CHEMOTACTIC CYTOKINES (CHEMOKINES) AND THEIR ROLE IN PHYSIOLOGICAL AND IMMUNOPATHOLOGICAL REACTIONS*

(chemokine / leukocyte trafficking / inflammation / RANTES / interleukin-8)

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Chemotactic cytokines or chemokines (Lindley et al., 1993) are members of a family of small, secreted polypeptides of molecular weight of 8000–11000 (Oppenheim et al., 1991). The ability of chemokines to selectively attract white blood cell subtypes suggests their involvement in leukocyte trafficking between the circulation and the tissues. Chemokines can also induce leukocyte activation and affect cellular proliferation. They are therefore implicated as regulators of important immune-mediated events, in particular of inflammation.

1. IDENTIFICATION OF CHEMOKINES

The majority of chemokines (Table 1) was described in the last decade. In some chemokines, e. g. interleukin (IL)-8, biochemical characterization preceded their

Abbreviations: IL – interleukin; RANTES, MIP, MCP, GRO, MGSA – see the legend to Table 1, cDNA – complementary DNA, TNF – tumor necrosis factor, VLA-4 – very late antigen-4, ICAM-1 – intercellular adhesion molecule-1, mRNA – messenger RNA, NF-κB – nuclear factor kappa B.

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Table 1. Human chemokines (modified according to Ahuja et al., 1994)

Group	Chemokine	Target cells
C-X-C chemokines (chromosome 4)	γIP-10	M, T
	Mig	?
	NAP-4	N
	PF-4	N, M, T
	ENA-78	N
	GCP-2	N
	GRO α, β, γ	N, Ba
	NAP-2	N
	IL-8	N, T, Ba, E
C-C chemokines (chromosome 17)	MCP-1	M, T, Ba
	MCP-2	M, T
	MCP-3	M, T, E, Ba
	MIP-1α	M, T, E, Ba, N, P
	MIP-1β	M, T
	RANTES	M, T, E, Ba
	I-309	M
C chemokines (chromosome 1)	SCM-1	?

Chromosome – localization of a chemokine gene in humans. Target cells – leukocytes which are the targets of chemotactic and activation effects of a chemokine.
Abbreviations:
γIP-10 – γ interferon-inducible protein 10, Mig – monokine inducible by γ interferon, NAP – neutrophil activating peptide, PF-4 – platelet factor 4, ENA-78 – epithelial cell-derived neutrophil-activating protein, GCP-2 – granulocyte chemoattractant protein 2, GRO – growth-related gene (equivalent name: MGSA; melanoma growth stimulatory activity), IL-8 – interleukin 8, MCP – monocyte chemoattractant protein, MIP – macrophage inflammatory protein, RANTES – Regulated on Activation Normal T-cell Expressed and Secreted, SCM-1 – single cysteine motif-1, M – monocyte, T – T lymphocyte, B – B lymphocyte, N – neutrophil, E – eosinophil, Ba – basophil, P – myeloid progenitor-cells.

molecular cloning. Other members of chemokine family, e. g. RANTES, were characterized directly at the cDNA level during searching for new activation genes in immune cells using the method of subtractive hybridization (Schall, 1991). The chemokine family currently comprises nearly 30 members (Schall and Bacon, 1994).

Two potential members of the family, the eosinophil attractant eotaxin (Jose et al., 1994) and the lymphocyte attractant lymphotactin (Kelner et al., 1994) were identified in animals and the existence of their human equivalents is being investigated.

2. STRUCTURAL CHARACTERISTICS

All chemokines are basic heparin-binding polypeptides with short N-terminal and long C-terminal sequences. A leader peptide sequence of 20–25 amino acids is cleaved from the chemokine precursors (92–99 amino acids) before the mature protein is secreted. Among individual members of the family there is considerable similarity: at the amino acid level, the sequence homology ranges from 30 to 80% (Ahuja et al., 1994). All chemokines possess a conserved motif of four cysteine residues, forming two characteristic disulfidic bonds in the tertiary structure of the proteins (Oppenheim et al., 1991). According to the presence of an intervening amino acid (X) between the first two cysteines (C) in the mature protein, chemokines have been subdivided into two groups, namely C-X-C (α) and C-C (β). Whereas the tertiary structures of human C-C and C-X-C chemokines are very similar, the quaternary structures are completely different: the IL-8 (C-X-C) dimer is globular, the MIP-1β (C-C) dimer is elongated and cylindrical (Clore and Gronenborn, 1995). This may account for some differences in biological activities of C-X-C and C-C chemokines, e. g. the lack of receptor cross-binding and of the reactivity between the two groups.

Very recently, Schall and Bacon (1994) have proposed that the nomenclature of the chemokine system may be expanded to include a third, so-called "C" branch. The prototypes of this novel chemokine class, the mouse chemokine lymphotactin (Kelner et al., 1994) and its plausible human homologue SCM-1 (Yoshida et al., 1995), lack two of the four characteristic cysteine residues, while they share a great deal of amino acid similarity with C-C and C-X-C chemokines.

3. CHEMOKINE GENES

The division into C-X-C, C-C, and possibly "C" chemokines corresponds to the fact that their genes are located on distinct chromosomes. The genes for C-X-C chemokines have been mapped to chromosome 4, q12-21 in humans; the genes for C-C chemokines have been located to human chromosome 17, q11-21 (Oppenheim et al., 1991). The gene encoding "C" chemokine lymphotactin maps to chromosome 1 in the mouse, contrasting with the location of the genes for the traditional C-X-C and C-C chemokines in the murine genome (Kelner et al., 1994). The gene for "C" chemokine SCM-1 is also distinctly mapped to human chromosome 1 (Yoshida et al., 1995).

The genes for human C-C chemokines show a conserved 3 exon/2 intron organization (reviewed by Schall, 1991 or Nelson et al., 1993). C-X-C chemokine genes,

Table 2. Principal functional properties of chemokines

a) Leukocyte chemotaxis
- Selective* potentiation of leukocyte adhesion
- Selective* transmigration of leukocytes through the capillary wall (actin polymerization, shape changes, increase in intracellular calcium)

b) Leukocyte activation
- Neutrophils (C-X-C chemokines with the exception of γIP-10): exocytosis of granules, increase in β_2-integrin expression, production of bioactive lipids, respiratory burst
- Eosinophils and Basophils (RANTES, MIP-1α, MCP-1, IL-8): histamine liberation, activation of cytolytic mechanisms
- Monocytes – Macrophages (C-C chemokines, γIP-10): release of lysozomal enzymes, O_2^- – release, cytostatic effects, respiratory burst

c) Regulation of cellular proliferation
- Malignant stem cells in the bone marrow: inhibition of proliferation by MIP-1α
- T lymphocytes: inhibition of αCD3-induced proliferation by MIP-1α
- Endothelial and smooth muscle cells: stimulation of proliferation (IL-8)
- Malignant cells: stimulation of melanocyte proliferation (GRO/MGSA)

*Selectivity of effect: for the target cells see Table 1.

except for the platelet factor-4 (PF-4) gene, possess an additional exon and intron (reviewed by Baggiolini et al., 1994). Similar organization including similarity in the splice junctions and in the sizes of the introns and exons (Baggiolini et al., 1994) suggests that the genes for the two chemokine subgroups have evolved by a gene duplication and chromosomal translocation from the same ancestral gene. Generation of chemokine multigene clusters by these mechanisms would probably benefit from DNA with high recombinatorial efficiencies such as repetitive and palindromic sequences. However, in the C-C subfamily, such sequences were found only in a limited number of genes, i. e. MCP-3, MIP-1α, and I-309 (Opdenakker et al., 1994).

4. CHEMOKINE FUNCTIONS

Though the chemokines do not have such pleiotropic properties as some other cytokines (e. g. IL-1 or TNF-α), they still possess a wide spectrum of activities, some of which go far beyond the boundaries of the immune system.

4.1. Chemokines and leukocyte migration

The primary function of chemokines is to selectively attract leukocytes by chemotaxis or haptotaxis including potentiation of binding interactions between leukocytes and the vascular endothelium (Table 2a). The emigration of leukocytes from blood vessels to a tissue is a complex four-phase process (Adams and Shaw, 1994). In the first phase, circulating leukocytes are slowed down by the effect of selectins and they start "rolling" along the vessel wall. During the second phase, leukocyte integrins, e. g. CD11b on neutrophils or VLA-4 (CD49d) on T lymphocytes, are activated. The activation leads to the third phase, which is a strong adhesion of leukocytes to the endothelial cells and to extracellular matrix around the endothelial junctions. The adhesion is mediated by the binding of leukocyte integrins to their ligands (e. g. ICAM-1 [CD54], VCAM-1 [CD106]) on activated endothelium. In the fourth phase, diapedesis leads to leukocyte transmigration between endothelial cells to the extravascular space.

Chemokines are actively involved mainly in the last phase of the migration process. The traditional view of leukocyte movement following a soluble chemoattractant gradient has encountered a number of problems, e. g. the instability of the gradient due to a rapid dilution of diffusible chemokines by the blood flow (Kunkel et al., 1994). Consequently, as an alternative mechanism, Rot (1992) has proposed that leukocytes migrate to tissues by "haptotaxis" – a directed movement of cells in response to the insoluble gradient of immobilized chemokines either bound to the endothelium or anchored to glycosaminoglycans of subendothelial extracellular matrix (ECM). At least with three chemokines – IL-8 (Rot, 1992), MIP-1β (Tanaka et al., 1993), and RANTES (Gilat et al., 1994) – their immobilization promoted adhesion of neutrophils and T lymphocytes to the endothelium. Proadhesive effects of RANTES and MIP-1β, immobilized to ECM, were abrogated by heparinase treatment of ECM (Gilat et al., 1994). This would suggest that the activity of ECM-degrading enzymes, such as heparinase, secreted by antigen- or cytokine-activated matrix-residing leukocytes, results in detachment of immobilized chemokines, therefore the leukocytes become mobile and migrate further on to the site of inflammation.

Leukocyte migration is initiated by the binding of chemokines to their specific receptors on leukocytes (see below). The ligand binding results in an increase of intracellular calcium ($[Ca^{2+}]_i$) and an activation of enzymatic pathways of signal transduction. Subsequently, the imbalance between globular and filamentous actin leads to actin polymerization, to elongation of the cell and polarization of its membrane (Vaddi et al., 1994). These cytoskeletal changes are the prerequisite for cellular movement and the transmigration to tissues.

Substantial degree of selectivity is the major difference between the effect of chemokines and the traditional chemoattractants such as the C5a complement component or bacterial products, e. g. formyl peptide (fMLP) (Baggiolini et al., 1994).

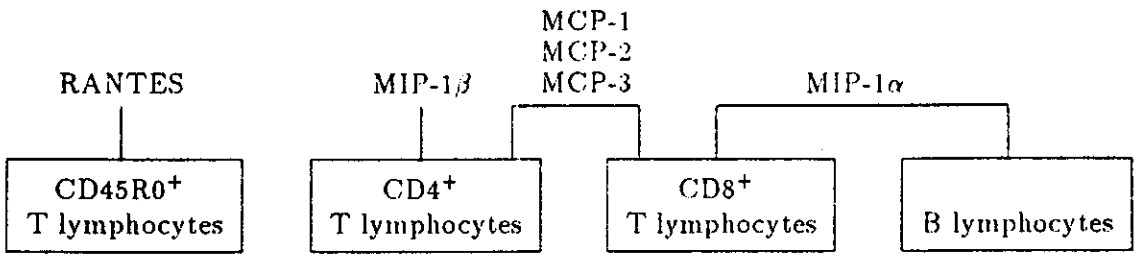

Fig. 1. Selective effects of RANTES, MIP-1α, MIP-1β, MCP-1, MCP-2 and MCP-3 on lymphocyte subsets. The target cells were defined in studies based on *in vitro* chemotaxis assays. The scheme was modified from Schall (1991) and updated by the results of Taub et al. (1993), Schall et al. (1993), and Loetscher et al. (1994).

In addition to the basic difference in specialization of C-X-C chemokines as neutrophil attractants and C-C chemokines as attractants for mononuclear cells, there are further significant distinctions between the effects of the members of the respective groups. For instance, some of the C-C chemokines select their targets among lymphocytes according to their phenotype (Fig. 1) and their activation state (Schall et al., 1993; Taub et al., 1993; Loetscher et al., 1994). The same authors reported that C-C chemokines with a similar selectivity promoted leukocyte adhesion to activated endothelium. It was proposed (Witt and Lander, 1994) that the selective binding of chemokines to distinct subsets of endothelial proteoglycans may be responsible for the aforementioned selectivity of attractant activity of chemokines. Another plausible control mechanism responsible for differential target-cell specificity of C-C and C-X-C chemokines may operate at the level of interaction of chemokine ligands with their receptors. This possibility would be in agreement with the recent observations that mutations of amino acid residues artificially introduced into the conserved region of the IL-8 gene coding for the ligand-binding site generated a novel monocyte, i. e. C-C chemoattractant, activity of this originally strictly neutrophil attractant C-X-C chemokine (Lusti-Narasimhan et al., 1995).

4.2. Chemokines and leukocyte activation

Chemokines often potentiate the effector functions of those cells which migrated to the tissue as a result of their activity (Table 2b). This is of significance especially in the case of basophils and eosinophils: histamine release from basophils and activation of eosinophil cytotoxic activity are features of the C-C chemokines MCP-1, MCP-3 and also of RANTES (Baggiolini and Dahinden, 1994). These properties can negatively contribute to the development of allergic inflammation, but could be beneficial for successful protection against parasite infections.

Of importance could also be the potentiation of monocyte and macrophage cytotoxicity by MCP-1. MCP-1 also supports the release of enzymes, O_2^- and induces respiratory burst. This chemokine, therefore, may be involved in immunological control of tumor growth by attracting monocytes to the site of malignancy and by their subsequent activation (Zachariae et al., 1990; Mantovani et al., 1992). Further support for the active role of MCP-1 in anti-tumor response has been provided by a recent study by Maghazzachi et al. (1994) showing that MCP-1, together with RANTES and MIP-1α, induces chemotaxis of antitumor effector natural killer (NK) and IL-2-activated NK cells.

4.3. Other functions of chemokines

Chemokines can also modulate proliferation (Table 2c) of lymphocytes, endothelial cells, smooth muscle cells and also of some malignant cells (Schall, 1991; Zachariae, 1993; Yue et al., 1994). The MIP-1α chemokine acts as a negative regulator of the cell cycle of normal myeloid precursors in the bone marrow. However, MIP-1α does not inhibit the cycle of leukemic cells (Schall, 1991; Eaves et al., 1993). Theoretically, inhibition of hematopoiesis by MIP-1α could be exploited during chemotherapy aimed at the interruption of the cell cycle of leukemic cells to ensure the protection of normal stem cells (Clark and Dexter, 1993; Eaves et al., 1993). Slightly controversial is the activity of IL-8: its proliferative effect on endothelial cells supports wound healing and neovascularization (Koch et al., 1992). By contrast, IL-8 mitogenic activity on smooth muscle cells can lead to the thickening of arterial intima (Yue et al., 1994).

5. CHEMOKINE RECEPTORS

There are several types of chemokine receptors differing in their binding specificity, affinity and kinetics of the receptor occupancy (Kelvin et al., 1993; Horuk, 1994). With one exception, all chemokine receptors belong to the superfamily of receptors coupled to GTP-binding proteins (G-proteins), which includes the rhodopsin receptor, adrenoreceptors and also the chemotactic receptors for C5a and formyl peptide (fMLP). The typical structural feature of members of this receptor family is the presence of seven transmembrane domains. According to Gerard and Gerard (1994), the receptor binding site is formed by the third extracellular loop and amino-terminal domain, and it is further complemented by portions of the membrane or the intracellular part of the molecule.

Binding of the ligand activates intracellular G-proteins starting the cascade of signal transduction events. These lead to the cell activation manifested by migration in response to chemotactic stimuli. The postreceptor events involve three separate limbs: (1) the phospholipase C/protein kinase C (PLC/PKC) pathway, (2) the

mitogen-activated protein (MAP) kinase cascade, and (3) the phosphatidyl inositol-3 kinases (PI-3) system. The latter system is a candidate for the signalling molecule, crucial to cytoskeletal remodelling (Dobos et al., 1992) and chemotaxis (Turner et al., 1995).

The function of chemokine receptors is not limited to signal transduction, but may involve other biological activities. Proteins showing considerable homology with the chemokine receptors can be encoded by the genome of some viruses, e. g., by the cytomegalovirus open reading frame (CMV-ORF) US28 (Neote et al., 1993) or the ORF of *Herpesvirus saimiri* ECRF3 (Ahuja et al., 1994). The latter authors and also Murphy (1994) or Kelvin et al. (1993) suggested that viruses could presumably exploit these homologues for evasion of antiviral host response by neutralization of chemokines, or perhaps by modulation of cytokine signalling.

Similarly as their ligands, chemokine receptors can be subdivided into two groups. The C-X-C receptor group comprises (1) IL-8 specific receptor A (IL-8RA), and (2) IL-8 receptor B (IL-8RB), which can, in addition to IL-8, bind also MGSA and RANTES. The genes coding for the IL-8 receptors are clustered on the human chromosome 2, q32-37 (Ahuja et al., 1992), closely linked to the candidate gene for *Nramp*, natural resistance-associated macrophage protein (White et al., 1994). The C-C chemokine receptor group is represented by CC-CKR1 ("CC chemokine receptor 1"), which binds the majority of C-C chemokines with varying affinity (Neote et al., 1993). Besides CC-CKR1, there are probably other receptors which, however, bind only some of the C-C chemokines (reviewed by Baggiolini and Dahinden, 1994). A typical functional feature of chemokine receptors is desensitization: the effect of one C-C chemokine (e. g. RANTES) makes the cell unresponsive to the subsequent stimulation by another C-C chemokine, e. g. MIP-1α (Neote et al., 1993; Baggiolini and Dahinden, 1994).

The last of the chemokine receptors is expressed on erythrocytes and binds promiscuously the C-C as well as C-X-C chemokines. This so-called "erythrocyte chemokine receptor" (ECKR) is identical with the "Duffy" blood group antigen (Neote et al., 1994). Horuk (1994) suggested that this receptor could function as a "clearance" receptor by binding the circulating chemokines and thus preventing their potential systemic effects. Moreover, this receptor may possess another physiological function: the Duffy blood group antigen is required by the malarial parasites *Plasmodium vivax* and *Plasmodium knowlesi* for invasion of human erythrocytes. Interestingly, binding of IL-8 blocks *in vitro* invasion of erythrocytes by *P. knowlesi*, suggesting potential clinical application in malaria (Horuk et al., 1993).

6. REGULATION OF CHEMOKINE EXPRESSION

6.1. Molecular mechanisms of regulation

The expression of chemokines is regulated on various levels. Recent studies implicated transcriptional regulation as the most important mechanism (Nelson et al., 1993; Rathanaswami et al., 1993). Promoter regions of chemokine genes contain a number of regulatory sequences necessary for their expression. These include, e. g., the response elements for lymphoid- and macrophage-transcription factor PU.1, regulatory factor IFN.1, and also for transcription factor NF-κB (Oppenheim et al., 1991; Schall, 1991; Danoff et al., 1994). Some of those *cis*-elements may be associated with stimulus and tissue-specific regulation of chemokine expression (Ueda et al., 1994). These gene regulatory sequences can be involved in regulation of both the induction (Nelson et al., 1993) and the suppression (Mukaida et al., 1994) of chemokine gene expression. Additionally, chemokine expression may be post-transcriptionally modified by (de)stabilization of the respective mRNA: e. g. the stability of IL-8 mRNA may be influenced by the RNA instability element, AUUUA, contained in IL-8 mRNA (Baggiolini et al., 1994).

6.2. Cellular regulatory mechanisms; cytokine–chemokine networks

A wide spectrum of cells can produce chemokines, including non-immune cells, e. g. epithelial and endothelial cells, fibroblasts, etc. Chemokine expression can be induced by a variety of stimuli ranging from physical and chemical stimuli or microorganisms to different mediators of immune reactions (for review see Oppenheim et al., 1991; Baggiolini et al., 1994). Responsiveness to the stimulatory signal in terms of expression of a particular chemokine gene can differ between various cell types (Strieter et al., 1994).

The regulation of chemokine production by other cytokines can be important for practical reasons. The expression of most C-C and C-X-C chemokines is induced by proinflammatory cytokines, i. e. IL-1, TNF-α (or TNF-α + IFNγ) in a *paracrine* manner (Oppenheim et al., 1991; Rathanaswami et al., 1993). Additionally, chemokine production can be potentiated by *autocrine* mechanisms (Oppenheim et al., 1993). On the contrary, other cytokines inhibit the chemokine production. These inhibitory cytokines belong to the Th2 type: IL-4 and IL-10 suppress the IL-8 production (Standiford et al., 1990a; de Wall Malefyt et al., 1991). IL-4 *per se* (Rathanaswami et al., 1993), or in conjunction with IL-13 (Marfaing-Koka et al., 1995), downregulates the production of the RANTES chemokine.

The relationship between chemokines and other cytokines is complex, involving cascades of their effects and resembling the character of a network. For example, according to Standiford et al. (1990b), Rolfe et al. (1991) and Strieter et al. (1994)

the following cytokine–chemokine network may operate in the lung: Primary stimulus, e. g., bacterial endotoxin, induces production of IL-1 and TNF-α in alveolar macrophages. These cytokines consequently activate macrophages and lung fibroblasts to the production of chemokines (MCP-1, IL-8). In the next step, mononuclear cells and neutrophils are recruited to the lung by the effect of the chemokines. Near the end of the network, further cytokines (e. g. TNF-α) or chemokines (e. g. MCP-1) can be produced by the recruited cells, thereby potentiating accumulation and activation of the lung leukocytes. A similar network may explain migration of leukocytes into the inflammatory synovium in rheumatoid arthritis (Koch et al., 1994; Strieter et al., 1994), or the glomerular and interstitial infiltration during various immunopathological processes affecting the kidney (Pattison et al., 1994; Stahl et al., 1994).

7. BIOLOGICAL EFFECTS OF CHEMOKINES

7.1. Physiology of chemokines

Chemokines together with other cytokines form a constitutive part of the regulatory mechanism of the organism. They dominate as the key mediators of both protective and pathological inflammation; selective effects of chemokines on distinct leukocyte subsets ensure that the "correct" cell types (i. e., those being the most effective for elimination of the triggering stimulus) are recruited to the inflammatory site at the appropriate phase of the process.

Chemokine-mediated accumulation of various leukocyte subsets in the inflamed tissue is manifested by the formation of cellular infiltrates. The composition of the infiltrates can be characteristic for certain types of inflammation, e. g. eosinophilic infiltration for allergy. However, cellular distribution in the infiltrate is not absolutely constant, but evolves with the development of the inflammatory process: a traditional example is the switch from neutrophilic to mononuclear infiltrate during transition from acute to chronic inflammation (for review see Strieter et al., 1994).

Several aspects of chemokine activity may be involved in the dynamic development and diversity of inflammatory infiltrates. First, the character of the infiltrate reflects the combined activities of a spectrum of several chemokines, rather than a separate action of a single mediator. Second, the pattern of expressed chemokines is subject to constant changes due to differing kinetics of chemokine expression, which is dependent also on the type of the producing cell: e. g. in lymphocytes, the MIP-1 production precedes that of RANTES, contrary to fibroblasts, which can produce both the chemokines concomitantly (Nelson et al., 1993). The last factor presumably involved in the development of inflammatory infiltration is the chemokine-secreting activity of polymorphonuclear granulocytes (PMN). Cassatella (1995) postulated that PMN, through the production of IL-8 and MIP-1α and β,

may be crucial regulators of the switch of the type of leukocyte infiltration from neutrophilic to mononuclear.

The concept of chemokines as physiological regulators of leukocyte trafficking to the inflamed tissue has been recently supported by some experimental findings *in vivo*: s. c. injection of recombinant RANTES to SCID (*severe combined immune deficiency*) mice together with i. p. transfer of human T lymphocytes elicited the migration of $CD3^+$ lymphocytes to the injection site (Murphy et al., 1994). Also the results of our clinical studies suggest the physiological importance of chemokines: mRNA for C-C chemokines was expressed in cells obtained from the lung of healthy people as well as from the lung of patients with interstitial inflammation and fibrosis (Petřek et al., 1994; Southcott et al., 1994). Moreover, the expression of RANTES mRNA correlated with the number of bronchoalveolar $CD45R0^+$ "memory subset" T lymphocytes, which represent the target of RANTES chemotactic activity *in vitro* (Petřek et al., unpublished). Consequently, together with Becker et al. (1994) we can speculate that the C-C chemokines constitute a part of the physiological defence by controlling the monocyte and T lymphocyte migration into the lung, where these cells can react to inhaled antigens and irritants.

Another aspect of chemokine physiology relates to their cell-activation properties. Chemokine-mediated activation of the effector (i. e. secretory, metabolic, and cytotoxic) functions of monocytes, neutrophils and also basophils can be beneficial for the control of tumor growth (Mantovani et al., 1992), and especially for the defence against infectious agents (e. g. Appelberg, 1992). In this context, recently George (1994) has hypothesized that, by analogy with their binding to endothelial proteoglycans, chemokines may bind to the peptidoglycans of bacterial cell walls, thereby "opsonizing" the bacteria. This would potentiate the effector mechanisms of phagocytes, namely phagocytosis and cytotoxicity.

7.2. Chemokines in immunopathology

If an imbalance develops between physiological defensive functions of leukocytes and their excessive chemokine-mediated activation, the outweighing negative effects alter the originally protective defence reaction into a damaging inflammatory process. Pathology results also from the situations when leukocyte accumulation surpasses physiological boundaries, e. g. in neutrophilia during endotoxin shock or in the increased number of macrophages in rheumatoid synovia.

Investigations searching for a potential link between chemokines and pathological inflammation brought data on chemokine expression in a number of acute and chronic inflammatory diseases (Table 3). The chemokine expression in the patients was increased in comparison to healthy controls. However, these studies cannot answer the question of whether the chemokine overexpression is due to (1) a primary role of chemokines in the pathological process, or (2) whether it is secondary to the inflammation. The arguments supporting the first possibility, i. e., the role of

Table 3. Immunopathology of chemokines

Expression of the chemokines increased above the level observed in normals detected (level of mRNA or secreted/membrane-bound protein) in clinical material from patients (cells, fluid from pathological sites, tissue homogenates, serum, etc.).

Acute conditions

Ischemia-reperfusion injury: IL-8 (Strieter et al., 1994)
Bacterial pneumonia in cystic fibrosis: IL-8 (Strieter et al., 1994)
Bacterial meningitis: IL-8 (Seki et al., 1993)
Pleural empyema: IL-8 (Strieter et al., 1994)
Gout: IL-8 (Zachariae, 1993)
Acute cell-mediated rejection of the kidney transplant: RANTES (Pattison et al., 1994)

Chronic conditions

Rheumatoid arthritis: MCP-1, MIP-1α, IL-8 (Koch et al., 1994; Strieter et al., 1994)
Fibrosing alveolitis: MCP-1, IL-8 (Carré et al., 1991; Antoniades et al., 1992; Southcott et al., 1995)
Sarcoidosis: MIP-1α (Standiford et al., 1993)
Pleural disease in tuberculosis: MCP-1 (Strieter et al., 1994)
Atherosclerosis: MCP-1, IL-8 (reviewed by Zachariae, 1993)
Glomerulonephritis: MCP-1, RANTES, IL-8 (Stahl et al., 1994)
Ulcerative colitis: IL-8 (Mitsuyama et al., 1994)
Endometriosis: RANTES (Khorram et al., 1993)
Psoriasis: IL-8, MGSA-GRO (Schröder et al., 1992)
Allergic disorders: MCP-1, IL-8 (Baggiolini and Dahinden, 1994)
RANTES (reviewed by Schall and Bacon, 1994, and by Zhang et al., 1994)

chemokines as the causative agents of inflammation, are given below. In the fibrosing alveolitis and in inflammatory bowel disease, the expression of IL-8 is limited only to the loci of inflammation, i. e., the lung or the intestinal mucosa (Mitsuyama et al., 1994; Strieter et al., 1994). Moreover, the expression of the neutrophil attractant IL-8 correlates with the number of neutrophils infiltrating the alveoli (Carré et al., 1991) and the intestinal mucosa (Mitsuyama et al., 1994), respectively. Further indication of the possible pathogenic role of IL-8 in fibrosing alveolitis is represented by the results of Southcott et al. (1995) who described association of IL-8 with the development of lung fibrosis as a complication of systemic sclerosis.

So far, the most convincing data confirming the causative role of chemokines in the pathogenesis of inflammatory disease have been provided by experiments with antibodies specific to chemokines. The anti-chemokine antibodies inhibited the chemotactic effect of these mediators *in vitro* (Strieter et al., 1994) and, most importantly, they were effective also *in vivo*: Anti-IL-8 antibody blocked the neutrophil accumulation at the site of s. c. injection of lipopolysaccharide (LPS) in rabbits (cited by McLean, 1994). The application of anti-IL-8 to rats prevented the development of experimental immune complex alveolitis (Mulligan et al., 1993). In rabbits, symptoms of acute immune complex-mediated glomerulonephritis were alleviated by administration of an anti-IL-8 antibody (Wada et al., 1994). Similarly, in rats, antiserum to the neutrophil attractant CINC (human GRO) inhibited acute inflammation induced by intratracheal instillation of endotoxin and cytokines (Ulich et al., 1995).

So far, it has not been explained why chemokines, instead of physiological "fine tuning" of the leukocyte migration, start to induce pathological accumulation and activation of these cells. This functional reversal could be partially explained by the results of studies in patients with fibrosing alveolitis [FA] (Strieter et al., 1994) and also patients with rheumatoid arthritis [RA] (Koch et al., 1994). Pulmonary fibroblasts of FA patients and rheumatoid synovial macrophages produced significantly higher amounts of IL-8 and MCP-1 than the fibroblasts and macrophages of healthy subjects. Moreover, the diseased fibroblasts and macrophages were insensitive to the signals regulating the chemokine expression in normal healthy cells: neither the stimulation of chemokine production by IL-1 and TNF-α, nor the inhibition due to the action of prostaglandin E_2 (PGE_2) or dexamethasone were effective in cells from the patients. Elevated chemokine production, escaping regulatory mechanisms, persisted in the fibroblasts even after repeated *in vitro* passages and it was accompanied by other functional and morphological changes. Strieter et al. (1994) therefore suggested that in chronic inflammation, the macrophages and fibroblasts might undergo a phenotypic change manifested by the dysregulation of chemokine production. Theoretically, the possibility cannot be ruled out that similar dysregulation may have an opposite outcome – a downregulation of chemokine expression and a decrease of the product level. However, the existence of an "immune deficiency" due to lack of a particular chemokine is, in our opinion, too speculative. Chemokines, as well as most cytokines, have overlapping activites meaning that the "missing" activity would be substituted by other similarly acting mediators.

8. PERSPECTIVE APPLICATIONS OF CHEMOKINES

8.1. New approaches to the diagnosis of inflammation

As chemokines act at the onset of the inflammation, their investigation may help to identify the early phase of the inflammatory process, before it reaches the

chronic, mostly irreversible, stage. Chemokines can be investigated at various levels: the detection of the transcripts of their genes can be achieved using either the Northern hybridization, or the more sensitive reverse transcription-polymerase chain reaction (RT-PCR) methodology; exploitation of those variants of the molecular techniques enabling mRNA quantitation is highly desirable (for review see e. g. Dallman et al., 1991; Trapnell et al., 1993; Siegling et al., 1994). Immunodetection of both secreted chemokines and membrane-bound proteins has become feasible with the development of polyclonal and monoclonal anti-chemokine antibodies. However, their applications necessitate strict specificity and lack of inter-chemokine cross-reactivity. Taken together, to date there is a sufficient technology enabling a critical evaluation of the significance of chemokines as diagnostic markers, or as "indicators" of activity of inflammatory disease states.

8.2. Immunotherapy

The current knowledge of potential therapeutical applications of chemokines is based on *in vitro* studies or on *in vivo* animal experiments. Theoretically, inhibition of chemokine production or the blockade of a chemokine or its receptor could interrupt the excessive accumulation and activation of leukocytes and thus prevent tissue or even systemic injury. As discussed previously, there are several reports on successful therapeutical usage of anti-IL-8 and anti-CINC antibodies in animal models of inflammation (Mulligan et al., 1993; McLean, 1994; Wada et al., 1994; Feng et al., 1995; Ulich et al., 1995). To our knowledge, antibodies specific to other, e. g. C-C chemokines, have not yet been tested. Other studies explored the potential therapeutical effect of chemokine receptor antagonists and investigated the effects of inhibitors of postreceptor events: e. g. Kemény et al. (1994) reported that cyclosporine A (CyA) inhibited the binding of IL-8 to cultivated keratinocytes and proposed that the blockade of IL-8 receptor might be an important mechanism involved in the action of CyA as an anti-psoriatic agent.

Chemotactic and especially activating effects of chemokines could be beneficial also for positive immunotherapy. Application of recombinant MCP-1 to healthy mice potentiated phagocytic and microbicidal functions of monocytes, so that the animals survived infection by a lethal dose of microbial pathogen; this effect was observed even in leukopenic animals (Nakano et al., 1994). Consequently, after verification in clinical trials, non-specific potentiation of immune response by application of carefully selected chemokines could supplement the therapy of severe bacterial infections, especially in patients with granulocytopenia (Nakano et al., 1994).

Progress in defining chemokine functions and also the delineation of the regulation of their expression brought new aspects into the mechanisms of action of some immunomodulators. For example, the phenomenon of potentiation of anti-bacterial resistance by IL-1 and TNF-α can be explained by the induction of MCP-1 and

other chemokines (Nakano et al., 1994). Chemokines may be presumably involved also in potentiation of non-specific immune mechanisms due to the action of traditional microbial immunomodulators such as membrane extracts from *K. pneumoniae* or the streptococcal immunomodulator OK432 (Luini et al., 1991; Tsuchiya et al., 1993). Finally, the molecular basis of chemokine expression has complemented the knowledge of mechanisms of anti-inflammatory action of glucocorticoids: e. g., dexamethasone inhibited the IL-8 gene transcription by interfering with the binding of transcription factor NF-κB to its binding site in the promoter region (Mukaida et al., 1994).

9. CONCLUSIONS

In the last decade, a great progress has been achieved in the chemokine area. Not only the chemokine family has been rapidly expanding, but there have been substantial extensions of our insight into the chemokine functions. From being originally regarded as specialized chemoattractants, chemokines have today become full-fledged members of the community of immunoregulatory cytokines. Undoubtedly, the forthcoming years will bring new findings about chemokines and their receptors and, especially, about *in vivo* relationships between chemokines, cytokines and immune cells. Finally, experimental models of transgenic animals, overexpressing genes for chemokines or their receptors, and animals with the knock-outed genes should help us to expand meagre knowledge of physiology and immunopathology of chemokines.

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Note added in proof

For a recent overview of past and present nomenclature of human, and also animal, chemokines see Prieschl, E. E., Kulmburg, P. A., Baumruker, T.: The nomenclature of chemokines. *Int. Arch. Allergy Immunol.* **107**: 475–483, 1995.