Viral antichemokines: from pathogenesis to drug discovery

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In a recent *JCI* Commentary, Steven Kunkel reviewed the organization of the chemokine system and its important role in the development, differentiation, and deployment of mammalian leukocytes (1). The system is massive (about 50 ligands and 18 receptors are known), reflecting the complexity of its major client, the immune system. Now in this issue of the *JCI*, Liu and colleagues remind us that an antichemokine system has evolved in viruses, presumably as a strategy to evade the immune system, and they provide the first glimpse of how antichemokines might be exploited clinically as "ready-made" anti-inflammatory drugs (2).

Antichemokines comprise a major group of virus-encoded chemokine modulators (Table 1) and consist of three subgroups based on structure and mechanism of action: (a) chemokine homologues that act as chemokine receptor antagonists (e.g., MC148R of *Molluscum contagiosum* virus [MCV]; ref. 3); (b) plasma membrane–expressed chemokine receptor homologues, which function as chemokine scavengers (e.g., US28 of human cytomegalovirus; ref. 4); and (c) three subtypes of secreted chemokine-binding proteins, which have unique structures and unknown ancestry but function as extracellular chemokine scavengers (examples include M3 of γ-herpesvirus 68 [5, 6] and various poxivirus proteins, including M-T7 of myxoma, investigated by Liu et al. in this issue [2]). Three other groups of virus-encoded chemokine modulators have quite different functions. They include (a) chemokine receptor homologues, such as open reading frame 74 of Kaposi's sarcoma–associated herpesvirus (7), which serves as a growth factor and angiogenic factor; (b) chemokine homologues that function as chemokine receptor agonists, including vMCK-1 of murine cytomegalovirus, which promotes viral dissemination via monocytes (8); and (c) nonchemokine agonists and antagonists of chemokine receptors, encoded by the HIV genome.

Viral chemokine modulators, in turn, are part of a larger group of viral proteins with obvious homology to host proteins. These primarily include immunomodulatory, growth factor, and cell cycle control proteins (9). Interestingly, M-T7 is a hybrid. It has one domain homologous to the extracellular region of the IFN-γ receptor, as well as a COOH-terminal chemokine-binding domain. Chemokines bind M-T7 via their COOH-termi-

Table 1
Viral chemokine modulators

Virus family	Virus	ORF	Common names	Chemokine or receptor class	Function
γ-Herpesviridae	*H. saimiri*	74	ECRF3	ELR+ CXCR	Calcium flux in vitro
	HHV8 (KSHV)	74	ORF 74		
		M3	M3	Chemokine binding protein	C, CXC, CC, CX3C chemokine scavenger
			KSHV GPCR	CC/CXCR (constitutively active)	Oncogenic Angiogenic
		K6	vMIP-I	CC chemokine	Angiogenic CCR8 agonist
		K4	vMIP-II	CC chemokine	Angiogenic HIV suppressive Th2 chemoattractant Antagonist at CCR1, CCR2, CCR3, CCR5, CXCR4, and CCR8
β-Herpesviridae	Human CMV	US28	US28	CC/CX3CR	Calcium flux Chemokine sequestration Smooth muscle cell chemokinesis HIV coreceptor
		UL146	vCXC-1	CXC chemokine	Neutrophil calcium flux, chemotaxis, and degranulation CXCR2-specific
		UL147	vCXC-2	CXC chemokine	NA

Table 1 (continued)

	Mouse CMV	m131/129	m131/129 MCK-1/MCK-2	CC chemokine	Virulence factor Blocks natural killer and T-cell response to MCMV in vivo; also proinflammatory early in infection. Mutant virus→reduced viremia Calcium flux (mouse peritoneal macrophages; human CCR3 transfectants)
	HHV6	U12 U83	U12 U83	CCR CC chemokine	Calcium flux in vitro THP-1 cell chemotaxis
Poxviridae	*Molluscum contagiosum* virus (MCV)	MC148	MC148R vMCC-1	CC chemokine	Blocks neutrophil monocyte, and T-cell chemotaxis induced by multiple CC and CXC chemokines Antagonist at CCR2 and CCR8 Blocks human hematopoietic progenitor cell proliferation
	Ortho- and leporipoxviruses	B29R (vaccinia)	T1 35-kDa protein vCCI vCKBP vCBP-I	CC chemokine-binding protein	Broad spectrum CC chemokine scavenger Anti-inflammatory in context of vaccinia infection and allergic airway inflammation in guinea pigs
	Myxoma	T7	T7 vCBP-II	C, CXC, and CC chemokine-binding protein	Broad spectrum chemokine and IFN-γ scavenger Virulence factor: anti-inflammatory in context of myxoma infection
Lentiviridae	HIV	Tat	Tat	CC chemokine mimic	Monocyte chemoattractant: CCR2, CCR3 agonist HIV suppressive factor at CXCR4 CCR8 antagonist Chemotactic agonist at CCR5
		env	gp120	Chemokine mimic	Neuronal apoptosis via CXCR4

All molecules listed, except for the poxvirus chemokine-binding proteins and HIV Tat, have conserved sequences with cellular chemokines or chemokine receptors   CCR, CC chemokine receptor; CXCR, CXC chemokine receptor; ELR+, subclass of neutrophil-targeted CXC chemokines; NA, not available; ORF, open reading frame; HHV, human herper virus, KSHV, Kaposi's sarcoma–associated herpervirus; CMV, cytomegalovirus; MCMV, mouse cytomegalovirus. See text and ref. 14 for primary citations.

nal glycosaminoglycan–binding (GAG-binding) domain (10). Fortuitously, M-T7 binds only to the rabbit form of IFN-γ, whereas for chemokines it crosses species barriers, thus permitting its use as a selective chemokine blocking agent in species other than rabbits.

Much of the rapid progress in this field can be attributed to major advances in viral genomics and to the advent of computer cloning. Examples continue to be found as additional viral genomes are sequenced and analyzed. However, in most cases it has been difficult to define relevant biological correlates, owing either to difficulty studying the virus (as in the case of MCV, which has not been grown in culture) or to the absence of animal models for the viral disease. Nevertheless, the general notion that chemokines act in antiviral host defense has solid support from studies with knockout mice lacking the chemokine macrophage inflammatory protein-1α (MIP-1α). These animals show decreased inflammatory responses to influenza A, Coxsackie B, and murine cytomegalovirus (11, 12). Thus it is tempting to speculate, for example, that the odd absence of inflammatory cells in pathologic lesions caused by MCV results from an antichemokine shield provided by secretion of its antichemokine MC148R (3). Myxoma virus, which causes a fatal systemic immunosuppressive disease known as myxomatosis in the European rabbit *Oryctolagus cuniculus*, is well suited to experimental analysis, and of all secreted myxoma gene products analyzed to date, deletion of M-T7 most profoundly attenuates virus virulence (ref. 13; G. McFadden, personal communication). However, whether this is due to blockade of IFN-γ, chemokines, both, or neither is still not clear.

Viral antichemokines typically have broad spectrum activity (e.g., M-T7 binds multiple CXC and CC chemokines, as well as the C chemokine lymphotactin), which suggests converse-

ly that the host mounts a broad antiviral chemokine response. This is consistent with studies of chemokine expression in patients with immunologically mediated diseases, such as multiple sclerosis, rheumatoid arthritis, and psoriasis. Yet, counterintuitively, knockout or neutralization of just one chemokine or receptor in vivo markedly attenuates pathology in numerous and diverse animal models of inflammation (reviewed in ref. 14). While detailed studies of the spatial and temporal aspects of chemokine expression during the course of inflammatory challenge are needed to more fully interpret these results, these successes may justify developing anti-chemokine therapies for the clinic.

Recently several potent small molecule antagonists of specific chemokine receptors have been discovered, and more are on the way (14). Some of these were obtained "off the rack" as HIV entry blockers, after the discovery that the chemokine receptors CCR5 and CXCR4 play a major role in this process (15). Others were found by drug discovery efforts targeting specific chemokine receptors. Additional blocking strategies underway include development of humanized neutralizing antibodies to chemokines and chemokine receptors. Preclinical studies and disease indications, eagerly awaited for all of these compounds, are now available for M-T7. As Liu et al. (2) report, a single intravenous injection of just 0.017 pg/g of M-T7 protein given to rats or rabbits immediately after balloon angioplasty attenuated atherosclerosis/restenosis injury, including reduction in both neointima formation and macrophage infiltration. Since M-T7 binds the chemokine monocyte chemoattractant protein-1 (MCP-1), the results fit well with recent reports of reduced pathology in atherosclerosis-prone mice when either MCP-1 or its receptor CCR2 is genetically disrupted (16, 17). Moreover, since M-T7 does not bind rat IFN-γ, the effects in rats cannot be due to scavenging of that cytokine. Indirect arguments suggest that the effects are not mediated by IFN-γ in the rabbit model either (2).

Whether the histological effects of M-T7 in this model are really due to blockade of MCP-1 (or some other chemokine) is still unresolved. The authors show that M-T7 treatment is associated with reduced detection of MCP-1, MIP-1α, and RANTES antigen in the medial layer of the arterial wall after angioplasty injury in the rat model, but they are careful to point out that this effect could occur by several mechanisms, including antigen masking. Moreover, the maximal concentration that one might achieve in vivo from the 0.017 pg/g dose of M-T7, even assuming distribution to be restricted to the intravascular space, is much lower than the reported K_d for chemokine binding. Finally, M-T7 could potentially bind to other extracellular regulators that contain GAG-binding domains, such as basic FGF (A. Lucas, personal communication). Despite these caveats about mechanism, the efficacy of M-T7 is quite striking and no toxicity was noted. Thus, restenosis injury may now be regarded as a disease indication for further clinical development of this compound. It remains to be seen whether M-T7 will provide a useful anti-inflammatory therapy for established inflammation or systemic disease. Timing of administration, bioavailability, pharmacokinetics, and antigenicity will pose bigger problems in those clinical settings than in the present experimental study.

The M-T7 results are similar to this group's previous reported results for SERP-1, a secreted serine proteinase inhibitor made by myxoma, in the same rat and rabbit models as well as in a rat aortic allograft model of transplant vasculopathy, and in a rabbit model of antigen-induced arthritis (18–20). These results persuaded the authors to launch a new biotechnology company, Viron Therapeutics Inc., London, Ontario, Canada, to explore the therapeutic potential of natural viral immunomodulators (21, 22). Ironically, now more than two decades after the eradication of smallpox, products of related poxviruses hold substantial promise for the treatment of human disease.

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