Review

The role of chemokine receptors in HIV infection

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A major breakthrough in HIV research 3 years ago was the identification of the elusive second receptors that the virus needs in addition to the CD4 molecule to get into human cells. A flurry of landmark papers in late 1995 and 1996 showed that members of the chemokine receptor family could be used by different HIV isolates for entry in to CD4+ cells (reviewed in Moore *et al*¹ and Horuk²), and that the chemokines which normally use these receptors can inhibit HIV replication in vitro, presumably by competing with HIV for the receptors.³ These observations opened up the possibility that the receptors or their ligands could provide new targets for antiretroviral therapy. Over the past few years awareness that the genes encoding these receptors can vary between individuals has led to studies of how such host gene polymorphisms shape the natural history of HIV infection (see fig 1). What now is our understanding of the role of chemokine receptors in HIV infection, and how can this information be used in clinical practice?

In early HIV infection, the vast majority of HIV isolates use the CC chemokine receptor, now referred to as CCR5, which in the blood is expressed predominantly on memory CD4+ T cells, such as those which have responded to a previously encountered pathogen. Moreover, CCR5 is largely expressed on the subset of CD4+ T cells which produce interleukin 2 (IL-2) and interferon gamma (IFN- γ)⁴; these are referred to as type 1 helper cells, and make a major contribution to the generation of cellu-

lar immune responses. Thus, from the earliest stages of infection HIV is undermining both the immunological memory of its host and the ability to coordinate a cellular immune response to a pathogen.

CCR5 is the major chemokine receptor expressed throughout the genital tract.⁵ The first targets of HIV infection acquired by sexual exposure are thought to be the CCR5 expressing dendritic cells in the mucosa.⁶ Dendritic cells are specialised cells of the immune system which are designed to pick up foreign antigens in the periphery and transport them to the lymph nodes, where they recruit T cells and initiate an immune response (reviewed in Austyn⁷). This system appears to have been subverted by HIV, which can infect dendritic cells at the mucosal surfaces and then hitch a ride to the lymph nodes. Once in the nodes, the virus is introduced by the dendritic cell to an array of activated susceptible T cells, and the infection of CD4+ T cells then takes off in an explosive manner (reviewed in Rowland-Jones⁸). It is probable that the requirement to infect dendritic cells at the very earliest stages of HIV infection is linked to the very restricted CCR5 usage of infecting isolates, even though the infection may have been acquired from a partner whose dominant virus populations use other coreceptors. This is supported by the observation that people who are homozygous for a 32 base pair deletion in their CCR5 gene (referred to as CCR5- Δ 32), which means their cells do not express this

Figure 1 Chemokine receptor use and the natural history of HIV infection.

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receptor (around 1% of white people), are almost completely resistant to HIV infection.⁹ Rare exceptions exist, where CCR5-Δ32 homozygotes have become infected with HIV, and it appears they have been infected with unusual viruses that have bypassed the initial requirement for entry through CCR5. In considering the worldwide epidemic of HIV, it is important to bear in mind that the CCR5-Δ32 mutation is largely confined to populations of European descent, so people in the parts of the world with the greatest burden of HIV infection do not derive any protection from lack of CCR5 expression.¹⁰

Although the earliest virus populations identified in HIV infection are very restricted in the parts of the viral envelope which interact with CCR5, the virus rapidly diversifies in the infected patient. In most people with progressive HIV disease a change takes place in the phenotype of the virus, from the non-syncytium inducing (NSI) isolates of HIV-1 which dominate in primary infection to strains of HIV which are cytopathic and syncytium inducing (SI) in culture.¹¹ This change in phenotype is now understood in terms of a switch in the chemokine coreceptors used by the virus.¹² The SI virus of late disease has acquired a number of changes in the viral envelope which allow it to use the CXCR4 chemokine receptor CXCR4, which is much more widely expressed, particularly on naive (not previously antigen exposed) T cells.¹³ Thus, the viral phenotype switch in late disease significantly increases the number of potential cellular targets for HIV infection, which goes a long way to explain the dramatic change in the rate of CD4+ cell decline often seen in late disease.¹⁴ Viruses which use CXCR4 (now abbreviated to X4 viruses, while CCR5 using viruses are referred to as R5) but are identical in every other respect to an R5 virus, cause substantially greater depletion of CD4+ T cells in tissue culture than R5 viruses.¹⁵ At the same time the virus is no longer susceptible to inhibition by the CCR5 using chemokines MIP-1α, MIP-1β, and RANTES, which are generated by CD8+ T cells,³ and are thought to be a major influence in the control of viral replication in asymptomatic HIV infection. Much less is understood about the ligand for CXCR4, called SDF-1, and how it may play a part in containing late stage virus. Unlike CCR5, CXCR4 is a very conserved receptor. Studies in knockout mice show that CXCR4 plays a critical role in a number of processes including haematopoiesis, cerebellar development,¹⁶ and normal vascularisation of the gastrointestinal tract,¹⁷ so that both the receptor and its ligand¹⁸ are crucial to survival. This makes the CXCR4 receptor a much less attractive target for therapeutic intervention. HIV isolates have been described which are able to use several other members of the chemokine receptor family for cell entry, including CCR2, CCR3, and STRL33, but these are relatively uncommon. A fascinating discovery was that members of the herpes virus family (KSHV and CMV) have genes that encode molecules resembling chemokine receptors,^{19 20} which presumably play a

part in subverting the immune response against these viruses. One of these, the CMV protein US28, can actually be used by HIV to enter cells¹⁹; moreover, it also appears to be able to bind and internalise the CC chemokines which has led to speculation that this particular decoy receptor might be relevant to the adverse effect of CMV infection on HIV disease progression.

Attention has recently turned to the role of polymorphism in the genes encoding the chemokine receptors used by HIV, together with those for the HIV suppressing chemokines, in determining disease outcome. The best characterised of these is the CCR5-Δ32 mutation, described above. Heterozygotes are not protected from acquiring HIV infection, even though their cells are harder to infect with HIV *in vitro* than those expressing wild type CCR5. However, several large cohort studies have demonstrated that HIV infected individuals who are heterozygous for CCR5-Δ32 exhibit significantly delayed disease progression,^{21 22} which is probably related to their reduced CCR5 expression. These observations prompted a search for additional polymorphisms in the genes encoding other chemokines and their receptors that could play a role in HIV pathogenesis. The next mutation to be described was a conservative mutation leading to a substitution of valine for isoleucine at position 64 in the first transmembrane domain of the CCR2 receptor, which is present at an allele frequency of 10–25% in different populations.²² The presence of this mutation (in both heterozygotes and homozygotes) has been associated with delayed progression to AIDS and death in most, although not all, cohorts. In contrast with the CCR5-Δ32 mutation, this polymorphism provides protection against HIV disease progression in races other than white people^{22–24}; indeed, in one African prostitute cohort, nearly half of a group of long term survivors owed their good outcome to the CCR2-64I mutation.²³ However, the mechanism of protection is rather obscure, since CCR2b is only rarely used as a coreceptor by HIV. One suggestion is that, since the CCR2 gene lies close to the CCR5 gene on chromosome 3 and is in linkage disequilibrium with it, there may be other mutations in the CCR2b-64I haplotype which affect the function of CCR5²²; however, a careful study has failed to demonstrate any alteration in either CCR2 or CCR5 expression or function in cells expressing the mutant genotype.²⁵ A polymorphism in the CCR5 promoter is very tightly linked to the CCR2-64I substitution,²⁶ but it is not yet clear that this has an impact on either CCR5 expression or function. Other polymorphisms in the CCR5 promoter region have been studied. Another polymorphism which is linked to both the CCR5-Δ32 and the CCR2-64I mutations had an independent effect on both CCR5 expression and HIV disease progression in one study,²⁷ but not in another.²⁴ Additional polymorphisms forming haplotypes in the CCR5 promoter region with an impact on HIV disease progression have recently been described.²⁸ A third polymorphism which apparently has a major impact on disease

progression was described early in 1998. This is a point mutation in the 3' untranslated region (UTR) of the SDF-1 α gene, for which homozygotes (approximately 1% of white people) showed a striking delay in the onset of AIDS and time to death.²⁹ Although the effect of this mutation on SDF-1 expression and function is not known, a potential mechanism could be increased production of SDF-1, which then blocks the interaction of the virus with CXCR4. However, protection from disease was not confirmed in another study; in fact, homozygotes for the mutant allele appeared to progress more rapidly to AIDS,²⁴ so the role of this mutation is controversial.

Although many of these genetic effects remain controversial or confusing, it is feasible that much of the heterogeneity that clinicians observe in the outcome of HIV infection in different people will ultimately be explained by their particular combination of coreceptor and chemokine genes.

What are the therapeutic implications of this rapidly expanding area of research? Since CCR5- Δ 32 homozygotes are apparently otherwise entirely healthy, it was reasonable to assume that agents which block CCR5 could be valuable both in HIV therapy and post exposure prophylaxis. Direct use of the CCR5 using chemokines would have a number of disadvantages, both from their potential to recruit and activate HIV susceptible cells and because RANTES can actually upregulate HIV replication in some cells, including macrophages.³⁰⁻³¹ An important finding was that analogues of the CC chemokines, such as truncated or modified RANTES molecules, are able to block HIV infection without activating the cell through the CCR5 receptor.³²⁻³³ One of these, amino-oxypentane (AOP)-RANTES has no effect on CCR5 signalling, yet is 10-fold more effective than native RANTES in inhibiting HIV entry;³² however, HIV infection of macrophages is still enhanced by this agent.³¹ Monoclonal antibodies have been used to map out the regions of CCR5 which are critical for HIV entry or important for its chemokine receptor activity,³⁴ so that antagonists can be targeted more precisely. One problem with these kinds of agents is that they tend to have a very short half life in plasma, and it is unlikely that they will be readily available in oral form.³⁵ More recently a number of low molecular weight (LMW) compounds have been described which are not related to the chemokines themselves, but are able to block HIV entry through the chemokine receptors. To date, several have been generated towards CXCR4,³⁶⁻⁴⁰ but LMW antagonists of CCR5 have been harder to find. None of these agents is yet available for therapy, but their potential is obvious. However, the ease with which HIV is able to adapt to resist most antiviral drugs used to date may limit their usefulness: viral variants which are resistant to inhibition by one of the CXCR4 inhibitors, a bicyclam called AMD3100, have already been derived in vitro.³⁷ The potential consequences of driving HIV evolution towards usage of an increasing repertoire of chemokine receptors will have to be carefully assessed.

In conclusion, in the 3 years since the identification of the chemokine receptors as coreceptors for HIV entry, many of the mysteries of HIV pathogenesis have become clearer, while the potential of these discoveries for therapy remains to be tapped.

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