Chemokine receptors – the new frontier for AIDS research

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CD4 is widely known as the HIV receptor, but is insufficient to allow viral infection. Recently, members of the family of chemokine receptors have been identified as the missing co-receptors, which act with CD4 to allow the virus to enter cells. These discoveries open up the possibilities of novel therapeutic strategies to combat HIV infection and AIDS.

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One of the central questions in AIDS research has been how the human immunodeficiency viruses, HIV-1 and HIV-2, fuse with and infect human cells. Early on in the study of these viruses, it was shown that HIV-1 primarily infects cells expressing the CD4 cell-surface marker (reviewed in [1]). CD4 is expressed predominantly on the surface of 'helper' T-cells and macrophages, cells that are known to be infected by HIV. The hypothesis that emerged was that the viral envelope glycoprotein gp120/gp41 forms a complex with CD4, leading to fusion of the membranous envelope of the virus with the plasma membrane of the host cell. But although CD4 and gp120 can form a complex, murine cells expressing human CD4 will not fuse with HIV-1. Clearly, the formation of this complex alone is not sufficient to allow viral fusion, suggesting that there may be a second or co-receptor molecule [2].

Understanding the viral fusion process has been further complicated by the fact that different strains of HIV-1 (and other primate lentiviruses, such as HIV-2 or SIV) show distinct cell tropisms. In other words, the viruses exhibit preferences for specific cell types. For example, HIV-1 strains that have been cultured in the laboratory in T-cell lines tend to infect primary T-cells, but not monocytes or macrophages, and are therefore known as T-cell 'adapted' strains. But many primary viral isolates infect monocytes, macrophages and primary T-cells, but not transformed T-cell lines.

The virus strains vary not only in tropism, but also in their ability to induce syncytia in culture (multinuclear cells formed by cell to cell fusion). These strains are preferentially isolated at late clinical stages of the infection and are a hallmark of late stage AIDS pathology. Within an HIV-positive individual, it seems that the strains of HIV-1 that initially establish a persistent infection are macrophage-tropic, non-syncytium-inducing (NSI) strains. Later, T-cell-tropic, syncytium-inducing (SI) strains of virus emerge [3]. These strains seem to be associated with progression to the clinical symptoms of AIDS.

These changes in tropism and the NSI/SI switch are associated with changes in the amino acid sequence of the envelope glycoprotein and in the entry and fusion properties of the virus [4,5], suggesting that different viral strains might use different co-receptors. But the nature of these co-receptors was, until very recently, a mystery.

The connection between chemokines and HIV

As early as 1988, it had been shown that $CD8^+$ T-cells (which do not carry the CD4 cell surface marker) could produce a soluble factor, $CD8^+$-derived antiviral factor (CAF), that inhibited HIV infection of other cells [6]. Recently, Cocchi *et al.* [7] examined a range of IL-2-stimulated, HTLV-1-transformed $CD8^+$ T-cell lines for the production of soluble factors that prevented infection of cells with $HIV\text{-}1_{BaL}$. This is a macrophage-tropic strain of the virus, with biological properties resembling those of some NSI primary isolates. The factors responsible for inhibition of infection were purified by high-pressure liquid chromatography and identified as the chemokines MIP-1α, MIP-1β and RANTES [7]. Purified recombinant chemokines were found to block the entry of NSI viruses into primary adherent macrophage populations [7]. Some individuals who are HIV-negative despite multiple high risk exposures to HIV also have high circulating levels of these chemokines [8], reinforcing the notion that these factors are relevant to the infection *in vivo*.

The co-receptor for HIV fusion has been avidly sought, but claims for its identification have not stood the test of time. But a novel cell–cell fusion assay, reported a few months ago, has finally made it possible to identify a co-receptor for SI strains of HIV [9]. The assay used vaccinia virus to express the HIV envelope glycoprotein on fibroblasts. These cells were tested for fusion with cells expressing CD4 and proteins encoded by a human cDNA library. The pools of cDNAs screened were progressively subdivided, until a single cDNA was identified that allowed fusion. The cDNA responsible has a high degree of sequence identity with the seven-transmembrane-spanning (7TM) G-protein-coupled receptor class of proteins. Because of its ability to support viral fusion, it was named fusin. It had previously been cloned as an orphan receptor, and was named HUMSTR, LCR-1 or LESTR

[10–12]. Its ligand has not yet been determined, despite intensive efforts by many laboratories [12]. The amino acid sequence for fusin shows highest identity with those of the chemokine receptors, and the average identity level is highest for the class of chemokines dubbed CXC (see Fig. 1). Fusin appears to be selective for those strains of HIV that are adapted to T-cell lines, and does not seem to act as a co-receptor for NSI viruses. But since MIP-1α, MIP-1β and RANTES can block the entry of NSI strains of HIV into target cells [7,8], it seemed plausible that a chemokine receptor might be involved here too.

What are chemokines?

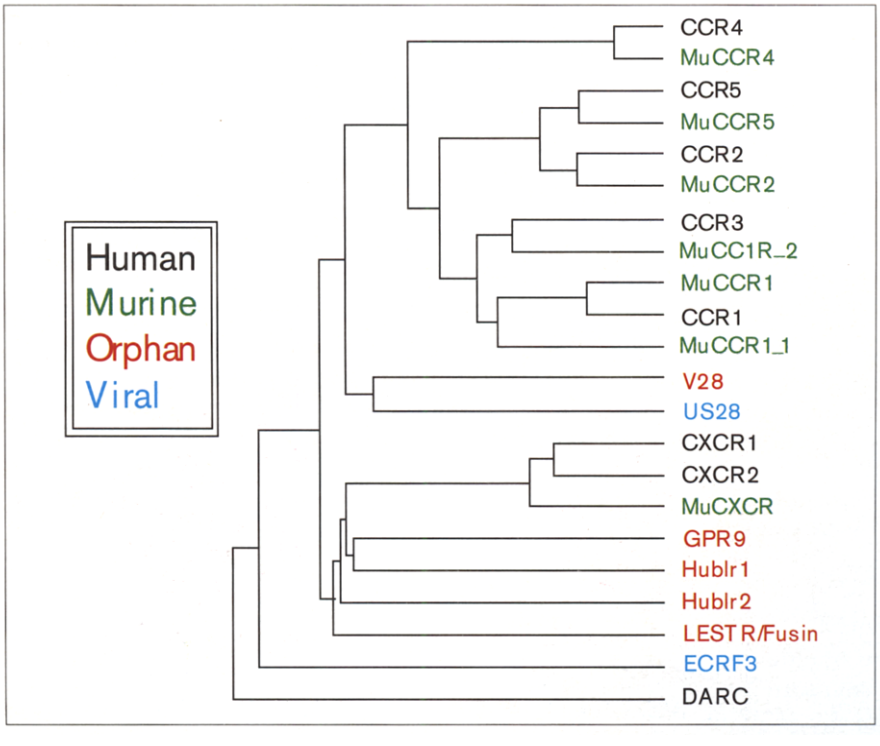
Chemokines are a superfamily of small proteins (with molecular masses of 8–10 kDa) that are involved in a number of inflammatory processes, including the selective activation and recruitment of leukocytes [13,14]. More than 30 human members of the superfamily have been identified so far. They show limited amino acid sequence identity — often as low as 20 % — but NMR spectroscopy and X-ray crystallographic studies show that their three-dimensional structures are well conserved. Almost all chemokines contain four distinctive conserved cysteine residues, forming two intramolecular disulfide bridges. The superfamily can be divided into three groups based on the spacing of these cysteine residues (see [14] for an alignment). In CXC chemokines, the first two cysteines are separated by one amino acid (X). The CXC chemokines seem to be primarily important in acute inflammation, and activate neutrophils. Two receptors for CXC chemokines have been cloned, CXCR-1 and CXCR-2 (also known as IL-8RA and B, respectively). In the CC chemokines, the first two cysteines are adjacent; these proteins do not generally appear to act on human neutrophils, but affect leukocyte populations such as monocytes, T-cells, eosinophils and basophils. They are thought to be involved in inflammatory diseases such as asthma, atherosclerosis and arthritis. MIP-1α, MIP-1β and RANTES are all CC chemokines. Five receptors for the CC chemokines have so far been identified (see Table 1 and [14]). Other chemokines such as lymphotactin, with only one disulfide [15], and another, with a CXXXC spacing (T.N.C.W. *et al.*, unpublished data), have been described, but their receptors are unknown.

Throughout this review, a simplified nomenclature for chemokine receptors, proposed at the recent Gordon Research Conference (Chemotactic Cytokines, June 23–28, 1996), will be used. Under this system, the CC chemokine receptors are termed CCR1–5, and the IL-8 receptors A and B are termed CXCR-1 and CXCR-2.

Towards a molecular hypothesis – the identification of CCR5

The finding that inhibition of infection by monocytotropic strains of HIV was associated primarily with RANTES, MIP-1α and MIP-1β and not other chemokines concentrated the search on a chemokine receptor shared by these three ligands (Table 1). The clone for human CCR5, when

Figure 1



Dendrogram showing the similarities between the different human, murine and virally encoded (human cytomegalovirus and herpes virus saimiri), and human orphan protein sequences. The sequences cluster in terms of the level of identity of pairs of amino acids. The further to the right that the branch points are shown on the diagram, the more similar the two proteins are. There is a clear grouping of the CC chemokine receptors. It is tempting to speculate that all the receptors in the lower half are CXC chemokine receptors, since the group spans known CXC chemokine receptors such as CXCR-1 and the virally encoded ECRF-3. This similarity would imply that the ligand for fusin is a CXC chemokine. Details of sequences used are given in Power and Wells [14].

Table 1

The human chemokine receptors and their murine homologues.

Receptor	Ligand (K_d)	Murine homologue
CXC chemokine receptors		
CXCR1 (IL-8RA)	IL-8 (1.7 nM)	none
CXCR2 (IL-8RB)	IL-8 (0.8 nM), GROa (1.2 nM), NAP-2	muIL-8R
CC chemokine receptors		
CCR1	MIP-1α (10 nM), RANTES (0.6 nM), MCP-3 (0.7 nM)	muCCR1
CCR2b	MCP-1 (0.26 nM), MCP-3 (6 nM)	muCCR2
CCR3	Eotaxin (0.5 nM), MCP-4, MCP-3, RANTES	muCCR3
CCR4	MIP-1α (14 nM), RANTES (9 nM), MCP-1	mCCR4A
CCR5	MIP-1α, MIP-1β, RANTES	mCCR5
Promiscuous and virally encoded receptors		
DARC	IL-8 (20 nM), GROα (24 nM), RANTES (42 nM), MCP-1 (34 nM)	mDARC
HCMV US 28	RANTES (3.4 nM), MCP-1 (6.1 nM), MIP-1α (2.5 nM), MIP-1β (5.1 nM)	–
HSV ECRF3	GROα, NAP-2, IL-8	–
Orphan receptor		
Fusin	?	mLCR

The CC chemokine receptors are given here as CCR1–5 in keeping with a recently proposed convention (Gordon Research Conference on Chemotactic Cytokines, June 23–28, 1996). For details of ligand binding and the receptor sequence alignments, see [14].

stably transfected in a CHO cell line, gave responses in a microphysiometer assay to MIP-1α and MIP-1β and RANTES, but not to other CC or CXC chemokines [16] and was thus a candidate for the co-receptor for primary NSI strains of virus.

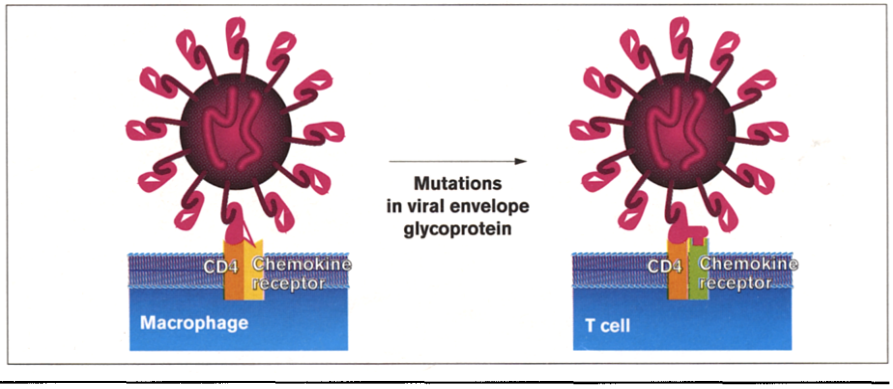
Studies by five groups [17–21] on the role of CCR5 in HIV infection appeared in the literature in the same week. These reports showed that, in the presence of CD4, macrophage-tropic NSI strains of HIV-1 such as JRFL, ADA and BAL can use CCR5 as a co-receptor for fusion. In contrast, CCR5 cannot act as a co-receptor for several T-cell-tropic, SI strains of the virus. Furthermore, expression of CCR5 plus CD4 allows the productive infection of nonprimate cells.

Switching chemokine receptors

The fact that NSI strains of HIV give way as the disease progresses to SI strains, in the process presumably switching co-receptors from CCR5 to fusin, raises a number of interesting possibilities about the way that the virus causes disease. Transmission of HIV by sexual contact is likely to lead to the infection of $CD4^+$ cells associated with the vaginal or rectal epithelium. Of the cell types in these tissues, Langerhans cells, a form of $CD4^+$ antigen-presenting dendritic cell that can migrate from the skin and other epithelia to the lymph nodes, are particularly interesting. These cells are known to be infected by HIV-1, and their migratory properties may well facilitate the transfer of virus to the lymphoid tissue; they may also act as reservoirs for the virus during the prolonged asymptomatic phase of the infection [22,23]. During this phase, virus production and infection of $CD4^+$ T-cells continues but is largely controlled by the immune system [24]; the viruses active in this phase may use CCR5 as the primary co-receptor. As infection progresses, however, mutant viruses, in particular viruses with changes in the V1, V2 and V3 loop regions of the gp120 subunit of the envelope glycoprotein, are continually generated and SI viruses that exhibit a clear preference for fusin as the co-receptor eventually emerge (Fig. 2). As few as two mutations are required to achieve this switch in tropism [8,25]. What drives this switch is unclear; it is possible that the switch in co-receptor may be required to allow the virus to escape the protective effects of circulating MIP-1α, MIP-1β and RANTES. Predictions based on the mutation rate of the virus suggest that viruses with an altered co-receptor preference will emerge within

Figure 2

Mutation in gp120 can change the tropism of HIV for particular cell types. The strains of HIV-1 that initially establish persistent infection are macrophage-tropic NSI strains and recognize CD4 and the co-receptor CCR5 on the surface of target cells. Mutation of as few as two amino acids in gp120 produces a T-cell tropic SI strain of virus.



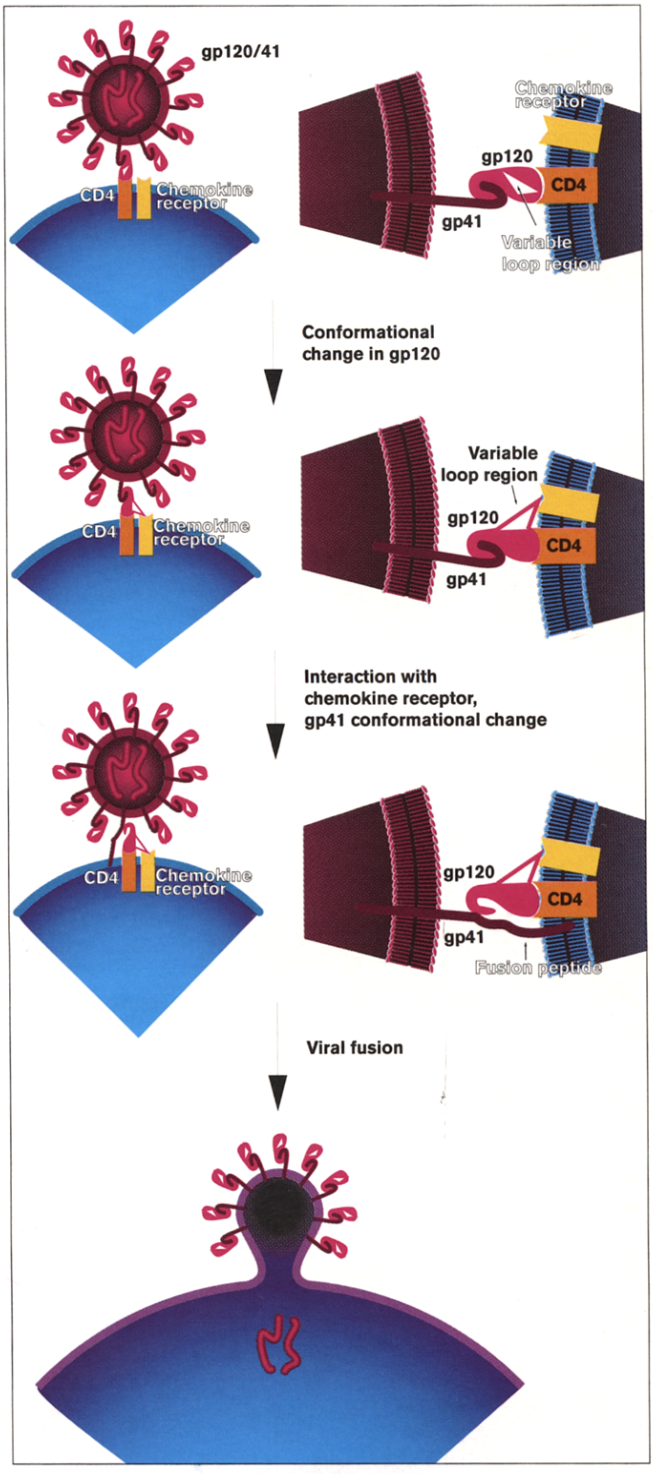
weeks of infection; why it takes several years or more for these viruses to become dominant is unclear.

The simple model that there are only two types of HIV, one using CCR5 and the other using fusin, is already breaking down, however. Some primary clinical isolates of HIV can use CCR3 as their co-receptor [18]. CCR3 was originally thought to be restricted to eosinophils, but is now known to be expressed on monocytes and T-cells as well. In addition, a dual tropic primary HIV-1 isolate, 89.6, has been identified that can induce cells expressing any one of a number of chemokine receptors (CCR3, CCR2b, fusin or CCR5) to form syncytia [20]. It is possible that some receptors may allow cell fusion and syncytium formation, but be less effective in allowing entry of a viral particle. This appears to be the case with strains of HIV-2, which also often show overlapping host cell tropisms and broader host cell ranges than those of HIV-1 strains. Fusion can be seen between cells bearing the envelope glycoprotein from HIV-2_{Rod} and those bearing CCR2b, CCR3, or CCR4, and to a lesser extent fusin or CXCR-2. But as yet only CCR3, fusin and CXCR2 have been shown to support infection by this strain of virus (R. Bron. *et al.*, unpublished data).

A model for the action of chemokine receptors in viral fusion and entry

At this stage, one can only speculate on how the viral envelope glycoprotein interacts with chemokine receptors and with CD4 to facilitate viral infection. It is known that when the viral envelope glycoprotein binds to CD4 there can be a conformational change resulting in the increased exposure of the V3 loop [26] (Fig. 3). The V3 loop is a disulfide-bridged, 30 amino acid region, which is exposed on the surface of the viral gp120 [27]. Since strains of virus that differ in sequence in this region can show different tropisms and may well use different co-receptors, one might expect the V3 loop to be important in the interaction with the co-receptor. Binding of gp120 to the co-receptor might cause a second conformational change, this time in the transmembrane subunit of the envelope glycoprotein (gp41), leading to exposure of the hydrophobic amino-terminal region of gp41 and its insertion into the host-cell membrane (Fig. 3). This mechanism for initiating membrane fusion may be similar to that of influenza virus, in which a conformational change in the haemagglutinin envelope protein is induced by low pH [28]. Insertion of the amino-terminal hydrophobic domain of gp41 into the cell membrane may provide the driving force to start the fusion of the viral and cell membranes. The interaction between gp120, CD4 and the chemokine receptor could be critical in the initiation of this process. This opens up the possibility that individuals who express abnormal levels or mutant forms of CCR5 would be resistant to HIV-1 infection, and may offer a further explanation of why some individuals who have

Figure 3



A model for the action of chemokine receptors in viral fusion and entry. Binding to CD4 can produce a conformational change in gp120, exposing the V3 loop and possibly other variable regions in the viral envelope glycoprotein. Binding to the co-receptor might cause a second conformational change, this time in gp41, leading to exposure of the hydrophobic amino-terminal region and insertion into the host-cell membrane. The fusion complex presumably involves multiple envelope glycoprotein–host cell interactions. This may provide the driving force to start the fusion of the viral and cell membranes.

had multiple high risk exposures to the virus do not become infected.

Given all of the above, why is HIV unable to infect non-primate cells? One would predict that the murine homologues of the chemokine receptors cannot function as fusion receptors, although this has not yet been shown. The murine CCR5 gene has been cloned, and its protein product has been shown to bind MIP-1α, MIP-1β and RANTES, just like its human counterpart [29]. In addition, it has 96 % amino acid similarity and 86 % identity to the human protein. Similarly, the human sequence for LESTR has 91 % identity to the rat receptor and 93 % identity to the bovine receptor. Most of the differences are clustered in the extracellular loops, but alignments of the various fusin and CCR5 sequences have not yet given insight into the question of why the rodent receptors should not act as a co-receptor. However, since only a few amino acid changes in gp120 are needed to change the viral tropism, as discussed above, perhaps the changes required to make CCR5 incompetent for gp120 binding are equally subtle. Mapping of the regions of the receptor essential for fusion might thus require analysis down to the level of individual amino acid changes.

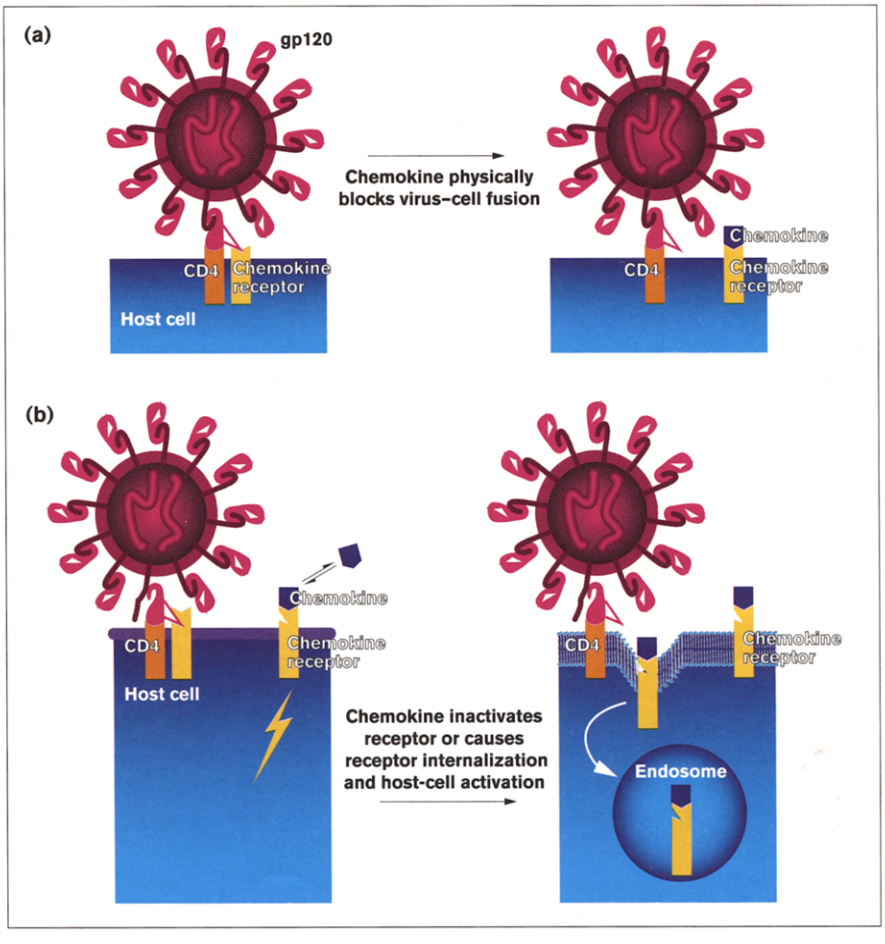
How do chemokine ligands block viral fusion?

Perhaps the most encouraging aspect of the discovery of the HIV co-receptors is that infection can be blocked by the use of the receptor ligands — MIP-1α, MIP-1β and RANTES for strains that use CCR5 as a co-receptor and eotaxin for those that use CCR3. The mechanism by which these chemokines can prevent HIV-1 from entering cells is still far from clear. The simplest explanation is that the binding site of the gp120 glycoprotein on the chemokine receptor overlaps with the ligand itself, and prevents the association of the chemokine receptor with gp120 or CD4 purely by steric means (Fig. 4a). However, the entry of some macrophage-tropic strains of HIV-1 into some cell lines could not be completely blocked with CCR5 ligands, suggesting that this interaction may not always be sufficient to block fusion [19]. Also, of the known ligands of CCR3 (eotaxin, MCP-4, MCP-3 and RANTES) only eotaxin has so far been shown to block the use of CCR3 by the YU2 strain of HIV-1 [18].

It is possible that inhibition of HIV-1 entry results instead from desensitization and down-regulation of the receptors (Fig. 4b). It is known that the chemokine receptors CXCR1

Figure 4

Blockade of viral infection by chemokines. There are two distinct mechanisms by which the addition of a chemokine ligand can block the infection by a virus. **(a)** A simple steric block. The chemokine prevents the formation of a productive complex by blocking off part of the chemokine receptor and preventing it from being available to the viral glycoprotein gp120 for complex formation. **(b)** The chemokine activates and then desensitizes the receptor (a process usually involving a conformational change and the phosphorylation of the intracellular regions of the protein), leading to internalization of the receptor. The result is that the chemokine receptor either cannot access the correct conformation to form a complex with gp120 and CD4, or simply is no longer present on the cell surface.



and 2 can be rapidly desensitized and internalized with a half life of 3 min at 37 °C following ligand binding [30]. We have seen similar effects for CCR1 (A.E.I.P. and Roberto Solari, unpublished data). It is tempting to speculate that the high levels of RANTES, MIP-1α and MIP-1β found in the plasma of nonprogressors may lower the level of active receptor present on the surface of macrophages. Once the ligand for fusin is identified, it will also be interesting to see whether it is over-expressed in individuals who are infected but progress particularly slowly to symptomatic AIDS. As fusin is specific for the SI strains, one would expect the fusin ligand to be more effective in inhibiting the later stages of disease progression than in blocking the initial infection in cases of transmission by sexual contact.

What is the ligand for fusin?

A large number of important questions remain to be addressed, including the identification of the natural human ligand for fusin. Early attempts to define a ligand for fusin (then called LESTR [12]) using radioligand binding assays, failed to show any significant binding by 11 known chemokines. However, it has been surprisingly difficult to identify ligands for chemokine receptors using assays of this kind. The ligands for CKR-2, -3, -4 and -5 were all identified in physiological response assays, such as calcium fluxes or microphyisiometry. Only after a ligand had been identified by these methods was it possible to determine conditions under which it was possible to show ligand binding and displacement. Furthermore, there are now at least 30 known human chemokines [31,14], including many that have only been identified as the products of expressed sequence tags (ESTs). It is possible that the fusin ligand could be one of these products. The alignment of the sequence of fusin with that of the CXC chemokine receptors suggests that the fusin ligand may be one of the recently identified, novel CXC chemokines.

Possibilities for therapeutic intervention

Before we can determine the type of chemokine-receptor-binding agents that would be therapeutically useful, we need to understand the mechanism by which some chemokines can block virus–target cell fusion. If chemokine ligands act by causing the desensitization and internalization of the receptor, that would suggest that a chemokine receptor agonist is required. As most of the chemokines were originally identified because of their roles in recruiting cells during an inflammatory response, the use of chemokine receptor agonists as anti-viral agents may have unwanted inflammatory side effects. But the injection of MIP-1α into patients does not cause inflammation [32], suggesting that the action of chemokines in recruiting and activating inflammatory cells requires that the cells are first 'primed' by other agents. The data of Paxton *et al.* [8] also suggest that some individuals may produce naturally high levels of chemokines, apparently without inflammatory side effects.

Traditionally, it has been very hard to find small molecules that block protein–protein interactions such as the interaction of cytokines with their receptors [33]. This is mostly because a typical protein–protein interface has a large surface area, with the contributions to the binding energy delocalized across this area. A small molecule would therefore have to cover a relatively large surface area to gain sufficient binding energy either to mimic, or to block the protein–protein interaction. In the case of the chemokines, however, the receptors are of the 7TM class. It is therefore far more likely that small molecules can be found to modulate the chemokine–receptor interaction, since other receptors of this class have proved amenable to such approaches. The case of C5a (complement factor 5a), a small protein of similar size to chemokines (75 amino acids) that binds to a 7TM receptor, is a good example [34]. C5a binds to its receptor in two steps, using two binding sites. The initial recognition uses one binding site, and triggers a conformational change in the flexible tail of C5a that allows binding to the main body of the receptor. It is this conformational change that presumably drives the activation of the G protein and the resultant signalling process. In the case of the C5a receptor, the residues important for signalling are localized in the flexible tail. It has been relatively easy to use this region of C5a to find small molecules that block the interaction between the ligand and its receptor. There are many parallels between the chemokine–receptor interaction and the C5a–receptor interaction, including suggestions of a two-site mechanism, and it is almost certainly also true that a conformationally flexible region, which contains the residues that are essential for the recognition of the receptor, changes on receptor binding [35]. Although no small molecule agonists or antagonists of chemokine receptors have been described to date, the importance of these receptors in the inflammatory process means that many groups are currently actively involved in the search. Thus, if the inhibitory effect of chemokines on viral fusion is due to activation and down-regulation, it seems very likely that some candidate inhibitors will be found.

If chemokines prevent viral fusion by a purely steric blockade of the chemokine receptor/gp120/CD4 complex, however, the problem is very different. The ability of a small molecule to block the interaction would then depend on just how localized the gp120-binding site on the chemokine receptor is. Under these circumstances, finding small molecule antagonists for the 7TM receptors may be no easier than it would be for other protein targets [35].

The other therapeutic question is which target to choose. Is it better to try to intervene at the primary infection stage, by blocking CCR5 and thus preventing the initial infection by NSI viruses, or to attempt to inhibit the spread of SI viruses via fusin? Both may have advantages. As SI viruses emerge late in infection and are associated with a more rapid clinical decline [3], preventing T-cell

infection by SI viruses may delay or prevent the emergence of symptoms in seropositive patients. On the other hand, an agent that blocks the infection of macrophages and dendritic cells might be effective early in the course of infection.

Any effort to find anti-HIV agents faces the problem that the virus may be able to mutate to a resistant form. Since there is a wide variety of chemokine receptors, it is reasonable to assume that when a therapy based upon chemokine receptors is tested clinically, mutant viruses will be selected that can use different chemokine receptors as fusion co-factors. The future therapy of HIV infection and AIDS will almost certainly continue to depend on the use of multiple treatments. But the addition of chemokine receptors as a novel, and somewhat unexpected, target clearly improves the chances of being able to maintain HIV-infected individuals in an asymptomatic state, and perhaps even to reverse the progression to AIDS.

Acknowledgements

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