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MINIREVIEW

Chemokines and Viruses: The Dearest Enemies

Paolo Lusso[1,2]

Unit of Human Virology, DIBIT–San Raffaele Scientific Institute, Via Olgettina n. 58, 20132 Milan, Italy

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The relation between viruses and the chemokine system is characterized by a complex blend of enmity and attraction. Chemokines are key regulators of innate and adaptive immune responses against invading microorganisms, including viruses. They act not only as immune system "traffic officers," controlling leukocyte migration under both physiological and pathological conditions, but also as fine orchestrators that modulate the induction, amplification, and cytokine-secretion pattern of antiviral responses. However, viruses have succeeded in turning the chemokine system into an ally. During the course of a long parallel evolution, viruses have captured from their hosts the genetic information for encoding chemokines and chemokine receptors and have reprogrammed it for evading the control of the immune system. Moreover, selected viral agents, most notably primate immunodeficiency retroviruses, have adopted chemokine receptors as essential gateways for entry into their target cells. The endogenous secretion of chemokines is thus emerging as an important *in vivo* mechanism of viral control, which is potentially inducible by effective vaccines. The deepening knowledge of the interactions between viruses and chemokines may lead to novel therapeutic and preventive strategies for the control of viral and inflammatory diseases.

Chemokines constitute a growing superfamily of intercellular messengers which play multiple roles in the development and homeostasis of different organ systems, particularly the hematopoietic system, as well as in the generation of both innate and adaptive immune responses. Moreover, chemokines are critically involved in angiogenesis and tissue repair mechanisms. Based on structural criteria, four families of chemokines have been recognized, each showing a distinctive N-terminal cysteine motif. Most known chemokines belong either to the CXC (or α) family, characterized by a two-cysteine motif with an intervening amino acid, or to the CC (or β) family, with two contiguous cysteine residues. In addition, a C (or γ) family, featuring a single-cysteine motif, and a CX_3C (or δ) family, in which two cysteines are separated by three intervening residues, have been described. A list of the human chemokines characterized to date is presented in Table 1. From a functional standpoint, two major groups of chemokines can be distinguished: housekeeping (HK) chemokines, which are generally expressed constitutively under physiological conditions and play essential roles in development and homeostasis, and proinflammatory (PI) chemokines, which are typically inducible and participate in the generation of both innate and adaptive immune responses. However, it is increasingly evident that some chemokines play a dual role according to their cellular and tissue distribution, as well as to the stage of development of the organism.

Most physiological activities of chemokines are mediated by the selective recognition and activation of cellular receptors belonging to the seven-transmembrane-domain, G-protein-coupled receptor superfamily (Table 1). The expression of functional chemokine receptors on target cells is as important as chemokine secretion for the efficiency of the system. Signaling through chemokine receptors is mediated by heterotrimeric G proteins which activate different cascades of intracellular signal conduction. Based on their ligand specificities, chemokine receptors can be classified into three major categories: specific (a single known ligand), shared (multiple ligands belonging to a single chemokine family), and promiscuous (multiple ligands of different families) (Premack and Schall, 1996). Similar to chemokines, chemokine receptors can be characterized as constitutive or inducible; as a general rule, constitutive expression is characteristic of housekeeping chemokine receptors, whereas inflammatory chemokines tend to recognize inducible receptors that are either expressed at low levels or absent on resting cells.

In addition to cellular chemokines and chemokine

[1] Also at Department of Clinical and Experimental Medicine, University of Bologna, 40138, Bologna, Italy.
[2] Address correspondence and reprint requests to author. Fax: +39-02-26434905. E-mail: paolo.lusso@hsr.it.

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TABLE 1

Human Chemokines and Their Receptors

Family	Chemokine designation: Common	Chemokine designation: Systematic[a]	Function[b]	Receptors
C (γ)	Lymphotactin α,β	XCL1,2	PI	XCR1
CC (β)	I-309	CCL1	PI	CCR8
	MCP-1	CCL2	PI	CCR2
	MCP-2	CCL8	PI	CCR1,CCR2,CCR5
	MCP-3	CCL7	PI	CCR1,CCR2,CCR3
	MCP-4	CCL13	PI	CCR2,CCR3
	MIP-1α	CCL3	PI	CCR1,CCR5
	MIP-1β	CCL4	PI	CCR5
	RANTES	CCL5	PI	CCR1,CCR3,CCR5
	Eotaxin	CCL11	PI	CCR3
	Eotaxin 2	CCL24	PI	CCR3
	HCC-1	CCL14	PI	CCR1
	HCC-2	CCL15	PI	CCR1,CCR3
	HCC-4	CCL16	PI	CCR1
	MDC	CCL22	HK	CCR4
	TARC	CCL17	HK	CCR4
	DC-CK1	CCL18	HK	?
	MIP-3β/ELC	CCL19	HK	CCR7
	MIP-3α/LARC	CCL20	HK?	CCR6
	SLC	CCL21	HK	CCR7
	TECK	CCL25	HK	CCR9
	CTACK/ESkine	CCL27	HK	CCR10
CXC (α)	GROα,β,γ	CXCL1,2,3	PI	CXCR2
	PF4	CXCL4	PI	?
	ENA-78	CXCL5	PI	CXCR2
	GCP-2	CXCL6	PI	CXCR1,CXCR2
	NAP-2	CXCL7	PI	CXCR2
	Interleukin-8	CXCL8	PI	CXCR1,CXCR2
	Mig	CXCL9	PI	CXCR3
	IP-10	CXCL10	PI	CXCR3
	I-TAC	CXCL11	PI	CXCR3
	SDF-1α,β	CXCL12	HK	CXCR4
	BCA-1	CXCL13	HK	CXCR5
	BRAK	CXCL14	?	?
	Lungkine	CXCL15	?	?
CX_3C (δ)	Fractalkine	CX3CL1	PI	CX_3CR1

[a] Based on chromosomal location.
[b] PI, inflammatory; HK, housekeeping.

receptors, several virus-encoded homologues have been identified, which attests to the dualistic relation that viruses entertain with the host chemokine system. Indeed, chemokines represent pivotal elements in the orchestration of effective antiviral immune responses; however, viruses have evolved strategies to subdue chemokines and chemokine receptors to their service, either by hijacking and reprogramming them to fight the immune system or by exploiting them as gateways for entry into cells. In turn, the human hosts have recently started to recapture the stolen information, by progressively unraveling the strategies used by viruses for transforming chemokines into effective anti-inflammatory agents. This review is focused on the complex interplay between chemokines and viruses, two "dearest enemies" which provide a unique paradigm of adaptive evolution for survival.

Chemokines as fine orchestrators of antiviral immune responses

Traditionally, chemokines have been viewed mainly as immune system "traffic officers", owing to their ability to direct the physiological recirculation of leukocytes, as well as to convene them at sites of tissue damage, inflammation, or infection. In recent years, however, this concept has evolved with the increasing awareness of the multiplicity of functions inherent to the chemokine

system. The regulated expression of chemokines and chemokine receptors influences not only the "space and time" of the immune responses, but also their magnitude and characteristics: chemokines selectively recruit both immature progenitor cells and functionally competent mature cells at specific anatomical sites (homing function), provide costimulation for cell activation (costimulatory function), induce or potentiate cellular effector mechanisms (effector function), and influence the cytokine-secretion pattern of immune responses (polarization function). Thus, chemokines can be envisaged as *bona fide* orchestrators of both innate and acquired immune responses.

Homing function. Chemokines play a critical role in leukocyte margination at the level of both adhesion to the endothelial surface and extravasation (Baggiolini *et al.,* 1994); in combination with selectins, integrins, and proteoglycans expressed on the endothelial lining, they identify the specific "address" of leukocyte destination. Paradoxically, however, the high level of specificity of the chemokine system is diminished by the remarkable redundancy and overlap which characterize its intricate web, particularly within the inflammatory subset: multiple chemokines bind and activate an individual receptor, while multiple receptors are bound and activated by an individual chemokine (Table 1); furthermore, multiple chemokines and chemokine receptors can be simultaneously expressed in individual cells. A possible key for interpreting this apparent relinquishment of specificity is the concept of "functional units" (Baggiolini, 1998). Some chemokines indeed display a unique array of specificities that, altogether, target a defined group of immune cells. Thus, they behave as "task-force instructors" that recruit and activate all the necessary roles for fulfilling a specific function.

The homing function of housekeeping chemokines is critical for the physiological development and homeostasis of the hematopoietic system. In lymphoid tissues, they are expressed constitutively, providing a steady attraction signal for naive T cells, B cells, and mature dendritic cells (DC). The essential, nonredundant function of housekeeping chemokines and their receptors is attested by the dramatic alterations of the architecture and function of lymphoid tissues caused by the deficiency or ablation of these genes, with striking similarities between the phenotypes resulting from the lack of receptors or of their specific ligands. A paradigm example is provided by mice lacking CCR7, which show a marked reduction of both lymphocyte and DC colonization of secondary lymphoid organs, associated with severe immunologic alterations (Forster *et al.,* 1999); interestingly, this condition is mimicked by a natural mutation (*plt*) in the gene encoding SLC, a CCR7 ligand (Gunn *et al.,* 1999). Similarly, a lethal phenotype, with abnormal development of multiple organs, results from either *CXCR4* or *SDF-1* gene knockout in mice (see below).

Inflammatory chemokines are mostly responsible for the recruitment of immune cells, including granulocytes, memory T cells, natural killer (NK) cells, monocytes, and immature DC, to sites of microbial invasion and inflammation. Due to the redundancy of the inflammatory chemokine system, deletion of individual genes does not generally cause the dramatic phenotypic alterations seen in the case of housekeeping chemokines. For example, MIP-1α knockout mice show a reduced inflammatory response against coxsackie virus infection; nevertheless, viral clearance consistently occurs, albeit delayed (Cook *et al.,* 1995). Another striking example is a congenital deficiency of human CCR5 (CCR5-Δ32) in populations of Caucasian origin, which is not associated with any evident phenotype, except for natural resistance to human immunodeficiency virus (HIV) infection (see below).

A wide variety of cell types produce inflammatory chemokines upon activation by stimuli of bacterial (e.g., LPS) or viral (e.g., double-stranded RNA) origin, as well as by early inflammatory cytokines (e.g., TNF-α, IL-1β, IFN-γ). The initial distress signal is emitted primarily by resident tissue cells, such as endothelial cells and fibroblasts, with subsequent amplification by professional antigen-presenting cells (e.g., DC and macrophages) and T cells. Among the latter, $CD8^+$ cytotoxic T lymphocytes (CTL) are increasingly recognized as an important source of immunomodulatory substances, including chemokines (Conlon *et al.,* 1995). As CTL can detect and kill virus-infected cells before the release of infectious particles, their chemokine-secreting activity may be essential during the early stages of infection, before the development of a full-blown inflammatory reaction, as well as during infection by noncytopathic viruses which induce limited inflammatory responses (Price *et al.,* 1999). Besides their ability to synthesize chemokines *de novo,* CTLs also possess a unique rapid-intervention mechanism, as their cytolytic granules are loaded with preformed chemokine pools that can be quickly discharged upon activation. For example, RANTES and MIP-1α are stored, along with granzyme A, within the cytolytic granules of $CD8^+$ T cells (Wagner *et al.,* 1998). Another major reservoir of RANTES in blood is represented by the α-granules of platelets, which release it almost instantly upon activation (Kameyoshi *et al.,* 1992). This peculiar feature of the RANTES physiology is still incompletely understood, but it is certainly relevant to the role played by platelets in antimicrobial host defense, as well as to the involvement of RANTES in tissue repair mechanisms and, possibly, in thrombus dissolution.

Costimulatory function. The physiological effects of chemokine-elicited intracellular signaling are not limited to the activation of a chemotactic program. Indeed, che-

mokines provide a costimulus that amplifies both proliferative and cytokine-secretive T-cell responses (Taub *et al.,* 1996). This function may allow antiviral immune responses to proceed beyond a critical threshold under conditions of low antigenic load or during infection by noncytopathic viruses. A separate observation is the unique T-cell mitogenic effect exerted by RANTES at micromolar concentrations (Bacon *et al.,* 1995), but the physiological significance of this phenomenon remains uncertain.

Effector function. Several lines of evidence indicate that signal transduction through chemokine receptors can directly activate cellular effector mechanisms. For example, different chemokines elicit the production of microbicidal oxygen radicals and bioactive lipids in monocytes or the release of cytoplasmic storage granule content, such as proteases from neutrophilic granulocytes and monocytes, histamine from basophils, and cytotoxic peptides from eosinophils (Baggiolini *et al.,* 1994). Chemokines were also shown to potentiate CTL effector functions by at least two independent mechanisms: enhanced degranulation (Taub *et al.,* 1996) and upregulation of Fas-ligand expression (Hadida *et al.,* 1999). Enhanced degranulation has also been demonstrated for NK cells, upon stimulation with a variety of CC chemokines (Loetscher *et al.,* 1996). These observations corroborate the concept that chemokines do not serve merely as road signs for directional migration, but rather as fine regulators of complex immune response programs.

Polarization function. A series of recent observations has linked the differential expression of chemokines and chemokine receptors with T-cell cytokine-secretion patterns, according to the T-helper (Th)1/Th2 paradigm. Following the demonstration of a preferred secretion of the CCR5 ligands, RANTES, MIP-1α, and MIP-1β, by Th1-polarized cell lines and clones (Schrum *et al.,* 1996), the finding that CCR3 is selectively expressed by Th2 cells (Sallusto *et al.,* 1997) provided the first evidence that chemokine receptors may represent markers of polarized cytokine responses. Other putative Th2 markers have subsequently been identified, including CCR4 and CCR8 (Bonecchi *et al.,* 1998; Sallusto *et al.,* 1998; Zingoni *et al.,* 1998), whereas CCR5 and CXCR3 have been implicated as Th1 markers (Bonecchi *et al.,* 1998; Loetscher *et al.,* 1998; Sallusto *et al.,* 1998). However, there are many potential caveats to a simplistic equivalence between the expression of certain chemokine receptors and Th1/Th2 responses. First, it must be emphasized that the expression of chemokine receptors is dramatically affected by the cell activation status, as illustrated by the equivalent levels of CXCR3 expression seen in freshly activated Th1 and Th2 cells (B. Moser, personal communication), as well as by the downmodulating effect of specific chemokine ligands; second, *in vitro* findings, using polarized T-cell lines or clones, may not reflect the actual chemokine-receptor expression *in vivo;* third, during polarized *in vivo* responses, specific chemokine receptors may be expressed primarily by cells other than $CD4^+$ T cells, as shown by the presence of CCR3 primarily on mast cells and eosinophils in tissues affected by systemic sclerosis, a Th2-dominated disorder (Annunziato *et al.,* 1999).

Despite the above caveats, the putative link between chemokines and cytokine-secretion patterns has important implications, as it suggests that the chemokine system may play a direct role in the generation and/or amplification of polarized responses. The results of DNA coimmunization studies in mice reinforce this hypothesis. Using plasmids encoding both HIV antigens and chemokines, a distinctive skewing effect was documented for individual chemokines, with RANTES and MCP-1 inducing Th1-skewed responses, associated with marked CTL activation, and MIP-1α seemingly favoring Th2 polarization (Kim *et al.,* 1998). By contrast, subsequent studies showed a Th1 predominance following MIP-1α coimmunization (Lu *et al.,* 1999), while MCP-1 knockout mice were found to be unable to mount Th2 responses (Gu *et al.,* 2000). These discrepancies emphasize the preliminary nature of these observations. Also, the mechanisms whereby chemokines may induce Th1/Th2 polarization remain unclear. An attractive hypothesis is that chemokine receptor-mediated signaling might directly activate specific polarization programs in naive cells. In support of this concept, *in vitro* treatment with MIP-1α or MCP-1 was shown to drive Th0 cells toward a Th1 or Th2 pattern, respectively (Karpus and Kennedy, 1997). However, a selective recruitment of functionally prepolarized cells is also likely to occur. Regardless of the mechanism involved, the expression of specific chemokines and chemokine receptors may represent a basic mode of amplification of Th1 and Th2 responses. These concepts raise the possibility of employing chemokines, either individually or in appropriate combinations, as specific "adjuvants" for modulating the pattern of immunity elicited by antimicrobial or antitumor vaccines.

"Molecular hacking:" Appropriation and reprogramming of chemokines and chemokine receptors by viruses

Over the course of a long parallel evolution, viruses have established with their natural hosts a relationship based on a wise balance between aggressiveness and respect. A rapid extinction of the host is not an effective survival strategy, as illustrated by the equally rapid extinction of the epidemic foci of certain zoonoses in humans, such as Ebola virus infection. Analysis of the genetic makeup of viruses and their multicellular hosts

TABLE 2

Chemokine Homologues, Chemokine-Binding Proteins, and Chemokine Receptor Homologues Encoded by Large DNA Viruses

Virus	Coding gene (gene product)	Cellular homologues	Proposed function
Herpesviridae			
mCMV	*m131* (MCK-1)	CC chemokines	Chemokine agonist (target cell recruitment?)
	M33	CCR1	Functional receptor? (infected cell dissemination)
hCMV (HHV-5)	*UL146* (vCXC-1)	IL-8	Chemokine agonist (target cell recruitment?)
	UL147 (vCXC-2)	IL-8	?
	US27	?	?
	US28	CCR1	Functional CC chemokine receptor HIV-1 coreceptor
	UL33	CCR1	?
HHV-6	*U83*	CC chemokines	Chemokine agonist (target cell recruitment?)
	U12	CC chemokine receptors	Functional CC chemokine receptor
	U51	CC chemokine receptors	Chemokine sequestration?
HHV-7	*U12*	CC chemokine receptors	?
HHV-8	*K6* (vMIP-I)	MIP-1α	Chemokine agonist on CCR8 (Th2 skewing?), angiogenesis
	K4 (vMIP-II)	MIP-1α	Chemokine agonist on CCR3 (Th2 skewing?), angiogenesis, broad-spectrum chemokine antagonist
	K4.1 (vMIP-III)	MIP-1β	Chemokine agonist on CCR4 (Th2 skewing?), angiogenesis
	ORF74	CXCR2	Constitutive signaling, cellular transformation
HVS	*ORF74/ECRF3*	CXCR2	Functional CXC chemokine receptor
Poxviridae			
MCV	*MC148R* (vMCC-1)	CTACK	Broad-spectrum chemokine antagonist
Variola virus	*G3R*	?	CC chemokine binding/inhibition
Vaccinia virus	*C23L* (B29R)	?	CC chemokine binding/inhibition
Myxoma virus	*MT7*	IFN-γ receptor	Broad-spectrum chemokine binding/inhibition
	MT1	?	CC chemokine binding/inhibition
Shope virus	*D1L*	?	CC chemokine binding/inhibition
Swinepox	*K2R*	CC chemokine receptors	?
Capripox	*Q2* (3L)	CC chemokine receptors	?
Cowpox	*D1L*	?	CC chemokine binding/inhibition

reveals the vestiges of a long and balanced chess game, during which each player has responded to the contender's attacks, move after move, with the implementation of adequate defense systems. One of the most remarkable stratagems enacted by viruses has been the hijacking of the genetic information encoding key host molecules involved either in antimicrobial immunity or in cell survival and proliferation. Often, the misappropriated genes have been modified over the course of the evolution, in order to better serve the viral purposes, such as reducing the efficacy of innate and acquired immune responses, enhancing the spread of the infection, or ensuring a longer life span to parasitized cells.

"Molecular hackers" have been hitherto recognized within three families of viruses: *Retroviridae, Poxviridae,* and *Herpesviridae.* Retroviruses have restricted their choice to cellular protooncogenes which govern the cell's survival and proliferation, whereas poxviruses and herpesviruses have selected multiple protein families, including fine immune response modulators. In particular, chemokines and chemokine receptors have represented prime targets of molecular appropriation by these large DNA viruses (Table 2), thus providing strong evolutionary evidence of the key role played by these molecules in antiviral defense mechanisms. The intense investigation in this area is driven not only by the interest in elucidating new paradigms of viral immune evasion, but also by the possibility of human exploitation of viral antichemokine strategies for the therapy of inflammatory diseases.

Viral chemokines. One of the main strategies of manipulation of the chemokine system by herpesviruses consists in the expression of viral chemokines, which generally show some degree of genetic and functional homology to their cellular counterparts (Table 2). These chemokines may act by different mechanisms, including recruitment of new target cells for infection, antagonism of cellular chemokines, and induction of "diverting" Th2-skewed or nonspecific inflammatory reactions. The importance of viral chemokines for pathogenicity is illus-

trated by the experimental ablation of the CC chemokine homologue MCK-1 in murine CMV, which results in a reduced tissue spread *in vivo,* with decreased inflammatory responses and rapid viral clearance (Fleming *et al.,* 1999). Human herpesviruses encode several chemokine homologues, including the CXC chemokines UL146 and UL147 of human CMV (Penfold *et al.,* 1999) and the CC chemokines U83 of human herpesvirus (HHV)-6 (Zou *et al.,* 1999) and vMIP-I, vMIP-II, and vMIP-III of Kaposi-sarcoma herpesvirus or HHV-8 (Boshoff *et al.,* 1997; Stine *et al.,* 2000). Most of these molecules act as functional agonists, at least on selected receptors; for example, vMIP-I is selectively active on CCR8 (Dairaghi *et al.,* 1999), vMIP-II on CCR3 (Boshoff *et al.,* 1997), and vMIP-III on CCR4 (Stine *et al.,* 2000). Since all of these receptors have been linked to Th2 responses, their activation may drive local immune responses toward a Th2-like pattern, thereby hindering Th1-polarized antiviral responses. Unlike other herpesvirus-encoded chemokines, vMIP-II also acts as a broad-spectrum chemokine antagonist, with the ability to reduce inflammatory responses *in vivo* (Chen *et al.,* 1998). Of potential relevance to the highly vascularized nature of Kaposi sarcoma, all the HHV-8-encoded chemokines exhibit some degree of angiogenic activity in the chick chorioallantoic membrane assay (Boshoff *et al.,* 1997).

Although less frequently than herpesviruses, poxviruses have also seized chemokine genes from their hosts. An example is the *MC148R* gene of molluscum contagiosum virus (MCV), which encodes a potent broad-spectrum chemokine antagonist (Damon *et al.,* 1998), with homology to the cellular CC chemokine CTACK.

Chemokine-binding proteins. Another viral strategy for counteracting chemokine activities is the adsorption and inactivation of chemokines by specific viral proteins or, less frequently, by viral chemokine receptors (Table 2). Several poxviruses encode soluble chemokine-binding proteins, belonging to two major classes, none of which shows homology with chemokine receptors or other host proteins (Lalani *et al.,* 2000). The first class of molecules is represented by myxoma virus MT7, which binds members of all chemokine families through their proteoglycan-binding domains, thereby hindering their interaction with the cell surface and the extracellular matrix. The second class, prototyped by MT1 of myxoma virus, encompasses several related proteins, 35–40 kDa in size, which bind CC chemokines with high affinity. Their mechanism of action involves direct inhibition of chemokine-receptor binding, thus limiting the recruitment of inflammatory cells to infected tissues. Finally, chemokine depletion can also be accomplished by adsorption to membrane-bound viral chemokine receptors (see below), as exemplified by the *US28* gene product of human CMV and the *U51* gene product of HHV-6, both of which were shown to bind and deplete extracellular RANTES (Billstrom *et al.,* 1999; Milne *et al.,* 2000).

Viral chemokine receptors. Several herpesviruses and poxviruses encode chemokine receptor-like molecules which maintain, in most cases, functional competence (Table 2). This suggests that chemokine-induced intracellular signaling and directional migration of infected cells may be instrumental for sustaining and disseminating the infection. Consistent with this concept, it has been shown that deletion of the *M33* gene, which encodes a chemokine-receptor homologue, markedly decreases the ability of murine CMV to grow in salivary glands, a privileged site of viral replication (Davis-Poynter *et al.,* 1997). Viral chemokine receptors are generally shared by several chemokine agonists, as illustrated by the human CMV *US28* gene product, which behaves as a broad-spectrum CC chemokine receptor (Neote *et al.,* 1993), or the herpesvirus saimiri (HVS) *ECRF3* gene product, which is activated by multiple CXC chemokines (Ahuja and Murphy, 1993).

The gene product of *ORF74* of HHV-8 provides a unique example of viral reprogramming of chemokine receptors. In spite of its high homology and colinearity with the *ECRF3* gene of HVS, as well as more distant relatedness with CXCR2, the HHV-8 receptor is functionally divergent because it displays a constitutive, ligand-independent signaling activity (Arvanitakis *et al.,* 1997); several chemokines act as inverse agonists on this receptor, causing a reduction of the basal signaling tone upon binding (Rosenkilde *et al.,* 1999). A single amino acid residue within the second intracellular loop has been fingered as the determinant of the constitutive activation of the *ORF74* gene product, since the same mutation was shown to induce constitutive activation of CXCR2 (Burger *et al.,* 1999). Transfection of *ORF74* promotes the growth of rodent fibroblasts (Arvanitakis *et al.,* 1997), suggesting an oncogene-like activity that may play a part in Kaposi sarcomagenesis.

The "accidental antagonists:" Chemokines as specific blockers of viral receptors

Although chemokines are implicated at several levels in the host mechanisms of antiviral defense, the chemokine system has not evolved as a specific form of antiviral protection. Nevertheless, selected members of the α and β chemokine families were "accidentally" transformed into specific antiviral factors when primate and feline immunodeficiency retroviruses, as well as at least one species of rodent poxvirus, "chose" chemokine receptors as critical components of their cellular receptor complex. The first close encounter between chemokines and immunodeficiency retroviruses occurred in 1995, with the identification of three members of the CC-chemokine family, namely RANTES, MIP-1α, and MIP-1β, as

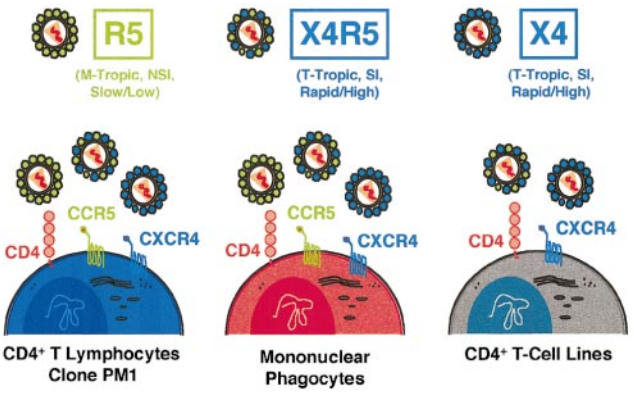

FIG. 1. Classification of HIV-1 variants based on coreceptor usage. Most human $CD4^+$ T-cell lines express CXCR4 but not CCR5; PM1 is a unique $CD4^+$ T-cell clone coexpressing CXCR4 and CCR5. CXCR4 is a functional HIV-1 coreceptor both in primary $CD4^+$ T cells and in *in vitro*-differentiated macrophages.

potent and specific $CD8^+$ T-cell-derived inhibitors of both human (HIV-1 and -2) and simian (SIV) immunodeficiency viruses (Cocchi *et al.,* 1995). Shortly thereafter, independent research led to the identification of a chemokine receptor-like molecule, CXCR4/fusin, as a critical cofactor (or coreceptor, along with the classic CD4 receptor) for HIV-1 envelope-mediated cell fusion (Feng *et al.,* 1996). Remarkably, CXCR4 was shown to serve as a coreceptor exclusively for T-cell line-tropic HIV-1 strains, whereas RANTES, MIP-1α, and MIP-1β displayed a selective inhibitory activity against non-T-cell line-tropic HIV-1 strains. The natural convergence of these two discoveries triggered an authentic chain reaction of events that in a few months led to the elucidation of several unsolved issues in HIV biology, opening new perspectives for the development of therapeutic and prophylactic strategies. Based on its ligand specificity for RANTES, MIP-1α, and MIP-1β, a second chemokine receptor, CCR5, was rapidly recognized as the major coreceptor used by non-T-cell line-tropic HIV-1 isolates (reviewed in Berger *et al.,* 1999). In addition, several minor HIV coreceptors were identified (e.g., CCR2b, CCR3, CCR8, CX_3CR1, GPR1, STRL33/Bonzo, GPR15/BOB, APJ), but their *in vivo* biological relevance remains uncertain. The differential usage of chemokine receptors has provided the long-sought-after physiological key for interpreting the biological variability among HIV-1 isolates. A novel classification of HIV-1 isolates based on coreceptor usage has therefore been proposed (Fig. 1), with the recognition of three major variants differing in their ability to use either CCR5 (R5), CXCR4 (X4), or both coreceptors interchangeably (X4R5). However, it must be emphasized that the biological and clinical significance of X4 and X4R5 strains is widely overlapping, which would reduce the major HIV variants to two, with CXCR4 usage as the main discriminative criterion ($CXCR4^-$ and $CXCR4^+$). The recent dramatic developments in the field have rendered obsolete all the previous nomenclature [i.e., syncytium-inducing (SI) vs non-syncytium-inducing (NSI), T-tropic vs M-tropic, rapid/high vs slow/low], since it has been documented that all viral variants do form syncytia, provided that target cells express sufficient levels of the relevant coreceptor; moreover, most, if not all, primary HIV-1 isolates, regardless of their coreceptor usage, can productively infect both primary $CD4^+$ T lymphocytes and mononuclear phagocytes. Indeed, at variance with previous suggestions, CXCR4 was recently shown to be a functional coreceptor for infection of human macrophages by primary $CXCR4^+$ strains, whereas the failure of long-term T-cell line-adapted X4 strains to grow in these cells is to be ascribed to postentry restriction factors (Verani *et al.,* 1998).

Recently, the focus on the relation between chemokines and viruses has widened, with the recognition that also a DNA virus, myxoma virus, can exploit chemokine receptors for infection of target cells (Lalani *et al.,* 1999). Similar to promiscuous HIV-1 strains, myxoma virus was shown to use at least three different chemokine receptors, namely CCR1, CCR5, and CXCR4; infection was specifically inhibited by the respective chemokine ligands. Whether additional receptor molecules, analogous to CD4 in the case of immunodeficiency retroviruses, are required for myxoma virus infection remains to be established.

In vivo evolution of HIV-1: There is AIDS without CXCR4. During the natural history of HIV-1 infection, a typical pattern of viral evolution has been documented (Fig. 2), with the invariable predominance of CCR5-dependent ($CXCR4^-$) strains during the early clinical

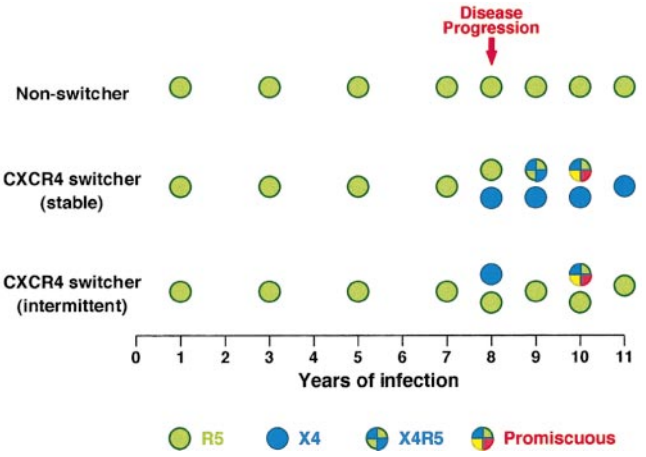

FIG. 2. Representative patterns of HIV-1 evolution *in vivo.* A significant proportion of patients never show any evidence of infection by $CXCR4^+$ HIV-1 despite progression of the disease. Promiscuous isolates can use interchangeably CXCR4 and CCR5, as well as some minor coreceptors. In uncloned viral isolates, usage of multiple coreceptors can be ascribed either to *bona fide* envelope promiscuity or, alternatively, to simultaneous infection by different biological variants.

stages, followed by the acquisition of CXCR4 usage and increasing coreceptor promiscuity, in parallel with the appearance of immunological and clinical signs of disease progression (Scarlatti *et al.,* 1997). Often, promiscuous isolates use interchangeably CXCR4 and CCR5, as well as minor coreceptors like CCR3, CX_3CR1, and/or others, reflecting either the presence of *bona fide* multitropic strains or a simultaneous infection by multiple biological variants. However, it is important to emphasize that this pattern of viral evolution is by no means the rule in the course of HIV-1 infection (Fig. 2). In fact, in approximately half the patients infected with clade-B and in up to 80% of those infected with clade-C HIV-1, usage of CXCR4 can never be documented throughout the course of the disease (Koot *et al.,* 1993; deRoda Husman *et al.,* 1999; Peeters *et al.,* 1999; Ping *et al.,* 1999), indicating that the emergence of $CXCR4^+$ variants is not an absolute requirement for the development of AIDS. Thus, despite a lower cytopathogenicity documented both in lymphoid tissue culture systems (Margolis, 1998) and in heterochimeric SCID-hu *thy/liv* mice (Camerini *et al.,* 2000), $CXCR4^-$ variants are sufficient to induce the full complement of immunologic defects leading to AIDS. Interestingly, however, late-stage $CXCR4^-$ biological clones from nonswitcher AIDS progressors seem to be inherently more pathogenic than early-stage isolates, as they were shown to induce thymocyte depletion in SCID-hu mice (Scoggings *et al.,* 2000); the increased pathogenicity of these strains is apparently unrelated to the use of additional, still undefined, coreceptors, as suggested by their inability to grow in cells from CCR5-deficient subjects (deRoda Husman *et al.,* 1999).

The above considerations raise some important questions. Being dispensable for the development of AIDS, do $CXCR4^+$ HIV-1 variants simply represent a peculiar type of opportunistic infection? Since small changes within the variable loops of gp120, most notably the V3 loop, are sufficient to confer CXCR4 usage, and in light of the remarkable infidelity of the viral replication machinery, a continuous emergence of $CXCR4^+$ variants would be expected; yet, $CXCR4^+$ viruses fail to appear for many years or never appear, in a high proportion of patients, throughout the disease course. Why? This apparent paradox can be explained by postulating a selective hindrance to the *in vivo* transmission and spread of $CXCR4^+$ variants in immunocompetent subjects. Several clinical observations corroborate this concept. First, horizontal transmission of $CXCR4^+$ HIV-1 appears to occur very rarely, as indicated by the extremely low infection rate in people with congenital CCR5 deficiency (see below). Second, in a few documented cases of *in vivo* transmission of a heterogeneous HIV-1 population (with admixed $CXCR4^-$ and $CXCR4^+$ variants), selective suppression of the $CXCR4^+$ component was documented after resolution of the primary infection (Cornelissen *et al.,* 1995; Lathey *et al.,* 1997). Furthermore, longitudinal follow-up of some patients undergoing viral phenotypic switch has demonstrated that, after their first appearance, $CXCR4^+$ variants do not rapidly and irreversibly become predominant, but rather tend to fluctuate and eventually disappear (Fig. 2), while $CXCR4^-$ variants remain consistently detectable (Ida *et al.,* 1997; Shankarappa *et al.,* 1999). All these observations imply the existence of negative selective forces acting against $CXCR4^+$ variants, the nature of which is currently unknown. A role has recently been suggested for the CXCR4 ligand, SDF-1, which was found to be highly expressed in the epithelium of the genital mucosae (Agace *et al.,* 2000). This observation could help to explain the preferential *in vivo* transmission of $CXCR4^-$ strains; however, given its low expression in blood cells and lymphoid tissues, SDF-1 is unlikely to explain the selective suppression of $CXCR4^+$ variants following the establishment of systemic infection. Do additional endogenous antiviral factors exist, which selectively target $CXCR4^+$ variants? Neutralizing antibodies are unlikely to play a part, as they do not seem to discriminate between HIV-1 variants based on coreceptor usage (Trkola *et al.,* 1998; LaCasse *et al.,* 1999). Could the cellular distribution of CCR5 and CXCR4 explain the different fates of the two viral variants *in vivo*? An intriguing hypothesis stems from the observation of a constitutive association of CD4 with CCR5 on the cellular surface (Xiao *et al.,* 1999). Thus, IL-2-stimulated, proliferating cells, albeit coexpressing CCR5 and CXCR4, would be preferentially infected by CCR5-using strains, resulting in a productive infection; conversely, $CXCR4^+$ viral particles would be predominantly internalized by resting $CD4^+$ T cells, which express CXCR4 but not CCR5, resulting in an abortive infection.

Use of chemokine receptors and pathophysiology of HIV infection. The use of chemokine receptors has critical implications for the pathogenesis, transmission, and immune control of HIV infection. The direct engagement of chemokine receptors by HIV may in itself represent a pathogenetic factor, as suggested by the observation that gp120-induced apoptosis of different cell types, including $CD8^+$ T cells and neurons, may be mediated by signaling through CXCR4 (Herbein *et al.,* 1998; Meucci *et al.,* 1998; Biard-Piechaczyk *et al.,* 2000).

Because the availability of functional chemokine receptors on specific cell types is a critical requisite for HIV entry, any genotypic or phenotypic host factors modulating their expression can markedly affect the viral transmission and tissue tropism. Several genetic polymorphisms of chemokines and chemokine receptors have been described, some of which can influence the susceptibility to or the course of HIV infection, either by reducing chemokine-receptor expression or by enhancing the production of suppressive chemokines. The first conclusive evidence that resistance to HIV can be ge-

netically determined was obtained with the identification of a homozygous 32-base-pair deletion within the coding sequence of the *CCR5* gene (CCR5-Δ32) in multiply exposed HIV-seronegative individuals (Samson *et al.,* 1996; Liu *et al.,* 1996); both $CD4^+$ T lymphocytes and macrophages derived from these subjects are inherently resistant to infection by $CXCR4^-$ HIV-1 strains, due to the absence of CCR5 on their surface membrane. By contrast, CCR5-Δ32 heterozygotes do not show a reduced risk of infection, but their disease course is delayed. This unique experiment of nature attests to the pivotal role of CCR5 in both person-to-person and cell-to-cell transmission of HIV-1 *in vivo,* downplaying the role of other viral coreceptors, including CXCR4, at least during the early phases of HIV-1 infection. Moreover, the lack of any apparent phenotype in CCR5-deficient people, while confirming the redundancy of the inflammatory chemokine system, provides a rational basis for the development of CCR5-targeted therapeutic approaches. Additional genetic polymorphisms of both chemokine receptors (e.g., CCR2, CCR5) and chemokines (e.g., SDF-1, RANTES) have subsequently been linked to a slower disease progression (reviewed in Berger *et al.,* 1999). However, some of these associations remain controversial. Conversely, a homozygous polymorphism in the CX_3CR1 gene was recently associated with accelerated progression of HIV-1 disease (Faure *et al.,* 2000). It is important to emphasize that multiple genetic polymorphisms may occur in the same individual, giving rise to a wide range of different phenotypes.

The choice of CCR5 as a primary coreceptor renders HIV vulnerable to the inhibitory activity of the CCR5 ligands RANTES, MIP-1α, and MIP-1β. Thus, the endogenous production of these chemokines in response to HIV infection represents a critical factor for the *in vivo* spread of this infection. A central paradox of HIV infection is indeed the fact that the virus induces the *in vivo* production of large amounts of its own natural inhibitors. In lymphoid tissue, which represents a primary site of viral replication, a dramatic expression of RANTES, MIP-1α, and MIP-1β was documented since the early stages of HIV-1 infection (Trumpfheller *et al.,* 1998). MIP-1α and MIP-1β were detected predominantly in $CD68^+$ macrophages within germinal centers, whereas massive amounts of RANTES mRNA were seen in the T-cell-dependent extrafollicular zone; strikingly, the levels seen in control lymph nodes, with mycobacterial infection or HIV-unrelated follicular hyperplasia, were markedly lower or null. Similarly, RANTES overexpression was seen in lymph node cell suspensions derived from HIV-1-infected subjects (Triozzi *et al.,* 1998), in plasma of patients with primary HIV-1 infection (Malnati *et al.,* 1998), and in mononuclear cells from intestinal lymphoid tissue (Ndolo *et al.,* 1999), lymph nodes, peripheral blood, and bronchoalveolar lavage (Cheret *et al.,* 1999) during primary SIV infection in macaques. Several important questions arise from these observations. What is the exact role of this sustained chemokine production in the natural history of HIV/SIV infection? Does it provide a physiological explanation for the inherently slow course of these *lenti*-viral infections? Is this a survival strategy for HIV/SIV, aimed at preventing widespread viral replication *in vivo,* which would lead to a rapid extinction of the host? Does it at the same time represent a pathogenetic factor which could, in the long term, compromise the immunologic function? What is the underlying mechanism? Is this mechanism specific for HIV/SIV infection or does it merely reflect a particularly robust nonspecific immune activation? Providing an answer to these questions will not only advance our understanding of the physiology of HIV infection, but also open new perspectives for the development of effective therapeutic and preventive strategies.

An unorthodox correlate of protection. For almost two decades, investigators have strived to identify correlate markers of vaccine-induced protection from SIV infection in experimental nonhuman primate models, as well as of natural protection in exposed–uninfected (EU) human subjects. However, the evidence obtained with the classic protection correlates has been inconclusive. Several recent observations suggest that the long-sought-after correlates may be provided by an unconventional type of immune response, associated with the release of high levels of CCR5-binding chemokines. Whether such chemokines play a direct protective role *in vivo* or, alternatively, are simply linked with other, still undefined, antiviral mechanisms remains to be established. Of note, CCR5 and its ligands are preferentially expressed in Th1-polarized responses (see above), which are essential in the control of intracellular pathogens like retroviruses.

The first indication that chemokine levels might correlate with vaccine-induced protection from SIV challenge came from studies in macaques immunized by the targeted iliac–lymph node route: in all the protected animals, as well as in a single naturally resistant one, mitogen-activated $CD8^+$ T cells were found to release increased amounts of soluble virus-suppressive factors, mainly consisting of CCR5-binding chemokines, compared to nonprotected animals (Lehner *et al.,* 1996). These observations were confirmed in subsequent SIV or SHIV vaccination studies using a variety of immunization protocols (Heeney *et al.,* 1998; Gaudin *et al.,* 1998; Wang *et al.,* 1998; Caufour *et al.,* 1999); a booster effect was seen upon secondary immunization, suggesting the involvement of memory $CD8^+$ T cells (Wang *et al.,* 1999). Although chemokine secretion is not unexpected among memory T-cell responses elicited by specific antigen stimulation (Moss *et al.,* 1997), the uniqueness of this phenomenon lies in the polyclonal nature of the $CD8^+$

T-cell response. Remarkably, a similar type of immune response may also be linked to natural HIV resistance, as suggested the increased prevalence of a high-RANTES/low-CCR5 phenotype in EU subjects, associated with Th1 polarization and decreased susceptibility of $CD4^+$ T cells to HIV-1 infection (Paxton *et al.,* 1998, and personal communication). All the above observations suggest the existence of a link between cognate and innate immune mechanisms, which seem to act in synergy for the control of HIV infection; nevertheless, the pathways whereby this connection is established remain obscure. The elucidation of such mechanisms might eventually lead to the development of novel vaccine strategies for the prevention of HIV infection.

Chemokines as new therapeutic principles. The discovery of the HIV-suppressive chemokines has opened new perspectives for the therapy of AIDS. Indeed, compounds capable of hindering the interaction between the viral envelope and the coreceptors might effectively complement the current poly-chemotherapy regimens, by aiming at a third molecular target, besides the two viral enzymes, reverse transcriptase and protease. According to the paradigm established for anticancer therapy more than two decades ago, such multiplication of molecular targets is essential for the long-term prevention of the development of drug resistance.

By virtue of the pivotal role it plays in the transmission and spread of HIV-1, the CCR5 coreceptor represents a prime target for new therapeutic strategies (Lusso, 1997), also in consideration of the apparent lack of immunologic alterations associated with its congenital deficiency. Different approaches have been proposed, including the use of natural chemokines, either in soluble form or with an endoplasmic reticulum-localization tag for intracellular CCR5 trapping (intrakines), full-length or small-peptide chemokine derivatives, and nonpeptide compounds. Particular emphasis has been posed on the need to produce nonsignaling molecules, in order to avoid potentially harmful inflammatory side effects, as well as putative enhancing effects on the replication of $CXCR4^+$ strains (Kinter *et al.,* 1998). Promising small-molecule nonpeptide CCR5 blockers have recently been developed, which are devoid of agonistic activity (Baba *et al.,* 1999; B. Baroudy, personal communication). Nevertheless, the perspective of employing CCR5-targeted agents in the therapy of HIV infection is stirring controversy, mainly related to the putative risk of exerting a selective pressure on the virus, thereby favoring the acquisition of CXCR4 usage. Although such a viral phenotypic switch was indeed observed in a proportion of infected SCID-hu mice treated with modified RANTES analogues (Mosier *et al.,* 1999), there are reasons to believe that this scenario will not occur in humans. As discussed above, the endogenous production of CCR5-binding chemokines in HIV-infected lymphoid tissue already provides a strong *in vivo* selective pressure in favor of CXCR4 usage (Trumpfheller *et al.,* 1998); this notwithstanding, $CXCR4^+$ HIV-1 strains emerge only in a limited proportion of patients and only after several years of sustained viral replication, suggesting the existence, *in vivo,* of negative forces that selectively restrain $CXCR4^+$ viruses. Thus, the exogenous administration of CCR5-targeted agents would be unlikely to induce the early emergence of $CXCR4^+$ HIV-1 strains; on the contrary, such agents should act in synergy with endogenous chemokines in suppressing the spread of $CXCR4^-$ HIV-1 strains, thereby also reducing the rate of spontaneous mutations which may favor the acquisition of CXCR4 usage.

In addition to CCR5, CXCR4 represents a primary target of new antiviral drugs, both for preventing the emergence of $CXCR4^+$ variants and for the treatment of patients with advanced disease. Small CXCR4-targeted molecules have been identified, including the bicyclam AMD3100 (Schols *et al.,* 1997) which was already recognized as an effective anti-HIV agent before the discovery of the HIV coreceptors. However, it is important to emphasize that a functional blockade of CXCR4 may cause severe side effects related to the crucial role of this housekeeping receptor in organ development and homeostasis. Indeed, both CXCR4 and SDF-1 knockout mice show a lethal phenotype, with severe defects not only in the B-lymphoid and myeloid compartments, but also in nonhematopoietic tissues, such as the heart and the brain (Nagasawa *et al.,* 1996; Ma *et al.,* 1998; Zou *et al.,* 1998).

In conclusion, the extraordinary advances in HIV biology during the past 5 years, in particular the elucidation of the complex relation between the virus and the chemokine system, have raised a cautious optimism on the possibility of developing effective new strategies for the control of HIV infection. Specifically, with the availability of specific blockers of the two main HIV coreceptors, CCR5 and CXCR4, it will be possible to design highly efficacious multidrug therapeutic protocols targeting three distinct steps in the viral life cycle: entry, reverse transcription, and maturation. The implementation of such protocols might eventually succeed in achieving the ultimate goal of anti-HIV treatment, which is still beyond the reach of the current therapeutic weapons: virus eradication.

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