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MINIREVIEW

Inflammatory and Physiological Roles of Chemokines

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Chemokines, members of the family of chemotactic peptides, have a well documented function in different inflammatory diseases where they induce leukocyte emigration into lesions. Several recent observations indicate that, in addition to pathological states, chemokines are also produced and secreted under physiological conditions by various exocrine glands in amounts sufficient for their full biological effect. The glands involved in chemokine production and secretion include eccrine sweat glands, lactating mammary glands, lacrimal and salivary glands. It is suggested that analogous to their role in inflammatory diseases, chemokines produced by the exocrine glands are responsible for the induction of homeostatic leukocyte migration into mucosal epithelia and skin and also, mammary glands and milk. In addition, the mechanism by which chemokines induce leukocyte homing under physiological circumstances is discussed. (Pathology Oncology Research Vol 2, No1–2, 16–20, 1996)

Key words: IL-8, MCP-1, RANTES, inflammation, sweat, milk, leukocytes

Introduction

Chemokines are members of an emerging family of cytokines with chemotactic activity (hence the name) for different leukocytes and other circulating and sessile cells.[1,2] Molecules are classified as chemokines based primarily on structural and not functional properties; they include peptides of 7-10 kD with four cysteines in conserved position within the molecule. Chemokines are divided into "C-X-C" or "α" and "C-C" or "β" subfamilies depending on the presence or absence of an intervening amino acid between the first two cysteines. More than thirty human chemokines have been described to date but only some of these molecules are well characterized (*Table 1*). Platelet factor 4 (PF4), the first chemokine, discovered over 40 years ago, has been clearly surpassed by interleukin-8 (IL-8) as the most widely studied α-chemokine; monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP 1α) and RANTES are considered to be the prototype members of the β-chemokine subfamily. Recent discovery of lymphotactin, a molecule with high homology to chemokines but containing only two cysteines in conserved position created the precedent of a peptide with less than four cysteines being called a chemokine; now lymphotactin is the sole member of a "C" or "γ" chemokine subfamily. The cellular effects of chemokines are mediated via specific G-protein-coupled receptors with seven transmembrane domains. Ten different chemokine receptors have been described. One receptor type can bind one, several or many different chemokines. Because leukocyte subsets can exclusively express one particular chemokine receptor, the most striking characteristic of chemokines is their ability to affect narrow subpopulations of leukocytes. Conversely, classical chemoattractants, e.g. formyl peptides, C5a, LTB_4, etc. are panchemotaxins; they affect a broad range of leukocytes. In general terms, α-chemokines, those containing the ELR sequence in particular, are neutrophil chemoattractants; whereas, members of the β-chemokine subfamily attract monocytes, eosinophils and basophils.[1,2] Lymphocyte chemoattractants can be found among members of all three subfamilies of chemokines.[1,2]

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In vivo inflammatory role of chemokines

Chemokines, IL-8 in particular, were shown to be produced by a wide variety of cells in different tissues upon stimulation by several members of a broad spectrum of inflammatory cytokines [from interleukin-1 (IL-1) to interleukin-17], by microorganisms and their products, by hypoxia/reoxygenation, inorganic crystals and other proin-

Table 1. **Human chemokines***

C-X-C (α) chemokines	*C-C (β) chemokines*	*C (γ) chemokine*
IL-8 (NAP-1)	MCP-1 (MCAF)	Lymphotactin
PBP→βTG→CTAP-3→ →NAP-2	MCP-2	
	MCP-3	
MGSA (Groα, NAP-3)	MIP-1α	
Groβ	MIP-1β	
Groγ	RANTES	
PF4	Eotaxin	
ENA-78	I-309	
γIP-10	HCC-1	
GCP-2		
γMIG		

* Additional twelve human peptides are classified as chemokines based on their DNA sequences.
Arrows "→" indicate N-terminal processing resulting in different biological activity. The most common alternative names are shown in brackets.

flammatory stimuli (reviewed in refs.1 and 2). Due to this and considering the potent in vitro chemotactic activity of IL-8 and other chemokines, it is not surprising that in the past several years these molecules have been profiled as agents responsible for inflammatory leukocyte recruitment into different pathological lesions.[1,2] Various experimental and clinical evidence suggests that chemokines generated in the inflamed tissues are the driving force behind the leukocyte egress from circulation.[1-3] When injected into humans and experimental animals, chemokines induce the accumulation of those leukocyte types and subsets in the injection sites that are capable of responding to that particular chemokine[1-3] Also, several clinical studies and experimental disease models have linked the production of chemokines to the pathogenesis of inflammatory and infectious diseases including septic shock, adult respiratory distress syndrome, chronic inflammatory bowel disease, arteriosclerosis, rheumatoid arthritis, gout, idiopathic pulmonary fibrosis, asbestosis, and different viral, bacterial, fungal and parasitic infections and infestations, etc (reviewed in ref. 1). As a result, a generally accepted view emerged that the in vivo production of chemokines is always associated with pathological conditions, mainly, various forms of inflammation and malignancy. However, many recent observations show chemokine production by secretory glands under physiological and not pathological conditions. This suggests the involvement of chemokines in homeostatic leukocyte emigration into the tissues bordering the external environment, e.g. mucosal surfaces and skin. The mechanisms by which chemokines induce physiological leukocyte homing are also outlined.

Chemokine secretion in eccrine sweat

In humans, the physiological process of sweating, secretion by eccrine sweat glands, is induced in a variety of stressful conditions with potentially hazardous outcomes for the individual, including thermal, physical, emotional and mental stress.[4] It is therefore possible that human sweat, in addition to its well-understood role in thermoregulation, also contributes to host defense. This is suggested by the fact that sweat contains molecules involved in host defense such as immunoglobulins (Ig) of the IgA subclass[5] and major inflammatory and host defense cytokines, IL-1 and TNF.[6-8] Recently, we investigated the leukocyte chemotactic activity of normal human eccrine sweat.[9] Sweating in healthy volunteers was induced by jogging outdoors; sweat was collected from the upper trunk into custom-made pouches during a 30 minute run at an approximate speed of 10-15 km/h. Sweat was aspirated from the pouch and immediately filtered through a 0.22 μm millipore filter. When sweat was tested in standard Boyden-type neutrophil and monocyte chemotaxis assays we observed that it was chemotactic for both neutrophils and monocytes.[9] The eccrine sweat was applied to a C4 reversed-phase high pressure liquid chromatography (HPLC) column and eluted by a gradient of acetonitrile, resulting in separation of the neutrophil chemotactic activity into several peaks. The number of peaks varied between three and seven, depending on the donor. One of these peaks was identified by ELISA as IL-8; others could possibly also be C-X-C chemokines since all the neutrophil chemotactic peaks lacked chemotactic activity for monocytes. Using a solid phase double ligand ELISA technique, IL-8 was found in sweat samples from five out of six donors at a mean level of 178 pg/ml of sweat. There was only one peak of monocyte chemotactic activity present in each sweat sample, eluting at the same position in all tested sweat samples from five different donors. This peak had no chemotactic activity for neutrophils, clearly indicating that it is not a formyl peptide, which is chemotactic for both monocytes and neutrophils.[9] The sweat-derived peak of monocyte chemotactic activity was subsequently identified by ELISA as monocyte chemotactic peptide-1 (MCP-1).

In addition, we showed that chemokines which appeared in sweat were derived from sweat glands rather than having been washed off the stratum corneum in the process of collecting sweat. To control for leukocyte chemotactic activity possibly present in the stratum corneum of the skin, epidermal scrapings were obtained from the heels of normal volunteers using a modification of a previously described method.[10] The extract of heel scrapings was applied to an HPLC column and the resulting fractions were tested for chemotactic activity. Stratum corneum-derived extract contained one broad peak of chemotactic activity for both monocytes and neutrophils,[9] suggesting that its nature was different from the chemotactic peaks found in sweat. This notion was further supported by the fact that the stratum corneum-derived chemoattractant did not show any heparin-binding on the heparin-Sepharose column, in sharp contrast to IL-8 and MCP-1. Immunohis-

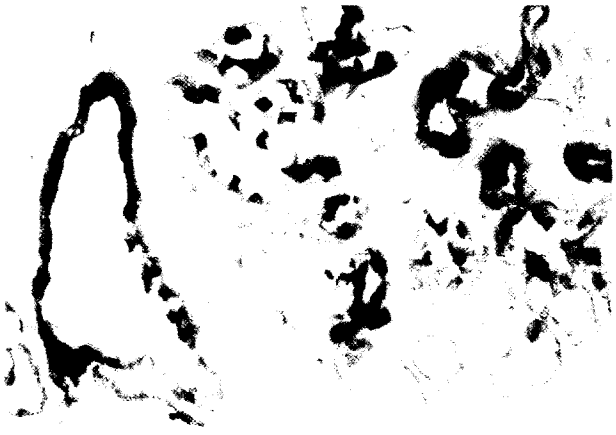

Figure 1. IL-8 immunoreactivity in small salivary gland of human oral mucosa

tochemistry provided further evidence clearly indicating that IL-8 is secreted by the sweat glands rather than being stratum corneum-derived. Frozen, acetone-fixed sections of normal human skin were stained with monoclonal anti-IL-8 antibody. Intense staining of the epithelium of the secretory and ductal portions of the coil of the sweat gland, ductal epithelial cells as well as intraluminal contents was observed. There was no staining of the epidermal layer of the skin, confirming the previously noted absence of IL-8 in the epidermis.[9] MCP-1 and melanoma growth stimulatory activity (MGSA/groα) have also been observed in normal eccrine sweat glands.[11] Chemokines secreted in sweat could be produced either by the sweat glands' epithelial cells or ultrafiltered from plasma after being produced elsewhere and carried to the sweat glands via blood. To distinguish between these two possibilities, in situ hybridization studies on samples of normal human skin were performed.[9] Using this method, abundant IL-8 mRNA was detected in the cytoplasm of epithelial cells of eccrine sweat glands but not other dermal or epidermal cells. This finding indicates that IL-8 is produced in situ by the epithelial cells of the eccrine sweat glands. In summary, normal human eccrine sweat contains multiple chemokines including IL-8 and MCP-1 and possibly others, e.g. MGSA/groα.[11]

Chemokines in colostrum and milk and other exocrine secretions

Chemokine production by sweat glands is not an isolated phenomenon. The presence of chemokines in the secretions of several other exocrine glands was recently tested for and revealed.[12,13] IL-8 and also RANTES, a member of the C-C subfamily of chemokines, were found in normal human colostrum and milk.[13] Titers for both chemokines were higher in colostrum (several ng/ml), but both chemokines could still be detected in milk several months after delivery. There was no statistical difference between chemokine concentrations in milk of mothers with term and preterm deliveries.[13] Both IL-8 and RANTES also appeared in secretions in galactorrhoea and in the witch's milk of the newborn. Using immunohistochemical staining IL-8 and RANTES immunoreactivity could be detected in the epithelial cells of the acini and ducts of normal mammary gland and in mammary tissue affected by several diseases, including ductal carcinomas and adenoic and cystic lesions of fibrocystic disease.[2,12] This, together with the fact that cultured primary mammary epithelial cells produce IL-8 (own unpublished observation), suggests that similar to the findings in sweat glands, the epithelium of the mammary glands is the source of the secreted chemokines. In addition to colostrum and milk, we detected IL-8 in tears which were induced in three normal healthy volunteers by exposing their faces to the vapors of freshly cut onion.[12] A mean value of 4.7 ng/ml for IL-8 was measured. Finally, immunohistochemistry detected IL-8 immunoreactivity in the epithelial cells of the parotid and submandibular salivary glands and the minor salivary glands of the oral mucosa *(Fig. 1)* suggesting the possibility that salivary glands also secrete chemokines.[12]

Possible physiological role of chemokines secreted by exocrine glands

Here, we provide various pieces of evidence indicating that chemokines are produced and secreted by exocrine glands of normal healthy humans *(Table 2)*. Different chemokines which are produced by different exocrine glands in different amounts and under different circumstances probably play a diverse, yet to be understood, physiological role. In an attempt to find a unifying motif in the secretion of chemokines by different glands, we postulate that the secreted chemokines could be responsible for homeostatic leukocyte recruitment into normal tissues bordering the external environment. This implies, on the basis of our current knowledge of principles guiding inflammatory leukocyte recruitment, that the homeostatic emigration of lymphocytes, and in some instances other leukocytes, into the normal tissues is also dependent on the presence of chemokines. In normal healthy individuals, lymphocytes leave the circulation and accumulate in

Table 2. **Evidence supporting chemokine secretion by exocrine glands**

Organ	*Biological activity*	*Chemokine purified*	*Chemokine mRNA*	*Immunoreactivity*
Eccrine sweat glands	+	+	+	+
Mammary gland	+			+
Lacrimal gland	+			–
Salivary gland				+

the mucosal organs and skin where they fulfill their role in immune surveillance and host defense. Similarly, lymphocytes and other leukocytes home to the epithelium of lactating mammary glands and after traversing the epithelial cell barrier also appear in colostrum and milk. While chemokines were known to be present in different pathological lesions, until now they were thought to be absent from the normal tissues and therefore not considered as potential inducers of lymphocyte accumulation into normal tissues. The direct proof of a concept linking chemokine secretion by exocrine glands and the physiological lymphocyte homing is still missing; however, some circumstantial evidence supports this theory. For example, it has been observed in human skin that lymphocytes accumulate in the epidermis around the acrosyringium, an area of the epidermis traversed by the eccrine sweat ducts where possible leakage of sweat into the epidermis could be envisaged.[14] It should be emphasized that the outcome of chemokine production by different exocrine glands could be very different. When secreted in sweat, chemokines induce lymphocyte accumulation in the epidermis but leukocytes do not appear in the sweat itself, whereas we suggest that, when secreted by the mammary gland, chemokines cause the accumulation of neutrophils, monocytes and lymphocytes in the mammary tissue and also in milk.[12,13] Such a difference is puzzling and may be explained by either the different concentrations of the secreted chemokines in sweat and milk or the different patterns of adhesion molecule expression by epithelial cells in skin and the mammary glands.

The mechanism of chemokine induced leukocyte emigration

Chemokines promote leukocyte emigration due to their ability to induce leukocyte adhesion to the endothelium and stimulate transendothelial migration. It was suggested previously that the firm adhesion of lymphocytes to the endothelium in the process of their extravasation, both into normal and inflammatory tissues, requires the activation of leukocyte integrins.[15,16] Chemokines, similarly to other chemoattractants, have been shown to induce very rapid activation of leukocyte integrins.[17] In addition, many of the chemokines were shown to induce the directional migration of lymphocytes and their subsets (*Table 1*, reviewed in ref. 1). In the process of leukocyte endothelial cell adhesion, the critical point of action of chemokines is after the first, selectin-mediated, adhesion step.[18,19] If chemokines stimulate leukocytes before their initial selectin-mediated interaction with the endothelium, leukocytes lose their ability to adhere and emigrate.[17] Thus, the soluble gradients of chemokines, postulated to be the driving force behind leukocyte emigration, inhibit rather than promote leukocyte endothelial cell adhesion. In order to achieve that integrins are activated on only those leukocytes which have already established their initial interaction with the endothelium, chemokines bind to the surface of the endothelial cells, instead of acting in solution, as suggested before.[17,18] Previously, we described such chemokine binding sites on the endothelial cells of the postcapillary venules,[19] the segment of the circulatory tree where both inflammatory and homeostatic leukocyte emigration takes place. It is possible that the proteoglycans present on the endothelial cell surface of postcapillary venules mediate such binding.[19-21] Endothelial cell proteoglycans upon binding chemokines, in turn, can enhance the chemokine induced leukocyte emigration.[22]

Conclusions

We present multiple pieces of evidence showing that, in addition to their well documented appearance in inflammatory lesions, different chemokines are produced and secreted in substantial amounts by several exocrine glands of normal healthy humans. These exocrine gland-derived chemokines should be considered a source of chemotactic activity responsible for accumulation of lymphocytes, dendritic cells and other bone marrow-derived blood-borne cells into skin, mammary glands, mucosal organs, etc. The mechanism by which chemokines induce homeostatic leukocyte emigration should closely emulate the process of inflammatory cell recruitment.

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