REVIEW ARTICLE

Chemokines: understanding their role in T-lymphocyte biology

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The chemokines are a complex superfamily of small, secreted proteins that were initially characterized through their chemotactic effects on a variety of leucocytes. The superfamily is divided into families based on structural and genetic considerations and have been termed the CXC, CC, C and CX₃C families. Chemokines from these families have a key role in the recruitment and function of T lymphocytes. Moreover, T lymphocytes have also been identified as a source of a number of chemokines. T lymphocytes also express most of the known CXC and CC chemokine receptors to an extent that depends on their state of activation/differentiation and/or the activating stimuli. The expression of two chemokine receptors, namely

CXCR4 and CCR5, together with the regulated production of their respective ligands, appears to be extremely important in determining sensitivity of T cells to HIV-1 infection. The intracellular events which mediate the effects of chemokines, particularly those elicited by the CC chemokine RANTES, include activation of both G-protein- and protein tyrosine kinase-coupled signalling pathways. The present review describes our current understanding of the structure and expression of chemokines and their receptors, the effects of chemokines on T-cell function(s), the intracellular signalling pathways activated by chemokines and the role of certain chemokines and chemokine receptors in determining sensitivity to HIV-1 infection.

INTRODUCTION

The chemokines (a shortening of **chemo**attractant **cytokines**) are a complex superfamily of small (6–14 kDa) secreted proteins that were initially characterized through their chemotactic effects on a variety of leucocytes [1–6]. To date, at least 40 distinct chemokines have been well characterized (some of which are listed in Table 1 along with their abbreviations), although this number is continually increasing. Chemokines have a plethora of overlapping functions and are produced by a variety of cell types during an inflammatory response. Generally, chemokines act on more than one type of leucocyte, and *in vitro* responses include chemotaxis, enzyme release from intracellular stores, oxygen-radical formation, shape change through cytoskeletal rearrangement, generation of lipid mediators and induction of adhesion to endothelium or extracellular-matrix proteins [4–6]. There is now considerable evidence to indicate that the co-ordinate expression of chemokines and adhesion molecules are responsible for selective trafficking of leucocyte populations from intravascular to extravascular compartments [1–6]. More recently, chemokines and their receptors have been shown to play a key role in the regulation of angiogenesis [7–11] and neuronal-cell regulation [12]. The pace of chemokine discovery has accelerated as a result of not only the establishment of bioinformatics-based analysis of the growing nucleotide databases, but also the finding that HIV-1 uses chemokine receptors for entry into cells. This finding was followed by an astonishing surge of research into this field that has seen great leaps forward in our general understanding of chemokine biology. Accordingly, chemokines and their receptors have now been implicated in several aspects of T-lymphocyte biology distinct from their

established role as directional cues for the movement of leucocytes in homeostasis and inflammation [1–6]. Hence, this review will focus primarily on the exciting recent advances in our understanding of the chemokine and chemokine receptor families and their effects on T lymphocytes, as well the biochemical events that may mediate these effects.

THE CHEMOKINE FAMILIES

Until very recently, only two groups of chemokines had been identified according to the position of four cysteine residues in highly conserved positions (Figure 1). These were termed the CXC (or α) and CC (or β) families according to the presence or absence of an amino acid (X) between the pair of cysteine residues nearest the N-terminus of the protein. The CXC family, which includes the prototypical CXC chemokine interleukin (IL)-8, generally attracts neutrophils and map to human chromosome 4 (Figure 1) [4–6]. In contrast, the CC group generally exert their effects on monocytes, lymphocytes, eosinophils and basophils, and these map to human chromosome 17 (Figure 1) [4–6]. However, the chemokine families are now much more complex than originally thought. Indeed, the CXC family contains distinct subfamilies based on sequence similarity, functional properties and chromosomal mapping of genes (Figure 1). For instance, within the CXC group there exists (i) the 'ELR' subset, members of which (e.g. IL-8), contain a glutamate-leucine-arginine amino acid motif near their N-terminus and which attract neutrophils [3]; (ii) the non-ELR subset (e.g. IP-10 and MIG) which lack the ELR motif and attract activated T lymphocytes [13–15], and (iii) SDF-1 α/β , which attracts lymphocytes, may represent a distinct subgroup of CXC chemokines, since it maps to human chromo-

Abbreviations used: CCR1 etc., CC chemokine receptors 1, 2, 3, 4 and 5; CXCR1 etc., CXC chemokine receptors 1, 2, 3, 4 and 5; CD28RE, CD28 response element; M-tropic, macrophage tropic; T-tropic, T-cell tropic; PI3K, phosphatidylinositol 3-kinase; PLC, phospholipase C; PLD, phospholipase D; PTK, protein tyrosine kinases; IL, interleukin (other chemokine abbreviations are defined in Table 1); PHA, phytohaemagglutinin; mAb, monoclonal antibody; NF- κ B, nuclear factor κ B; TNF- α , tumour necrosis factor- α ; MAP kinase, mitogen-activated protein kinase; AOP, amino-oxy-pentane.

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Table 1 Selected chemokines and their abbreviations

This is a selected list of chemokines and is not an exhaustive list of known chemokines. For additional lists of known chemokines that are not indicated, refer to cited references. Alternatively, an updated list is maintained on the World Wide Web on <http://cytokine.medic.kumamoto-u.ac.jp/CFC/inex.html>

Abbreviation	Definition
CC chemokines	
DC-CK-1 or PARC	Dendritic cell-derived chemokine-1 or pulmonary and activation-regulated chemokine
ELC	EBI1-ligand chemokine
LARC	Liver and activation-regulated chemokine
MCP-1	Monocyte chemotactic protein-1
MDC	Macrophage-derived chemokine
MIP-1 α	Macrophage inflammatory protein-1 α
SLC	Secondary lymphoid tissue chemokine
RANTES	Regulated on activation, normal T cell expressed and secreted
TARC	Thymus and activation-regulated chemokine
TECK	Thymus-expressed chemokine
CXC chemokines	
BCA-1	B-cell-attracting chemokine -1
GRO α	Growth-related oncogene- α
IL-8	Interleukin 8
IP-10	Interferon- γ -inducible protein
Nap-2	Neutrophil-activating protein-2
MIG	Monokine induced by interferon- γ
PF-4	Platelet factor-4
SDF-1	Stromal cell-derived factor-1

some 10, whereas the other CXC chemokine genes map to human chromosome 4 (5 in mouse) [4–6]. At least two of these CXC chemokine subgroups can be further distinguished functionally, since the ELR-containing CXC chemokines generally exert angiogenic effects, whereas the non-ELR CXC chemokines generally exert angiostatic effects [7–11].

Subfamilies within the CC family may also exist, on the basis of gene location, cell targets, function and receptor specificity. Whereas the prototypical CC chemokine genes such as MCP-1, RANTES, MIP-1 α and MIP-1 β cluster at human chromosome 17 [4–6], genes for several other newly identified CC chemokines, such as ELC (MIP-3 β) and the lymphocyte-specific SLC, map to chromosome 9p13 [16–19]. Furthermore, SLC has a unique 36- or 37-amino-acid (murine or human respectively) C-terminal extension not seen in any other chemokine family member and contains the expected four cysteine residues characteristic of the CC chemokines plus two additional C-terminal cysteine residues [17–19]. Since SLC appears to be restricted in expression to lymphoid organs in humans and selectively stimulates chemotaxis of T lymphocytes, it may represent another distinct subfamily of CC chemokines [17–19]. The genes for other novel CC chemokines have also been mapped to other chromosomal locations. For instance, TARC [20,21] and MDC [22] have both been mapped to chromosome 16, whereas LARC (MIP-3 α) has been mapped to human chromosome 2 [23–25]. The gene for another novel CC chemokine, TECK, is uniquely located on chromosome 8 in mouse, although the chromosomal location in humans has not yet been identified [26].

Figure 1 Schematic diagram of the structure and chromosomal locus of chemokine family members

The four families of chemokines are distinguished by the pattern of cysteine residues near the N-terminus. The CXC family has an amino acid between these two cysteine residues, the CC family has none, whereas the C family has only one cysteine residue at the N-terminus. The CX₃C family, which to date is made up solely of fractalkine, has three amino acids between the two cysteine residues. The extracellular stalk and cytoplasmic domain are shown in black; the transmembrane (TM) segment is shown in white. See the text for further details and references.

In addition to the classical CXC and CC chemokine subfamilies, there are two more chemokine subfamilies. Hence lymphotactin, which attracts lymphocytes but not monocytes, defines the C (or γ) family of chemokines, since it has only a single cysteine residue near the N-terminus and the lymphotactin gene maps to chromosome 1 [27,28]. A recently identified chemokine, fractalkine (also termed neurotactin), defines the CX₃C (or δ) chemokine family, which possess fundamentally different properties [29,30]. This chemokine has three intervening amino acids between the N-terminal cysteine residues. In addition, whereas most chemokines terminate at about 70–80 amino acids, fractalkine continues for a further 241 residues [29,30]. Moreover, an 18-amino-acid hydrophobic sequence near the C-terminus is suggestive of a membrane-anchoring motif, and subsequent studies have confirmed that CX₃C protein is detected on the cell surface [29,30]. Accordingly, the structure of this protein has been proposed to be that of a chemokine-like domain present at the end of a stalk which extends from the cell membrane [29,30].

CHEMOKINE RECEPTORS

The specific effects of chemokines are known to be mediated by seven-transmembrane-spanning G-protein-coupled receptors (reviewed in [4]), which are similar to many other seven-transmembrane-spanning receptors, but chemokine receptors generally share some defining features such as the DRYLAIVHA motif in transmembrane region 3 and the second intracellular loop domain [4]. Although over 40 chemokines have been identified to date, considerably fewer chemokine receptors have been cloned, but these exhibit distinct or overlapping specificity or tissue expression (Table 2). CXC chemokine receptors have around 36–77% sequence identity, while CC chemokine receptors have 46–89% identical amino acids [4]. Moreover, the CC chemokine receptors CCR1–CCR5 as well as the CX₃CR are closely linked on chromosome 3p21 [31,32], whereas CXCR1 and CXCR2 map to chromosome 2q35 and CXCR4 maps to chromosome 2q21 [33–35]. In addition, a new locus for chemokine receptors has recently been identified, since CCR6 maps to chromosome 6q26–27 [36].

The known CC chemokine receptors all appear to exhibit overlapping specificities (Table 2), albeit with variable affinity [4–6]. Moreover, several CC chemokines can bind more than one receptor, such that MIP-1 α binds to CCR1 and CCR5, while RANTES binds to CCR1, CCR3 and CCR5. In contrast, eotaxin apparently only interacts with CCR3, but CCR3 also binds MIP-5, RANTES, MCP-2, MCP-3 and MCP-4. Thus each chemokine may recruit multiple types of cells even if they express different types of receptors, whereas each cell may respond to multiple types of chemokines even by expressing a single type of receptor. The exact physiological meanings of such promiscuous and complex relationships between chemokines and their receptors are still unclear, but partially overlapping specificities may have advantages in acute inflammatory responses, where similar leucocyte subsets have to be rapidly recruited in a wide variety of settings and microenvironments. CCR2 occurs in two forms that are the results of alternative splicing and are termed CCR2A and CCR2B, and differ only in their C-terminal intracellular tails and have similar binding specificities for MCP-1, MCP-3 and MCP-4 [37]. Both CC and CXC chemokine receptors are widely distributed and can be found in various immune cells as well as neuronal cells [4–6].

Although the chemokine/ligand promiscuity does not usually cross CC versus CXC boundaries, one exception to this rule is

Table 2 Human chemokine receptors and ligand specificity

Receptor	T lymphocyte expression*	Ligand
CC chemokines		
CCR1	Yes	MIP-1 α , RANTES, MCP-2, MCP-3, MIP-5 (leukotactin-1)
CCR2A/B	Yes	MCP-1, MCP-2, MCP-3, MCP-4
CCR3	Yes	Eotaxin-1, eotaxin-2, MIP-5, RANTES, MCP-2, MCP-3, MCP-4
CCR4	Yes	TARC
CCR5	Yes	RANTES, MIP-1 α , MIP-1 β ,
CCR6	Yes	LARC
CCR7	Yes	ELC, SLC
CCR8	Yes	I-309, TARC, MIP-1 β
D6† (putatively CCR9 or CCR10)	Unknown	MIP-1 α , MIP-1 β , RANTES, MCP-1, -2, -3, -4, eotaxin
CXC chemokines		
CXCR1	Yes	IL-8
CXCR2	Yes	IL-8, GRO α / β / γ , NAP-2
CXCR3	Yes	MIG, IP-10
CXCR4 (LESTR/fusin)	Yes	SDF-1 α / β
CXCR5	No	BCA-1
C chemokines		
Unknown	Yes‡	Lymphotactin
CX₃C chemokine		
CX ₃ CR1 (V28)	Yes	Fractalkine
Miscellaneous chemokine receptors		
DARC	Unknown	IL-8, GRO α , RANTES, MCP-1, TARC
Putative chemokine receptors		
TYMSTR	Yes	Unknown

* Expression may vary between T-cell subsets and/or T-cell lines or activation state of the T cells.

† Sequence of human D6/CCR9 (EMBL/GenBank™/DBJ accession number Y12815) is identical with sequence submitted for human CCR10 (accession number U94888). Hence, designation of human D6 as CCR9 awaits confirmation.

‡ Receptor has not been cloned, but its expression is indicated by virtue of chemotactic response to the chemokine indicated.

the Duffy antigen receptor for chemokines (DARC), which is the same as the Duffy blood-group antigen found on red blood cells. DARC has less than 20% amino acid identity with CXC and CC chemokine receptors and can bind both CXC and CC chemokines [38,39]. Although initial molecular-modelling approaches predicted the presence of nine transmembrane-spanning domains, subsequent analysis has predicted DARC to have seven transmembrane-spanning domains [38,39]. However, DARC lacks the highly conserved DRYLAIVHA motif in the second cytoplasmic loop, is not coupled to heterotrimeric G-proteins and has not been reported to elicit any detectable signal-transduction events [38,39]. Furthermore, DARC does not bind MIP1 α , MIP1 β or lymphotactin [38,39]. The biological role for DARC is unclear, although it has been proposed to act as a 'sink' for chemokines, which keeps their circulating levels low [38,39]. There are also several virally encoded chemokine receptors (e.g. US28 and ECRF3) as well as a growing number of putative chemokine receptors such as TYMSTR (T lymphocyte expressed seven transmembrane domain receptor) which has 33–36% sequence identity with CXCR2 and CCR1 and is expressed on T lymphocytes [40]. Although TYMSTR does not bind any known chemokine, the TYMSTR gene is located on chromosome 3, suggesting that the natural ligand for TYMSTR might be a CC chemokine, since this is the same

chromosomal location of the genes for all five CC chemokine receptors [31,40].

CHEMOKINES REGULATE MOVEMENT OF T LYMPHOCYTES

An established role for several members of the CXC and CC chemokine families is to provide directional cues for the movement of leucocytes in development, homeostasis and inflammation [1–6], and this is thought to involve a series of co-ordinated interactions between leucocytes and endothelial cells that requires highly regulated interplay between selectins, integrins and chemokines [41,42]. The continuous circulation of lymphocytes through the blood, tissues and lymphatics in an organized manner brings naïve lymphocytes into the lymph nodes, where they encounter antigen and are transformed into memory lymphocytes that migrate into inflamed tissue to ensure immunity. Furthermore, the dramatic increase in the secretion of chemokines during inflammation results in the selective recruitment of leucocytes (including T lymphocytes) into inflamed tissues. Moreover, the potential of activated T lymphocytes to produce multiple chemokines (as discussed later), suggests that T cells themselves may play an active role in the recruitment of other leucocytes to sites of infection or inflammation. Certainly, most of the known members of the CXC, CC, C and CX₃C chemokines have been reported to attract various subsets of T cells as well as B cells (Table 3).

T LYMPHOCYTES ARE A SOURCE OF CHEMOKINES

Stimuli required for chemokine production

Traditionally, the main products of activated T cells have been regarded as cytokines such as IL-2, IL-4, IL-13 or interferon- γ [72]. However, it is now clear that chemokines also represent a major product of various lymphocyte populations upon activation. After activation therefore, IL-8, MIP-1 α , MIP-1 β , lymphotactin and RANTES have all been reported to be up-regulated at either the mRNA or protein level, and chemokines appear to be among the most widely and abundantly expressed activation-inducible genes in lymphocytes as well as in other cell types [28,73–75]. The mitogen phytohaemagglutinin (PHA) elicits only small, temporal amounts of MIP-1 α/β and RANTES, whereas higher sustained amounts are elicited by CD3 ligation [74]. Co-stimulation of anti-CD3 monoclonal antibody (mAb) stimulated T cells with anti-CD28 results in a major augmentation of chemokines over levels elicited by CD3 ligation alone, although other co-stimulatory receptors such as CD2, CD4 or CD5, can also co-stimulate the production of these chemokines, albeit less effectively than CD28 [74]. Furthermore, in genetically modified murine systems, full production of MIP-1 α is dependent on CD28 co-stimulation interactions and is controlled by pathways different from those regulating CD28-dependent Th1 and Th2 differentiation [76]. However, in contrast with findings using human T cells [74,75], murine T-cell production of RANTES

Table 3 Effect of chemokines on T lymphocytes

Chemokine	Chemotactic effects	Other effects	References
C-C chemokines			
RANTES	Yes (CD45RO ⁺ memory cells)	Activation of T lymphocytes; co-stimulates proliferation/IL-2 production; up-regulates CD25 expression; stimulates uropod formation; inhibits M-tropic HIV entry	[15,43–48]
MIP-1 α	Yes	Inhibits/co-stimulates T-cell proliferation/IL-2 production; inhibits M-tropic HIV entry	[15,49–51]
MIP-1 β	Yes	Inhibits M-tropic HIV entry	[15,49–51]
MIP-3 α (LARC/exodus-1)	Yes		[23,52]
MIP-3 β (ELC/exodus-3)	?		[24]
MIP-4 (PARC/DC-CK-1)	Yes (CD45RA ⁺ T cells)		[53,54]
MIP-5 (leukotactin-1)	Yes		[55]
Eotaxin	Yes (CCR3 ⁺ T _H 2 cells)		[56]
Eotaxin-2	No		[57]
MCP-1	Yes (CD45RO ⁺)	Co-stimulates proliferation and IL-2 production; up-regulates CD25 expression	[15,46,58–60]
MCP-2	Yes (CD5RO ⁺)		[15,61]
MCP-3	Yes (CD5RO ⁺)		[15,61]
MCP-4	Yes		[62]
TARC	Yes		[63]
SLC (exodus-2/C6kine)	Yes		[17,18]
TECK	Yes (thymocytes)	Potentially involved in T-cell development	[26]
MDC	Yes (CD45RA ⁺ or activated T cells)	Inhibits HIV entry	[22,64]
C-X-C chemokines			
IL-8	Yes		[15,65–67]
Gro α	No reported effect		
Gro β	No reported effect		
Gro γ	No reported effect		
IP-10	Yes		[14,15]
MIG	Yes (activated T lymphocytes only)		[13]
SDF-1	Yes (CD45RA ⁺)	Inhibits T-tropic HIV entry	[68–71]
C chemokines			
Lymphotactin	Yes		[27,28]
CX ₃ C chemokines			
Fractalkine	Yes		[29,30]

appears to be constitutive and independent of anti-CD3 and anti-CD28 co-stimulation [76]. Hence, the regulation of RANTES production may differ between human and murine cells. Interestingly, IL-2 cannot induce chemokine production [76], even though it can induce T-cell expression of several chemokine receptors such as CCR1 and CCR2 [77]. However, stimulation of these T cells with anti-CD3 and anti-CD28 mAbs reduced CCR1 and CCR2 expression [77]. Thus, T-cell production of chemokines and their responses to secreted chemokines appear to be regulated by the co-stimulatory signals that are available. If CD28 is ligated, T cells produce chemokines as well as cytokines such as IL-2, but as a consequence they lose responsiveness to chemotactic molecules. It might be predicted, therefore, that cells specific for the presented antigen will remain at the site of antigen and will recruit other cells that have not yet encountered antigen to that location, thus serving to ultimately amplify the immune response.

Transcriptional regulation of chemokines in T lymphocytes

In terms of transcriptional regulation of chemokines in T cells, RANTES is the best studied CC chemokine. It is constitutively expressed at low levels (< 800 pg/ml) in T cells and can be up-regulated during mitogen or CD28-dependent T-cell activation. The RANTES promoter contains four nuclear factor (NF)- κ B-binding sites (at positions -30 , -44 , 213 and -579 relative to the transcription start site), one of which (-579) serves as a CD28-responsive element (CD28RE) similar to that described in the promoters of a number of cytokine genes such as IL-2, whereas another site (-213) is an NF-AT binding site that also has weak affinity for NF- κ B [78–83]. A CD28RE has also been identified in the IL-8 promoter [82]. Indeed, stimulation with anti-CD3 mAb alone is able to induce RANTES and IL-8 promoter activity, whereas co-stimulation with anti-CD3 and anti-CD28 mAb further increased the activity. Moreover, mutation of the CD28RE prevented RANTES promoter activity induced by CD28 ligation [73,80]. RANTES is up-regulated late (3–5 days) after activation by PHA in normal T lymphocytes, whereas in fibroblasts, renal epithelial and mesangial cells and smooth-muscle cells, RANTES mRNA is up-regulated quickly (24–48 h) by tumour necrosis factor- α (TNF- α) [84–86]. It is noteworthy, therefore, that several proximal *cis*-acting elements (termed A–E) and their associated transcription factors identify early-acting and late-acting transcriptional regulatory pathways that contribute to RANTES gene expression. The 'A' region binds Rel family members including p50–p65 heterodimers and p50–p50 homodimers and novel non-Rel factors. These non-Rel 'A'-site-binding proteins are up-regulated in T-cell nuclear extracts on day 3 after activation and are coincident with RANTES mRNA induction. This binding complex was termed R(A)FLAT (for RANTES A-site binding factor of late-activated T cells) [79]. The purine-rich 'C' site does not conform to known transcription-factor consensus binding sites and the nuclear protein complex recognizing this site [designated R(C)FLAT] has yet to be grouped into any of the known transcription-factor families. It, too, has unusual kinetics after activation and is strongly induced between days 3 and 5, coincident with the onset of RANTES mRNA up-regulation. Another site binds NFIL6, which accumulates in the nucleus within the first 24 h after T-cell activation and rapidly declines thereafter [78]. This factor may be partially responsible for a small, early peak of RANTES mRNA expression sometimes detected in isolates of human peripheral-blood T lymphocytes as well as transient expression of RANTES mRNA observed in response to cytokines such as TNF- α and IL-1 β in many other cell types. Thus there appears to be a switch in

the transcriptional regulatory machinery as a T cell progresses from early to late activation processes, such that early activated nuclear factors (e.g. NFAT and NF-IL-6) are replaced by the novel late-activated factors R(A)FLAT and R(C)FLAT, which regulate the RANTES promoter [78,79].

T-CELL EXPRESSION OF CHEMOKINE RECEPTORS

Given the known functional outcomes of chemokines on T lymphocytes such as chemotaxis, it was a natural assumption that T cells expressed specific chemokine receptors and there has been intense effort to determine the precise chemokine receptors that are expressed on T cells. Studies relating to the expression of chemokine receptors in T lymphocytes have largely focused on mRNA expression, owing to the limited availability of specific antibodies to determine surface expression. Hence, the measurement of mRNA expression may not necessarily reflect events occurring at the cell surface. These approaches have indicated that T cells can certainly express most of the known CC, CXC and CX₃C chemokine receptors (Table 2).

The precise pattern of chemokine receptor expression depends on the activation state of the T cell. For instance, expression of a number of chemokine receptors such as CCR1, CCR2, CCR5, CXCR4 and CX₃CR1 is markedly up-regulated after mitogenic stimulation and/or prolonged treatment of T lymphocytes with IL-2 [77,87,88]. Moreover, some receptors, such as CXCR3, are restricted to activated T cells and this correlates well with the observation that its ligands, IP-10 and MIG, both attract activated T cells [87]. Indeed, for the most part, chemokine receptor expression does correlate well with the known chemotactic effects of the respective ligands on T lymphocyte subsets. For example, CXCR4 (the receptor for SDF-1), is expressed predominantly on CD45RA⁺ naive T lymphocytes [69]. This finding is consistent with the view that SDF-1, constitutively expressed in a broad range of tissues, is involved in basal trafficking of naive lymphocytes. In contrast, CCR5 is expressed mainly on the CD45RO⁺ memory subset, which migrate in response to RANTES, the major ligand for CCR5 [69]. Furthermore, CCR2 expression on the memory T cell subset correlates with the response of these cells to MCP-1 in chemotaxis assays [60]. Some controversy surrounds the expression of the two IL-8 receptors, CXCR1 and CXCR2, on T cells, since they have been reported to be restricted to the NK-like cells within the T-cell lineage which are recruited by IL-8 [60]. However, other groups have clearly demonstrated high-affinity binding of IL-8 to T-cell populations that have not been enriched for NK cells [65,67]. If there were a restriction of CXCR1 and CXCR2 to NK cells, it is unlikely that the chemotactic responses of CD4⁺ and CD8⁺ T cells to IL-8 would have been detected [65–67].

AN EXPANDING ROLE FOR CHEMOKINES IN T-LYMPHOCYTE BIOLOGY

Although chemotaxis of T lymphocytes is undoubtedly an important role for most chemokines, progress in the identification and characterization of chemokine receptors seen in the last 3–5 years, has led us to acknowledge that the role of chemokines and their receptors in T-cell biology may be much more complex and wide-ranging than originally thought. Some of the new roles in which chemokines and their receptors have been implicated will now be discussed.

Chemokine receptor expression defines T-lymphocyte subsets

There is increasing evidence to support the idea that chemokine receptors are expressed on T lymphocytes depending on their

state of activation or differentiation. For example, expression of CCR4 and CCR3 (and perhaps CCR7) has been linked to the program of Th type 2 differentiation *in vitro* [56,89,90]. In contrast, CXCR3 and CCR5 are preferentially expressed on human Th1 cells and are present on T lymphocytes recovered from the synovial fluid of rheumatoid joints which exhibit a Th1 phenotype, whilst CXCR4 and CCR2 (and perhaps CCR1) are expressed equally on both Th1 and Th2 cells [89,91,92]. This pattern of receptor expression correlates with the efficient attraction of Th1, but not Th2, cells by the appropriate CCR5 ligands (e.g. MIP-1 α , MIP-1 β and RANTES). Moreover, SDF-1 and MCP-1, which bind to CXCR4 and CCR2 respectively, exerted chemotactic effects on both Th1 and Th2 cells [93]. Chemokine receptor expression and association with Th1 and Th2 phenotypes can be affected by other cytokines known to affect T lymphocyte polarization, such as α -interferon (promotes Th1 phenotype) and transforming growth factor β (promotes Th2 phenotype). Hence, transforming growth factor β inhibited CCR3, but enhanced CCR4 expression, whereas α -interferon inhibited CCR3, but up-regulated CXCR3 expression [94]. These observations imply that T-cell differentiation may well require migratory capacity as well as distinct cytokine production patterns. In addition, chemokines may be part of effector and amplification mechanisms of polarized Th1- and Th2-mediated immune responses, and their receptors might serve as Th1 versus Th2 markers as well as targets for selective modulation of T-cell-dependent immunity. There is certainly evidence that differential expression of chemokine receptors may influence functional T-cell responses other than chemotaxis, as evidenced by the demonstration that certain CC chemokines (e.g. RANTES, MIP-1 α and MCP-1) promote lymphocyte activation [46,95]. However, MIP-1 α has actually been shown to inhibit proliferation driven by anti-CD3 mAb and IL-2 production by splenic lymphocytes [51]. Hence, the role of chemokines in promoting lymphocyte activation/differentiation remains unclear and highly contentious.

The co-stimulatory T-cell molecule CD28 can regulate the expression of a number of chemokine receptors (e.g. CCR5) as will be discussed in further detail later [88]. One possibility, therefore, is that the differential expression of chemokine receptors during T lymphocyte differentiation may be related to the differential immune regulation that is stimulated by the CD28 ligands B7.1 and B7.2, which have been demonstrated to promote Th1 and Th2 differentiation respectively [96]. In this respect, eotaxin (the major ligand for CCR3), is up-regulated in tissues known to be sites of allergic reactions, such as the airways, and is important for the attraction of eosinophils leading to lung eosinophilia [97]. The generation and maintenance of an allergic reaction requires Th2 cells as a source of IL-4 and IL-5 that serve as growth and stimulation factors for basophils and eosinophils. Hence, the presence of CCR3 on both eosinophils and Th2 cells, as well as the observation that T cells co-localize with eosinophils in diseased tissues, may suggest a possible mechanism for T-cell recruitment in the airways during the pathogenesis of allergic lung disease and may also provide a potential target for therapeutic intervention. Given that CD28-dependent co-stimulation has been demonstrated to be required for induction of a lung mucosal Th2 immune response and airway eosinophilia, a close relationship between CD28 and regulation of CCR3 expression is an exciting possibility [98,99].

The role of chemokine receptors in HIV-1 entry

HIV-1 is the aetiological agent of AIDS which results from the destruction of CD4⁺ lymphocytes in infected individuals. The

ability of HIV-1 to infect different types of cells varies from isolate to isolate and is referred to as 'cellular tropism'. Many primary isolates replicate well in monocytes but not in transformed T cell lines and are classified as monocyte/macrophage-tropic (M-tropic), although they can infect primary T cells [100]. Other isolates, particularly those that have been passaged in lymphoid cells *in vitro* (T-cell-adapted strains) infect primary T lymphocytes, but not monocytes or macrophages, and are referred to as T-cell tropic (T-tropic) viruses [100]. The entry of HIV into target cells is mediated by the viral envelope glycoproteins such as gp120, which binds the cellular receptor CD4, although CD4 expression alone on target cells is not sufficient for viral entry [100]. An exciting and influential series of discoveries in recent chemokine research was the finding that certain chemokine receptors can act as co-receptors for the HIV-1 virus. This was based on observations that a number of CC chemokines (e.g. RANTES, MIP-1 α and MIP-1 β) can suppress infection of T cells with the M-tropic, but not the T-tropic, HIV-1 strains [47]. Several groups subsequently showed that CCR5 (the receptor for RANTES, MIP-1 α and MIP-1 β) is the co-receptor for M-tropic HIV-1, whereas CXCR4 (which binds SDF-1) is the co-receptor responsible for the efficient entry of T-tropic strains of HIV-1 into target cells and that SDF-1 blocked infection with T-tropic, but not M- or dual-tropic HIV-1 [70,71].

The importance of chemokine receptors in the events leading to HIV-1 infection were reinforced by the discovery that homozygosity for a 32 bp deletion in the human *CCR5* gene (*CCR5* Δ 32 allele), which produces a mutant truncated protein that is not expressed on the cell surface, was found to confer resistance to infection by HIV-1 with no obvious deleterious phenotype [101,102]. Another much rarer mutation, *CCR5-m303* (which contains a premature stop codon at position 303) has also been reported to confer resistance to HIV-1 infection [103]. Although CCR5 mutations clearly confer a very high level of resistance to HIV infection, it seems likely that there are other important resistance factors. Indeed, later during the course of infection, T-tropic HIV-1 variants emerge which can also use CCR3, CCR2 and CCR5 in addition to CXCR4 [104]. The emergence of these viruses has been suggested to coincide with a less favourable clinical prognosis. Furthermore, a mutation (V64I) in the transmembrane region of CCR2 also has a significant protective effect on HIV disease progression. The precise mechanism by which this *CCR2-64I* mutation influences protection against HIV-1 remains unclear, although it has been speculated that it may be related to CCR5 heterozygosity, since there is strong linkage disequilibrium between *CCR2-64I* and a mutation (*CCR5-59653T*) in the regulatory region of the closely linked CCR5 gene [105]. It is also noteworthy that other CC chemokines, such as MDC (binds CCR4) and the recently identified putative chemokine receptor TYMSTR (ligand unknown) [40], can inhibit HIV-1 infection of T lymphocytes, whereas I-309 (binds CCR8) inhibits CCR8-dependent infection of monocytes by diverse HIV-1 strains, further suggesting that a number of chemokine receptors are involved in HIV-1 entry [64,106].

Chemokines and lymphocyte development

During their development and differentiation, lymphocytes move through different tissue compartments, although the mechanisms by which this complex cellular trafficking is regulated are not fully understood. However, a group of recently identified chemokines including TARC [20,21], ELC [16], SLC [17–19], LARC [23–25] and DC-CK-1 [53,54] are constitutively expressed at high levels in the thymus, lymph nodes and other lymphoid tissues and/or attract T lymphocytes which bear selective

receptors, namely CCR4 (predominantly binds TARC), CCR6 (binds LARC) and CCR7 (binds ELC and SLC) (Table 2). DC-CK-1 is produced by dendritic cells of germinal centres and T lymphocyte areas of secondary lymphoid organs and is chemotactic for naïve T lymphocytes [53,54], whereas TECK is produced by thymic dendritic cells and is chemotactic for macrophages, dendritic cells and thymocytes [26]. The restricted and constitutive production of these chemokines in lymphoid tissues and/or their selective binding to chemokine receptors that are expressed by T lymphocytes, suggests that they may be heavily involved in the regulation of lymphocyte trafficking during development. Such a role for chemokines is certainly supported by observations in mice lacking CXCR5, which is highly expressed on B lymphocytes. In such mice there is a loss of inguinal lymph nodes and a defective formation of primary follicles and germinal centres in the spleen and Peyer's patches, reflecting the inability of B lymphocytes to migrate into B-cell areas [107]. Furthermore, there is substantial evidence that SDF-1 may attract progenitor B cells into the microenvironment of stromal cells where growth and differentiation factors are released, since mice lacking the SDF-1 gene have severely impaired lymphopoiesis and low numbers of B lymphocyte and myeloid bone-marrow precursors [108]. In addition, SDF-1 is chemotactic for pro- and pre-B cells which depend on stromal-cell contact for growth and differentiation [109].

Chemokines and antigen presenting dendritic cells

Dendritic cells are most potent among antigen-presenting cells and are believed to be indispensable to the initiation of a primary immune response [110]. Dendritic cell precursors are thought to originate in the bone marrow and subsequently migrate into the peripheral tissues and lymph nodes. The trafficking of dendritic cells from blood to tissues and then to lymph nodes is identified in part by members of the CC chemokine family (MCP-3, MCP-4, RANTES, MIP-1 α/β , MIP-5 and LARC) as well as the CXC chemokine SDF-1 [52,110–113]. Indeed, a number of chemokine receptors have been detected in dendritic cells, including CCR1, CCR2, CCR3, CCR5 and CCR6 as well as CXCR1, CXCR2 and CXCR4 [52,111,114,115]. Dendritic cells also produce a number of chemokines such as IL-8 [116] MIP-1 γ [117], MDC [22], MCP-1, RANTES and MIP-1 α [111] as well as DC-CK-1 and TECK, which both appear to be preferentially expressed in dendritic cells [26,53,54]. Since both dendritic cells and T cells act as a source and target for numerous chemokines, one can envisage a complex and finely balanced interplay of chemokine release and production during interaction of dendritic cells and T cells. In its simplest form, this model depends initially on migration of dendritic cells to regional lymph nodes which is partially dependent on locally produced chemokines as well as encounters with antigen. Once the migratory 'processing' dendritic cells mature to 'presenting' cells, they interact with and activate naïve T cells. The initial interaction with naïve T cells may be promoted by one or more of the chemokines known to be released by dendritic cells, particularly SDF-1, since naïve T cells express CXCR4 [69]. However, other chemokines such as DC-CK-1, for which receptors have not been characterized, may also be involved. Upon activation, the T cells themselves produce a number of chemokines that may (i) bind to their respective receptors on dendritic cells and act to sustain the dendritic cell-T cell interaction by promoting or sustaining further migration of dendritic cells to activated T cells and/or (ii) promote T-cell activation responses by binding to up-regulated chemokine receptors on activated T cells in an autocrine or paracrine manner.

SIGNALLING PATHWAYS ACTIVATED BY RANTES IN T LYMPHOCYTES

The biochemical events underlying the chemotactic effect as well as other biological roles of chemokines, have been a major focus of interest in many cell types for several years, initially in the hope of discovering novel therapeutic anti-inflammatory target(s). In this respect, one of the best characterized chemokines in terms of both functional influence on T lymphocytes and regulation of signal-transduction mechanisms is the CC chemokine RANTES. As a result, RANTES serves as a useful prototype for studying signalling events elicited by other chemokines in T lymphocytes. RANTES is an extremely versatile chemokine, since it influences a number of T-cell consequences including uropod formation (a cytoplasmic projection indicative of cellular polarization), expression of adhesion molecules during chemotaxis, cytokine release and T-cell proliferation as well as protection against HIV-1 (Table 3) [15,43–48]. It is therefore critical to understand the discrete 'biochemical wiring' that ensures these events occur.

Structural requirements for RANTES–receptor interaction

Studies in other systems have indicated that chemokines interact with their receptors via two distinct sites, one necessary for binding and the other for activation of signal transduction [118,119]. Hence, residues near the N-terminus of RANTES are required for signalling, since N-terminal truncation mutants of RANTES could bind target cells, but they did not induce chemotaxis or a calcium flux and could act as receptor antagonists [120–122]. Thus RANTES must interact with its receptor(s) via residues other than those at the N-terminus. Whereas the two-site model of interaction between RANTES and its receptors appears appropriate, mutagenesis of RANTES has revealed that it can interact with each of its receptors (CCR1, CCR3 and CCR5) in a distinct and specific manner. Thus Arg-17 is necessary for RANTES binding to CCR1, Phe-12 for binding to CCR3, whereas Phe-12 and Ile-15 are required for binding to CCR5 [123]. In addition, specific residues near the N-terminus of RANTES have been identified that are involved in activating signal transduction via CCR1 (Pro-2, Asp-6, Thr-7), CCR3 (Pro-2 and Tyr-3) and CCR5 (Tyr-3 and Asp-6) [123]. In terms of identifying the important regions of the RANTES receptor(s) that are required for signalling, CCR5 appears to be best characterized. In this respect, a key region of CCR5 involved in its specific interaction with RANTES, MIP-1 α and MIP-1 β and its subsequent activation of signalling events, lies within the second extracellular loop and possibly adjacent transmembrane segments [124].

Activation of G-protein and protein tyrosine kinase-dependent signals by RANTES

RANTES stimulates biphasic calcium mobilization in a CD4⁺ tetanus-toxin-specific T-cell clone, with the initial transient peak mediated by a heterotrimeric-G-protein-coupled pathway that is associated with chemotaxis [95]. The second, sustained, peak of calcium influx is dependent on protein tyrosine kinases (PTK) and is associated with Ca²⁺ channel opening, IL-2 receptor expression, cytokine release and T-cell proliferation [95]. In contrast, other CC chemokines that attract T cells namely MCP-1, MIP-1 α as well as the CXC chemokine IL-8, induce only the initial transient increase in Ca²⁺ [95]. Interestingly, RANTES-induced calcium mobilization depends on the expression of CD3, but the full significance of this observation has yet to be

demonstrated, although it may imply that RANTES may engage the TCR complex as a way of effecting cellular activation [125]. Given the partial sensitivity of RANTES-stimulated calcium mobilization to PTK inhibitors, it is perhaps surprising that RANTES stimulation has not been reported to induce tyrosine phosphorylation of phospholipase C (PLC), even though it induces the tyrosine phosphorylation and activation of a number of other substrates, including the PTKs ZAP-70, p125^{FAK} and the related Pyk-2 [95,126]. Activation of these PTKs by RANTES facilitates its access to a number of downstream biochemical events, and so it can potentially influence T-cell activation, gene transcription, cell-cycle progression and even cell survival. Indeed, immunoprecipitates from cells stimulated with RANTES reveal that this chemokine induces a functional molecular complex consisting of p125^{FAK}, ZAP-70 and the focal adhesion protein paxillin that is required for T-cell focal adhesions formed in response to RANTES stimulation [127]. Furthermore, RANTES stimulates the tyrosine phosphorylation and activation of STAT1 and STAT3, which correlates with the induction of gene expression of the STAT-inducible proto-oncogene *c-fos* in the T-cell lines Jurkat and MOLT-4 [128]. It is noteworthy that MIP-1 α and MIP-1 β , which both exhibit receptor specificity similar to that observed to RANTES, also stimulate tyrosine phosphorylation of Pyk-2, STAT1 and STAT3 [126,128]. Although the precise mechanisms involved remain obscure, RANTES clearly stimulates PTK activation and in this respect RANTES is similar to other G-protein-coupled receptors such as bombesin and vasopressin receptors which can also couple to PTKs [129]. The apparent activation of both G-protein-dependent and PTK-dependent signalling pathways by RANTES supports the notion that different functional events are mediated by different signalling pathways [95]. Certainly, RANTES induced homotypic aggregation has been shown to be herbimycin-sensitive and pertussis-toxin-resistant, whereas lymphocyte uropod formation induced by chemokines can be completely inhibited by pertussis-toxin treatment [48,130,131].

Activation of phosphatidylinositol 3-kinase (PI3K) by RANTES

RANTES stimulates the activation of the class I_A PI3K in peripheral-blood-derived T cells [45,132]. Coupling of receptors to this heterodimeric p85/p110 PI3K is known to require interaction of SH2 domains within the p85 regulatory subunit with specific phosphotyrosine-containing binding motifs [(p)YXXM] located in several growth-factor receptors or adaptor molecules such as insulin receptor substrate-1 [132]. Since no such recognized motifs are present in known RANTES receptors, it is unclear how the receptor couples to this PI3K, although other G-protein-coupled receptors, such as formylmethionyl-leucylphenylalanine ('fMLP') receptors, are able to activate the heterodimeric p85/p110 PI3K [133,134]. In light of the demonstration that both PTK-dependent and G-protein-dependent signalling events are triggered by RANTES in T cells, it is noteworthy that the p85/p110 PI3K is synergistically activated by tyrosine-phosphorylated peptides and $\beta\gamma$ subunits of G-proteins [134]. Given the well-established role for RANTES as a chemotactic agent and evidence that RANTES promotes T-lymphocyte activation, it seems entirely appropriate that RANTES can activate a PI3K-dependent signalling cascade, since this cascade has been implicated in both the regulation of chemotaxis and as a pivotal signal involved in T-cell activation [135,136]. Indeed, RANTES-induced chemotaxis and polarization of normal human T lymphocytes are inhibited by pretreatment with the PI3K inhibitor wortmannin [45].

There are several outstanding questions relating to the activation of PI3K by RANTES. For instance, the effect if any, of RANTES on the G-protein-coupled form of PI3K (PI3K γ) has yet to be elucidated. Certainly, there is evidence that both the p85/p110 heterodimer and PI3K γ can be activated by a single receptor stimulus, since stimulation of the THP-1 pre-monocytic cell line with either concanavalin A [137] or MCP-1 (S. Turner, S. Ward and J. Westwick, unpublished work) can activate both the p85/p110 heterodimer as well as a distinct pertussis-toxin-sensitive PI3K. It will therefore be important to assess the effect if any, of RANTES on either PI3K γ or other distinct PI3Ks such as the recently identified class II PI3K-C2 α or the class III PtdIns-specific 3-kinase [132]. In addition, the possible activation by RANTES of downstream effectors of the PI3K-dependent signalling cascade, such as protein kinase B, has yet to be reported. Finally, recent studies have revealed that RANTES also activates phospholipase D (PLD) in Jurkat cells and this activation is dependent on the GTP-binding proteins ARF and RhoA [138]. PI3K activity is known to be essential for receptor-mediated activation of the related GTP-binding protein Rac in mammalian cells, and the PI3K homologue TOR2 controls Rho activation in *Saccharomyces cerevisiae* [139,140]. One possibility, therefore, is that PI3K may be involved in regulating RhoA and hence PLD activation. Rho has been implicated in regulating actin-filament organization and cell motility, so it is certainly possible that Rho may play an important role in mediating the chemotactic effects of RANTES [141]. PLD and the ARF family of proteins have been implicated in the vesicle formation and retrograde transport of proteins between Golgi and endoplasmic reticulum, and they may therefore operate a mechanism for receptor down-regulation [142]. In this respect it is noteworthy that PI3K has been shown to be involved in growth-factor-receptor down-regulation and that agonist-stimulated receptor internalization is a feature of several chemokine receptors, including CCR5 [143,144]. It is therefore tempting to speculate the involvement of PI3K(s) in agonist-stimulated chemokine-receptor internalization.

Intracellular signals elicited by other chemokines

In contrast with RANTES, signalling by other CC and CXC chemokines in T lymphocytes has not been studied in comparable depth. Nevertheless, several studies have indicated roles for phosphoinositide hydrolysis, protein kinase C activation, trans-membrane calcium flux and PLD activation as signalling events underlying T lymphocyte and NK-cell responses to IL-8 [65–67]. Interestingly, IL-8 does not stimulate PLD activation in neutrophils, such that IL-8 may discriminate between its cellular targets by differentially engaging distinct signalling pathways in neutrophils and T lymphocytes [65]. Given that RANTES has been demonstrated to activate PI3K in normal T lymphocytes [45], it is noteworthy that IL-8-stimulated migration of neutrophils is inhibited by the PI3K inhibitor wortmannin [145]. However, the effects of IL-8 on PI3K activity in T lymphocytes remain unknown. Finally, IL-8 stimulation of mouse lymphocyte cell lines expressing CXCR1 results in guanine nucleotide exchange on RhoA, thus indicating the activation of RhoA [146]. Given that IL-8 is known to activate PLD in T lymphocytes [65], and by analogy with the RhoA-dependent activation of PLD by RANTES [138], it is possible that IL-8 may also activate RhoA in normal T lymphocytes, although this has yet to be confirmed. Finally, the CXC chemokine SDF-1 induces tyrosine phosphorylation of Pyk-2 [126] and has also been shown to activate PI3K (Y. Sotsios, S. Ward and J. Westwick, unpublished

work) as well as the mitogen-activated protein (MAP) kinase cascade in peripheral-blood-derived T lymphocytes and T cell lines (K. Bacon, personal communication). There is therefore sufficient evidence to indicate that there is at least some overlap in the signalling pathways utilized by both CXC and CC chemokines.

CHEMOKINE RECEPTOR SIGNALLING AND PROTECTION AGAINST HIV-1 ENTRY ARE SEPARABLE FUNCTIONS

The molecular mechanisms by which chemokine receptors facilitate HIV-1 entry are a complex phenomenon involving the formation of a heterotrimeric complex of the HIV envelope glycoprotein, CD4 and the co-receptor on the surface of cells [147–150]. Using a panel of CCR5–CCR2B receptor chimaeras, it has been determined that regions of CCR5 involved in chemokine ligand specificity and in the cofactor usage for various HIV-1 strains are not identical. For instance, chemokine selectivity is mediated by the second extracellular loop, whereas cofactor usage is dependent on the N-terminus and first extracellular loop [151,152]. Consistent with this observation, antibodies binding to the N-terminal region of CCR5 block HIV-1 infection, but have no effect on chemokine activity. However, one antibody which recognizes the second extracellular loop of CCR5, blocks both the binding and the biological activity of the ligands RANTES, MIP-1 α and MIP-1 β as well as infection by M-tropic and dual-tropic HIV-1 [153]. Thus, whereas the specificity of CC chemokine binding to CCR5 is determined by a single domain, the gp120-binding site is more complex and probably involves at least two domains, although changes in the N-terminus can be sufficient to disrupt significantly gp120 binding and HIV entry. In addition, there are several lines of evidence to indicate that chemokine signalling and HIV-1 entry are separable functions of CCR5. For instance, pertussis toxin, genistein and herbimycin A are all able to inhibit RANTES signalling, but do not block the ability of RANTES to inhibit HIV-1 infection [95,154,155]. Furthermore, truncation mutants of CCR5 (which are unable to elicit intracellular calcium fluxes and chemotaxis in response to RANTES, MIP-1 α and MIP-1 β), or certain CCR5–CCR2 chimaeras (which are lacking domains necessary to signal in response to their natural chemokine ligands), are still able to function as HIV-1 co-receptors [151,152,156,157].

RELEVANCE OF CHEMOKINE RECEPTOR INTERNALIZATION TO HIV-1 ENTRY

Both CCR5 and CXCR4 are endocytosed upon ligand binding. Although the mechanisms of agonist-stimulated chemokine receptor internalization are not fully understood, this event is also pertussis-toxin-insensitive [143,154,155,158]. However, it seems that internalization is not required for the co-receptor function of either CCR5 or CXCR4 for HIV-1 entry, since mutations of these receptors that prevent agonist-stimulated internalization do not impair HIV-1 entry. In the case of both CXCR4 and CCR5, although HIV-1 entry was unaffected, the impairment of receptor internalization correlates with a decreased potency of their respective ligands as inhibitors of HIV-1 infection [143,154]. Thus the ability of a co-receptor to internalize is not required for HIV-1 entry, although it may contribute to the HIV-1 suppressive effect of CXC and CC chemokines, possibly by reducing the density of co-receptors. PI3K isoforms may have some role to play in this respect, given the recognized role of PI3K in mediating growth-factor-receptor internalization and the ability of some chemokines to activate PI3K [45,159]. Our knowledge of the mechanisms required for activation of the p85/p110 hetero-

dimeric PI3K or PI3K γ , together with the lack of effect of PTK inhibitors and pertussis toxin on HIV-1 entry would appear to argue against this possibility. However, the growing diversity within both the PI3K family of enzymes and the functional consequences known to require PI3K activation suggests that this possibility warrants further investigation.

DO HIV-1 ENVELOPE PROTEINS ELICIT BIOCHEMICAL SIGNALS VIA CHEMOKINE RECEPTORS?

There is growing evidence to indicate that HIV-1 envelope glycoproteins may be more versatile with respect to their interaction with signalling pathways than hitherto anticipated. For instance, HIV-1 envelope proteins such as gp120 can induce the rapid pertussis-toxin-sensitive tyrosine phosphorylation of the PTK Pyk-2 [126]. Since Pyk-2 can feed into both the MAP kinase and Jun kinase signalling pathways and can modulate ion-channel function, there may be a link between HIV-1 binding to chemokine receptors and several signalling cascades known to regulate cell growth, cell survival and cell differentiation. Furthermore, recombinant gp160 envelope proteins from M-tropic (but not T-tropic) HIV-1 can actually induce a calcium signal through CCR5 on CD4⁺ T cells and envelope-mediated signal transduction through CCR5 induces chemotaxis of T cells [160]. Hence, although signal transduction through CCR5 may not necessarily be required for efficient fusion and entry of HIV-1, it is possible that this envelope-protein-stimulated chemotactic response may contribute to the pathogenesis of HIV *in vivo* by chemoattracting activated CD4⁺ cells to sites of viral replication and/or that CCR5 signalling may enhance viral replication by increasing the activation state of the target cells.

THE RELATIONSHIP BETWEEN CD28, CHEMOKINES AND HIV RESISTANCE

In view of the fact that HIV-1 infection of T lymphocytes is critically influenced by CC chemokines [47], and because several chemokines are strongly up-regulated upon CD28 ligation [33], the effect of CD28 ligation on HIV-1 infection of T cells has been investigated by June and co-workers in a series of elegant studies. This group have demonstrated that activation of T cells from uninfected donors with immobilized antibodies to CD3 and CD28 renders these cells highly resistant to HIV-1 infection [161]. Moreover, similar treatment of T cells from HIV-1 infected donors also induces a potent anti-HIV effect, but still promotes the long-term polyclonal proliferation of CD4⁺ T cells in the absence of exogenous cytokines or feeder cells [161]. Immobilization of CD28 mAb is crucial to the development of HIV resistance, as cells stimulated with soluble CD28 mAb are still highly susceptible to HIV infection. The resistance of anti-CD3 and anti-CD28 co-stimulated T cells to some strains of HIV-1 *in vitro* is paradoxical, however, since antigen presentation *in vivo* leads to increased viral replication, indicating that viral presentation *in vivo* is much more complex and dependent on many other cellular events.

The induction of HIV-1 resistance is specific to CD28 and cannot be mimicked by other accessory molecules such as CD2, CD4 or CD5, even though these receptors can produce levels of CC chemokines similar to those produced by CD28 ligation. Thus these results suggest that the CD28-dependent antiviral effect cannot be explained just by the enhanced production of CC chemokines RANTES, MIP-1 α and MIP-1 β which block infection by M-tropic isolates [47,74] and that other additional anti-HIV-1 action(s) are provided by CD28. Indeed, CD3/CD28

Figure 2 CD28 ligation promotes T-lymphocyte resistance to M-tropic HIV-1 infection

CCR5 is the major co-receptor for M-tropic HIV-1 strains and their entry is inhibited by its CC chemokine ligands (RANTES, MIP-1 α and MIP-1 β). CXCR4 is the major co-receptor for T-tropic strains, entry of which is inhibited by its ligand SDF-1. CD28 co-stimulation results in up-regulation of CC chemokine production and release, as well as down-regulation of CCR5 cell surface expression. In contrast, the outcome of CD28 ligation on CXCR4 expression is controversial. These effects of CD28 ligation on CCR5 and CXCR4 expression are consistent with the observation that only the M-tropic viruses are susceptible to the CD28 antiviral effect (denoted by the crossed arrow), whereas co-stimulated cells remain sensitive to the T-tropic virus (denoted by the uninterrupted arrow).

stimulation causes a down-regulation or prevention of induction of CCR5 HIV-1 receptor mRNA expression [88]. Moreover, CD28 stimulation results in up-regulation of CXCR4 mRNA expression, whereas PHA-activated cells express both CCR5 and CXCR4 mRNA [88]. Furthermore, CD4 expression and the

binding of HIV-1 gp120 to CD28-stimulated cells are normal, indicating that the antiviral effect occurs after binding and before the initiation of reverse transcription [88]. M-tropic viruses bind to the cells, but do not enter, whereas T-tropic viruses are able to enter CD28-stimulated T cells. Furthermore, CD28 co-stimulated

cells are resistant to fusion with cells expressing high levels of M-tropic envelope and sensitive to fusion with cells expressing T-tropic envelope, indicating that infection with M-tropic virus is blocked at the levels of membrane fusion [88]. The effects of CD28 ligation on CCR5 and CXCR4 expression are consistent with the observation that only the M-tropic viruses are susceptible to the CD28 antiviral effect, whereas co-stimulated cells are sensitive to the T-tropic virus [88]. More recently, however, another study has demonstrated that surface expression of CXCR4 is also down-regulated by CD28, indicating that other mechanisms may be required to confer resistance to T-tropic entry [162]. Taken together these studies indicate that T-cell proliferation can be uncoupled from HIV entry cofactor expression and that CD28 co-stimulation *in vitro* induces a form of cellular resistance to HIV-1 infection that is similar to that recently described in individuals with a genetic disruption of the CCR5 gene (Figure 2) [101,102].

The CD28 homologue CTLA-4 plays a critical inhibitory role during T-cell activation [163]. Interestingly, antibodies to CTLA-4 which inhibit T-cell proliferation also up-regulate CCR5 expression, suggesting that CTLA-4, in contrast with CD28, promotes susceptibility to HIV-1 infection. Moreover, both the natural ligands for CD28 and CTLA-4, namely B7.1 and B7.2, also appear to up-regulate CCR5 expression (C. June, personal communication). To date there are no reports concerning the effects of CTLA-4 on CC chemokine production. However, the identification of the CD28-dependent regulation of CC chemokines and CCR5 may have important therapeutic implications both for preventing infection and for limiting viral spread in patients with HIV-1 infection.

CHEMOKINES AS A THERAPEUTIC STRATEGY TO PREVENT HIV INFECTION

Given that a number of CC chemokines [47,64] and the CXC chemokine SDF-1 [70,71] inhibit HIV-1 infection of primary lymphocyte cultures, it is unsurprising that attempts have been made to use derivatives of CC chemokines in therapeutic settings. Generally, the strategy that has been adopted is to use modified chemokines that bind to CCR5 but which fail to signal, thus minimizing the risk of non-specific reactions. Several such derivatives that act as receptor antagonists and do not induce chemotaxis, have been shown to inhibit M-tropic strains of HIV-1. These include RANTES(9–68) (which lacks the N-terminal eight amino acids), Met-RANTES (which has a single extra methionine residue at the N-terminus) and amino-oxypentane(AOP)-RANTES (which has chemically added hydrocarbons at the N-terminus) [164,165]. AOP-RANTES and RANTES both induce down-modulation of cell-surface CCR5. When RANTES is removed, the receptor is recycled to the cell surface, whereas the receptor internalized in the presence of AOP-RANTES is retained in the endosomes [166]. Hence, the differences between AOP-RANTES and RANTES in their effect on receptor down-regulation and recycling suggest a mechanism for the potent inhibition of HIV infection by AOP-RANTES and supports the notion that receptor internalization and inhibition of receptor recycling present new targets for therapeutic agents to prevent HIV infection. There are, however, several drawbacks to using chemokine antagonists, since their use could be generally immunosuppressive. This could be a particular problem with derivatives of RANTES because RANTES binds to several chemokine receptors. In this respect it may be more beneficial to target MIP-1 β derivatives, as MIP-1 β appears to be more specific for CCR5 than RANTES.

There have been recent promising advances in the development of effective chemokine-receptor inhibitors. For example, three low-molecular-mass compounds, previously known for their inhibitory effects on HIV replication, have been shown to block entry of T- but not M-tropic strains by interacting with CXCR4 [167–169]. These compounds are the bicyclam AMD3100 [167], an 18-amino-acid residue peptide termed T22 [168] and an oligopeptide termed ALX40-4C [169]. Although all three compounds also inhibit SDF-1-stimulated calcium mobilization, this effect generally requires higher concentrations than those required for antiviral activity [167–169]. Hence, the antiviral activity is likely to depend on competition for co-receptor binding by the virus. There is also encouraging evidence relating to the viral chemokine-like protein vMIP-II as a possible lead for the development of an antagonist that inhibits entry of both M- and T-tropic HIV-1. In this respect, vMIP-II displays a broader spectrum of receptor activities than any other mammalian chemokine, since it binds to the virally encoded US28 (which has been demonstrated to act as a cofactor for HIV entry into cells), as well as CCR1, CCR2, CCR3, CCR5, CXCR4 and, to a lesser extent, CXCR2 [170–172]. Binding of vMIP-II does not result in calcium mobilization, but rather blocks calcium mobilization and chemotaxis induced by other chemokines [171]. Of particular interest, however, is the observation that vMIP-II inhibits cell entry of HIV-1 mediated through CCR3, CCR5 or CXCR4 [171,172], indicating that this protein may serve as a lead for development of broad-spectrum anti-HIV agents. Finally, genetically modified CC (e.g. RANTES and MIP-1 α) and CXC (e.g. SDF-1) chemokines have been targeted to the endoplasmic-reticulum lumen to bind intracellularly the newly synthesized CCR5 or CXCR4 respectively and which consequently prevents their transport to the cell surface. The T lymphocytes expressing these ‘intrakines’ were subsequently found to resist M- and T-tropic HIV-1 infection [173,174]. This gene-based intrakine therapy using genetically modified lymphocytes should have a potent and longer-lasting anti-HIV effect than that achieved by the administration of chemokines, which have a short half-life *in vivo* (< 10 min). Consequently, chemokine-based therapy would require frequent administration into infected individuals throughout their lifetime, which may be ineffective and may induce inflammatory reactions.

CONCLUDING REMARKS

In a matter of a few years there has been an explosion of interest and understanding of what chemokines are and what they do in a number of functional settings, and it is now clear that their role in T-lymphocyte biology is not restricted simply to cell attraction. An intriguing image of the biochemical events underlying chemokine receptor ligation in T cells is also beginning to emerge. The generation and use of transgenic and knock-out mice models of chemokines and/or chemokine receptor(s) should ultimately prove essential tools in adding to our knowledge of the precise functional importance of individual chemokines. Recent use of MCP-1 transgenic mice has determined that MCP-1 is sufficient to cause the accumulation of both monocytes and lymphocytes *in vivo* and that MCP-1 potentiates, but is not sufficient, to cause an inflammatory activation [59]. In addition, CCR5^{-/-} mice exhibit enhanced production of type 1 (e.g. interferon- γ) and type 2 (e.g. IL-4) cytokines, increased delayed-type hypersensitivity reaction and increased humoral responses to T-cell-dependent antigenic challenge, indicating a novel role of CCR5 in down-modulating T-cell-dependent immune responses [175]. In view of the identification of several new chemokine

receptors in lymphoid tissues and of lymphocyte-selective chemokines, as well as the increasing number of T-cell functional consequences being attributed to chemokines, continued research in this exciting area is assured for many years to come. The long-term benefit of this work should hopefully manifest itself in providing novel therapeutic avenues for immune deficiencies, autoimmune diseases and transplantation.

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