peptide and bicyclam compounds targeted to CXCR-4 that prevent T-tropic HIV infection have been described (88-90). However, all of these compounds also block signalling through CXCR-4. A potentially even more serious obstacle in the development of coreceptor targeted threrapeutics is the plasticity of envelope coreceptor interactions. It is quite conceivable that coreceptor targeted compounds would simply select for strains that use an alternative coreceptor, or different regions of the same coreceptor. Furthermore, since AIDS has been documented in CCR-5 delta32 homozygotes, it is unlikely that even total ablation of the CCR-5 entry pathway will achieve much more than retardation of disease progression, once infection has been established. It is even possible that CCR-5 targeted therapy might accelerate the course of disease by selection of viral strains that use alternative coreceptors (most notably CXCR-4, but also including CCR-2b and CCR-3) whose occurrence is associated with disease progression (91). It remains possible, however, that combinations of agents that ablate the coreceptor function of both CCR-5 and CXCR-4 would have a significant impact on the ability of HIV to propagate in vivo.

6.2 Changing patterns of coreceptor usage during HIV-1 disease progression - cause or consequence of immune suppression?

It is interesting to speculate as to why primate lentiviruses have evolved plasticity in their interactions with coreceptors. The ability to use utilize multiple functionally redundant contacts with coreceptors could conceivable facilitate immunological escape. Thus, in the face of a neutralizing antibody response (which, in significant part, is directed against V3 sequences (92), a major modulator of coreceptor interaction), the selection of variants with altered envelope sequences would be permitted without compromising the ability of the virus to use a given coreceptor. Furthermore, changes in envelope sequence that enable the virus to use additional coreceptors, while retaining the ability to interact with CCR-5, could be tolerated. It appears that dua-tropic strains that use both CCR-5 and CXCR-4 are less tolerant of perturbations in CCR-5 sequence than are M-tropic strains (71, 72), suggesting that acquisition of the ability to utilize CXCR-4 might involve the sacrifice of a degree of functional redundancy and/or affinity in the envelope/CCR-5 interaction.

During the early, asymptomatic phase of HIV-1 infection M-tropic strains predominate. It is quite possible that this is the simply consequence of a 'founder effect': It is likely that the major route of transmission of HIV-1 is via infection using CCR-5 as a coreceptor, given the observation that CCR5 delta32 homozygous individuals are much less likely to be HIV infected than other genotypes. However, why do strains that also use CXCR-4 tend to arise only late in the course of infection, when in many cases they retain the ability to use CCR-5? While it has often been noted that SI viruses are associated with poor prognosis it is important to emphasise that the occurrence of SI viruses that can use CXCR-4 (and usually CCR-5) is only a correlate of disease progression (91, 93). We can as yet, draw no conclusions about causality. Although it is possible that a presumptive expansion in tropism might accelerate the course of HIV-1 disease, particularly since CXCR-4 is expressed on the naive T-cells subset from which CCR-5 is largely absent (94), it is equally possible that CXCR-4 utilizing viruses arise as a consequence of, rather than being the cause of immunosuppression. It appears that in addition to the selective transmission of M-tropic viruses, that there may, in fact, be an advantage to the virus in using CCR-5, at least early in infection. Indeed, viruses isolated from a laboratory worker who was accidentally infected with the exclusively T-tropic, IIIB strain became M-tropic with time (95). Although the coreceptor utilization profile of these strains have not yet been documented, a selective advantage for CCR-5 utilizing viruses early in infection is suggested. In addition, a chimeric virus (containing HIV-1 derived envelope sequences in an otherwise SIV genome) replicates to higher titers in macaques if the envelope is derived from the dua-tropic 89.6 strain than does a chimera containing the envelope of the T-tropic strain IIIB (96). Since precisely the opposite observation was made upon cultivation of these viruses in cultured macaque PBL, it is clear that the selective pressures on coreceptor utilization are different in vivo as opposed to in vitro. At least two scenarios could be envisaged that provide CCR-5 utilizing viruses with a selective advantage: Firstly, it is conceivable that a major selective influences are the result of envelope directed neutralizing immune responses, which could impose a restriction on the nature of coreceptor utilization. Indeed, neutralization phenotype and viral tropism (and therefore, presumably, coreceptor selection) are not independent properties of the viral envelope (97). A. general observation is that primary HIV-1 isolates (which tend to use CCR-5 but not CXCR-4) are somewhat more resistant to neutralization by naturally occurring HIV-1 antibodies than are laboratory adapted strains (98). Thus, it is possible that immunological constraints are placed on envelope sequence and conformation that could negatively influence the ability of the virus to use CXCR-4 as a coreceptor. Therefore, the occurrence of CXCR-4 utilising variants late in the course of disease might be a reflection of the infected individuals compromised ability to mount immune responses to new variants that are capable of utilising CXCR-4 that would have been readily neutralized earlier during the infection.

Conversely, a selective advantage for viruses which use CCR-5 may be immune response-independent. An equally plausible hypothesis is that CXCR-4 utilizing strains only become prevalent late in disease as a result of the selective depletion of CD4+ memory T-cells (the subset that preferentially expresses CCR-5). These are replaced at a high rate by naive counterparts which, (at least in uninfected individuals) express higher levels of CXCR-4 than of CCR-5 (94), thus providing T- or dual-tropic strains with a selective advantage.

Regardless of the selective pressures on coreceptor usage, the occurrence of SI viruses in only about 50% of patients with advanced disease indicates that the ability of the virus to use CXCR-4 is by no means necessary for the onset of immune suppression. Conversely, the fact that infected CCR-5 delta32 homozygotes also have progressive disease (43-46) indicates that disease causation cannot be universally ascribed to viruses that use this coreceptor either.

7. CORECEPTOR - GP120 INTERACTIONS: WHAT HAPPENS NEXT?

It is known that gp120 CD4 interaction induces conformational changes in envelope that expose previously concealed epitopes (99). These changes are not sufficient to induce membrane fusion, rather they are likely to facilitate coreceptor binding, Presumably coreceptor binding results in additional conformational modification, ultimately resulting in exposure of the gp41 N-terminal fusion peptide, although this remains to be proven. Are these events accompanied by conformational changes in the coreceptor, (potentially resulting in signal transduction) or does the coreceptor simply serve as a relatively rigid binding site with the bulk of the conformational change occurring on the viral side of the membrane fusion reaction?

The physiological function of 7TM receptors is to transduce signals via coupling to G-proteins. In several cases where post signalling events have been studied (using predominantly the beta 2 adrenergic receptor as a prototype) signalling is rapidly followed by receptor phosphorylation (mediated by specific G-protein coupled receptor kinases). Phosphorylation results in desensitization of the receptor and recognition by arrestins which are necessary for the subsequent internalisation and recycling of the phosphorylated receptor (100). We and others have investigated whether any of these processes are coupled to the function of CCR-5 as an HIV-1 coreceptor (101-103). Studies using mutant receptors which fail to couple to G-proteins and transduce chemokine signals reveal that signalling is fully dissociable from the role of CCR-5 as a coreceptor. Similar findings have been reported for CXCR-4 (65). In addition, over-expression of arrestin (which enhances chemokine induced internalization) or dominant negative arrestin mutants (which block chemokine induced internalization) had no effect on the ability of CCR-5 to function as an HIV coreceptor (103). Nevertheless, it remains possible that gp120 CCR5 interaction might result in signalling, even though this is not a necessary event for HIV-1 entry to occur.

Clearly much remains to be learned about the nature of viral envelope-coreceptor interactions. The functional sequences of the first wave of coreceptors are only partially characterized and no data have yet been published relating to those of the newly identified coreceptors. On the other side of the equation the identity of, and to what extent, non-V3 sequences influence recognition of the expanding array of coreceptors is yet to be determined. It is also unclear to what extent coreceptors other that CCR-5 and CXCR-4 are truly utilized in vivo, For example do viruses present in CCR-5 delta32 homozygotes utilize exclusively CXCR-4, or other coreceptors such as BOB/GPR15 and Bonzo/STRL33? Is the frequency of viruses using alternative receptors more frequent in individuals who express low levels of CCR-5? Virtually nothing is known about how the expression of coreceptor genes is regulated, and whether or not this might be a potential target for therapeutic intervention. Given the many unanswered questions, there can be no doubt the already substantial number of publications on the subject of primate lentivirus coreceptors will continue to grow for the forseable future.

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Key words: HIV, SIV, Chemokines, Receptors, Viral infection, AIDS, Immunodeficiency

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