The chemokines: cytokines that direct leukocyte migration

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INTRODUCTION

In a previous editorial review in this *Journal*, Appleton[1] discussed the involvement of cytokines in wound healing and the role of chemoattractants in promoting a leukocyte infiltrate. However, factors such as C5a, leukotriene B4, platelet-activating factor and the bacterial N-formyl peptides do not have sufficient specificity to explain the distinct nature of the inflammatory infiltrate seen under different conditions. Over the past decade, considerable progress has been made in characterizing a subfamily of chemotactic cytokines, the chemokines. They direct the migration of specific leukocyte subsets and, as a group, share a number of structural and functional similarities[2,3]. Chemokines are all small proteins (8–10 kd) and are often glycosylated. Their amino acid sequences show 20–80% homology, and their monomeric tertiary structures have considerable similarity[4].

One of the defining characteristics of the chemokines is the presence of two pairs of highly conserved cysteine residues; the amino terminal pair are either separated by a single amino acid (c-x-c, α chemokines) or are adjacent (c-c, β chemokines). The genes for c-x-c chemokines are located on chromosome 4 in man whereas those for c-c chemokines are found on chromosome 17. The genomic structure of many c-x-c chemokines contains four exons whilst there are usually only three for c-c chemokines. Many c-x-c chemokines are neutrophil chemoattractants but c-c chemokines tend to be active on monocytes and T-cells. Certain c-x-c chemokines contain a characteristic amino acid sequence consisting of glutamic acid, leucine and arginine (ELR motif) at the amino terminus. The ELR motif appears to be important for signal transduction involved in neutrophil chemotaxis and is absent in c-c chemokines[5].

So far at least 15 chemokines have been characterized for each group. Some of the better known c-x-c chemokines include interleukin-8 (IL-8), melanocyte growth-stimulating activity (MGSA/Gro-α, β and γ), platelet basic protein (PBP) and its derivatives β-thromboglobulin (β-TG), connective tissue activating peptide III (CTAP-III) and platelet factor 4 (PFG4), γ inducible peptide-10 (IP-10) and epithelial neutrophil activating peptide 78 (ENA-78). Well-known members of the c-c family are monocyte chemoattractant proteins 1 (MCP-1), 2 and 3, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β, RANTES, and eotaxin. Lymphotactin is the only chemokine that does not fit into the c-x-c or c-c family[6]. It is structurally similar to other chemokines, but the first and third cysteine residues appear to have been lost. The gene for human lymphotactin is found on chromosome 1. Activated $CD8^+$ T-cells are a source of this chemokine, which appears to act specifically on lymphocytes.

THE MECHANISMS OF ACTION OF CHEMOKINES

Chemokines in solution are capable of stimulating the directed migration of white cells. Evidence, however, is accumulating that chemokines are bound *in vivo* to proteoglycans on the surface of vascular endothelium where they are presented to leukocytes[7,8]. We do not know whether chemokines exist physiologically as monomers or dimers[9,10], but it is likely that receptor binding promotes leukocyte adhesion by triggering the activation of integrins[11].

Several chemokine receptors have been cloned[12,13]. They all consist of an extracellular amino terminus, seven transmembrane domains and a cytoplasmic carboxy-terminal portion. They thus share structural homology with receptors for other leukocyte chemoattractants, such as the complement component C5a, as well as receptors for other signalling molecules, for instance adrenaline and angiotensin II. Various approaches, such as the use of protein chimeras, site-directed mutagenesis and truncated proteins, have been employed to study the details of receptor-ligand interactions.

Chemokine receptors are defined by the chemokines that they bind. Thus, the MCP-1 receptor appears to be specific for MCP-1 while the IL-8 receptor B is shared within the c-x-c group between IL-8 and Gro-α[13]. A promiscuous receptor that binds chemokines from both groups has been characterized as the Duffy blood group antigen[14]. Ligand binding to this receptor is not associated with a calcium flux, which normally appears to be required for signalling. The main role of the Duffy antigen is proposed as a sink for free chemokine molecules in order to prevent leukocyte adhesion at inappropriate sites. However, this receptor may have other roles since it is also expressed on post capillary venules[15]. Many orphan receptors are known. These molecules have sequence homology with known chemokine

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receptors but their ligand has not yet been identified[12]. Some viruses, for instance cytomegalovirus[16], also express receptor molecules that bind multiple chemokines.

Ligand binding is usually followed by a rapid rise in intracellular calcium and may be inhibited by pertussis toxin, which implies a G protein linked signalling pathway[17,18]. More recently, chemokine receptor activation has also been shown to occur in the absence of a calcium flux, and a wortmannin sensitive phosphoinositide 3-kinase pathway has been proposed[19].

It is becoming apparent that chemokines are not necessarily specific for individual leukocyte subtypes: MCP-1, for instance, will have a chemoattractant effect upon monocytes[20], T-cells[21], basophils[22] and NK cells[23]. Their activity is not limited to chemoattraction. IL-8 will stimulate neutrophil degranulation and the production of the respiratory burst[24] in addition to chemotaxis. There is also some evidence that IL-8 plays a role in new blood vessel formation[25].

LEUKOCYTE ACCUMULATION IN RESPONSE TO CHEMOKINES

Chemokines cause leukocytes to accumulate *in vivo*. IL-8 elicits a neutrophil infiltrate when injected in the skin of experimental animals[26] and intradermal injection of MCP-1 causes a monocyte accumulation[27]. This association of specific infiltrates with specific chemokines has been exploited in characterizing novel members of the group; for instance, the c-c chemokine eotaxin was characterized from broncho-alveolar lavage fluid in a guinea-pig model of asthma associated with massive pulmonary eosinophilia[28].

Immunohistochemical studies and *in situ* hybridization have revealed chemokines in human pathology. MCP-1 is associated with the monocyte infiltrate which occurs in atherosclerotic plaques[29], rheumatoid arthritis[30], idiopathic pulmonary fibrosis[31] and a variety of tumours[32–34]. IL-8 has been shown to be present where neutrophils accumulate in infectious[35] and inflammatory conditions[36]. Both IL-8 and Gros-α have been detected within psoriatic plaques[37].

Chemokines can cause the migration of specific leukocyte subsets *in vitro*, and there is now evidence from both animal models and the study of human diseases that this reflects their role *in vivo*. The 4th International Chemokine Symposium was held this year. It is very clear that enormous progress has been made, but many important fundamental questions remain, ranging from chemokine structure to the nature of the intracellular signalling pathways. The importance of these molecules in every disease state associated with an inflammatory (leukocyte) infiltrate cannot be overestimated.

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