CONTINUING MEDICAL EDUCATION

CHEMOKINES IN THE SKIN

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In last few years, focus has shifted from cytokines which have pleiotropic biologic properties to chemokines with target cell selective activity. The separation has led frequently espoused proposition that chemokines are involved in the pathogenesis of disease having specific infiltrates and point to possible role in chronic skin diseases. Depending upon the structure these chemokines are divided into three subfamilies, two major subfamilies: CXC and CC, and one putative subfamily C with only one member known as lymphotactin. A recent insight into chemokine physiology comes from demonstration of interaction between chemokines and their cloned receptors. These chemokine receptors are members of the transmembrane spanning (7-TMS), G-protein- coupled receptor family. So far CXC chemokine receptors and seven CC receptors have been cloned. Recently, the importance of selective chemoattractant activity of chemokines has been overshadowed by chemokine receptors emerging as new targets for anti- HIV therapy as the connection between chemokines and HIV-1 had been established. Among the CXC chemokine receptors, CXCR4, and among the CC chemokines receptors, CCRI, CCR2b, CCR3, and CCR5 have been implicated as HIV-1 coreceptors.

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In last few years, focus has shifted from cytokines which have pleiotropic biologic properties to chemokines with target cell selective activity. This specificity for leucocyte subset is separates chemokines from other chemoattractants. To date more than 40 members of this family have been identified and it is striking to recall that such a large family of proteins and their receptors was unknown less than 10 years ago.[1]

Chemokines are small with molecular weight in the range of 8- 12 KD and has been further subdivided into three subfamilies based on structural and genetic considerations.[2] One major chemokine subfamily is called as "C-X-C" because the two amino acids nearest the N- termini of these proteins are separated by a single amino acid.[3,4] They are chemotactic for neutrophils and/or T- lymphocytes in vitro, but are not chemotactic or minimally chemotactic for monocytes, eosinophils or basophils. In contrast, "C-C" subfamily shows two adjascent cysteines near the amino terminus as a conserved structure. They are not neutrophil attractant but are chemotactic for monocytes, T lymphocytes, basophils and eosinophils. Recently lymphotactin which is a potent attractant for T- lymphocytes, but not monocytes has been described.[5] It has only two cysteines and it has been suggested that it belongs to a third chemokine subfamily denoted C because of the lone cysteine in the N- terminal domain.

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Chemokine receptors are members of the 7- transmembrane spanning (7- TMS), G-protein-coupled receptor family through which various chemokines act.[6,7] So far, four CXC chemokine receptors and seven CC receptors have been cloned.[1] The understanding of chemokine physiology has been made perplexing by the " promiscuous relationship" between chemokine receptors and chemokines although it does not cross CC versus CXC boundaries. Recently it has been demonstrated that chemokine receptor expression can be regulated, IL -2 strongly upregulates expression of CCR1 and CCR2 in circulating T cells.[8]

CXC chemokines

The prototypic CXC chemokine is IL-8 , synonymous with monocyte- derived neutrophil chemotactic factor, is a novel cytokine that induces chemotaxis, degranulation, respiratory burst, adherence, shape change, Ca^{++} mobilisation, and regulation of CD 11b/CD18 glycoprotein in human neutrophils.[9,10] IL -8 is produced by monocytes, alveolar macrophages, endothelial cells, fibroblasts, epithelial cells when these cells are stimulated with lipopolysaccharides (LPS), TNF-α or IL -1β.[11] In vitro experiments have shown that IL-8 is produced by a variety of cell types including cultured keratinocytes, dermal fibroblasts, endothelial cells and melanocytes in a time and concentration dependent manner.[3,4] Only IL-1α could be shown to be stored in normal skin and there is absence of iL Iβ bioactivity in the epidermis therefore suggesting keratinocytes rather than bone -marrow derived cellular source.[11] Depending upon the type of stimulus there can be two different types of IL-8, the most abundant and potent form is 72 amino acids long which is secreted upon stimulation with TNF-α and second form is 77 amino acids variant also known as endothelial IL - 8 because of synthesis by these cells which is secreted upon primary cytokine stimulation.[12,13] Significant amount of 72 amino acid variant amounts of IL -8 were shown in psoriatic scales and in the lesional skin of patients with psoriasis, and keratinocytes may be the producer cells.[14] Therefore this chemokine could play a role in keeping up the inflammatory pattern seen in psoriasis, and it may be linked to epidermal hyperproliferation.[15] It was further shown that in the presence of arachidonic acid, IL-8 stimulates neutrophils to produce large amounts of LTNB 4 thus providing a distinct loop for amplification of inflammation.[16] There are two receptors that bind IL-8 with high affinity. The one CXCRI which recognizes IL-8 only and second CXCR2 which recognizes GRO, NAP-2, ENA-78 along with IL-8.[17]

Table 1. CXC chemokines

Name	Chemotactic for	Stimuli	Receptors
IL-8	PMN*,T lymphocytes basophils	IL-1 TNF-α	CXCR1 & CXCR2
GRO-α	PMN,Melanoma cell	IL-1 TNF-α	CXCR2
GRO-β	PMN,? End. Cell	IL-1, TNF- α	CXCR2
GRO-γ	PMN,? End. Cells	IL-1 TNF-α	CXCR2
IP-10	T lymphocytes, TILO	IFN-γ	CXCR3
MiG	T lymphocytes, TIL	IFN-γ	CXCR3
Platelet factor 4	fibroblasts	?	?
SDF-1α	lymphocytes	?	CXCR4
GCP-2	PMN	IL-1. TNF-α	?

* PMN-Polymorphonuclear leukocytes, * End Cells- Endothelial cells, ?TIL - Tumor infiltrating lymphocytes.

Another important chemokine in the skin is IP-10, the product of an interferon -γ . A variety of cell types express IP -10 in vitro including mononuclear cells, keratinocytes, fibroblasts, endothelial cells and T lymphocytes.[18,19] It is a poor neutrophil chemoattractant and activator. IP-10 has been detected in psoriatic plaques,[20] leprosy lesions and during the development of cutaneous delayed cellular immune responses.[21] Recently it has been proposed that epidermotropism of CTCL could be explained by cytokine loop involving secretion of chemokine IP-10 by keratinocytes and IFN-α by the lymphoid infiltrate.[22] The basis of this hypothesis has been the overexpression of IP -10 shown in skin biopsies from patients with mycosis fungoides. Taub et al have shown that human interferon -inducible protein-10 induces mononuclear cell infiltration in mice and promotes the migration of human T-lymphocytes into the peripheral tissues.[23] Some of the T-cell dependent effect may be mediated by another IFN-α inducible protein isolated from macrophages known as MIG. It has chemoattractant activity in vitro for tumour infiltrating lymphocytes.[24] Both IP-10 and MIG share the same receptor and this receptor CXCR3 has recently been cloned.[25]

GRO-α (so named because of its initial description as the product of gene differentially expressed in transformed hamster cells that had suffered loss of growth control), also known as MGSA or melanoma growth stimulatory activity, because of its mitogenic effects on melanoma cell line, is a neutrophil - specific chemoattractant secreted by activated mononuclear cells along with IL-8 and having similar potency.[26] GRO-α has been detected in psoriatic scale material in amounts comparable to those of IL-8.[27] In normal cultivated cell types, the relative amount of GRO-α released was found to be 5% to 20% of the amount of IL -8 secreted whereas in diseased skin equal amount of IL -8 and GRO-α can be detected.[14] The different ratios of the chemokines in disease could be due either to another potent and yet unknown source of GRO-α or the presence of performed GRO-α that is released under disease condition.[27] GRO-β and GRO-γ are closely related proteins that are also potent neutrophil attractants. When the mRNA of different GRO forms was investigated semiqantitatively in psoriasis lesions, GRO-α was the most abundant form whereas six-fold and 25 times lower amount of GRO-β and GRO-γ were detected , respectively.[28,29] All the three forms of GRO bind to CXCR2 receptors.[30]

The other chemokines without well- defined and clear activity in skin are shown in Table I.

C-C chemokines

MCP-1 is the prototype of the C-C chemokine subfamily, purified from different sources with chemoattractant and activator properties. MCP-1 is potent proinflammatory protein, as effective as the complement component C5a in allergic diseases, induces high levels of histamine release from human basophils, and stimulates exocytosis. However little is known about factors that induce the migration, activation, and mediator release of basophils/mast cells at the site of inflammation. Keratinocytes, dermal fibroblasts and endothelial cells are capable of producing MCP-1.[3] The accumulation of monocytes in the epidermis and along the dermo - epidermal junction in several different inflammatory disorders can be party explained by this monocyte specific chemokine. In vitro

study has shown that MCP -1 is similarly potent attractant for activated CD4 and CD8 memory T lymphocytes. [31] In assays using keratinocytes and fibroblasts, it has been reported to attract NK cells. MCP-2 and MCP3 are other novel monocyte chemoattractants but are less potent and efficacious than MCOP-1.[32] The newest members of the family are MCP-4 and MCP-5.[33,34] MCP-4 has similar structure as MCP-3 and MCP -5 has so far only identified in the mouse.

Another C-C chemokine with important role in cutaneous inflammation is RANTES (regulated upon activation, normal T expressed and secreted) RANTES is a protein of 8-10 kD,

Table II. CC Chemokines

Name	Chemotactic for	Stimuli	Receptors
MCP-1	Monocytes,memory T Lymphocytes,basophils NK cells	IFN-γ, IL-1, TNF-α	CCR2 CCR4
MCP-2	Monocytes,memory T lymphocytes basophils NK cells, Eosinophils	IL-1, TNF-α	CCR3
MCP-3	Monocytes,memory T Lymphocytes,basophils NK cells, Eosinophils	Allergen+IgE	CCR1,CCR2, CCR3
MCP-4	Monocytes, T lymphocytes,Eosinophils	?	?CCR3
MCP-5 (mouse only)	Monocytes, T lymphocytes,eosinophils	?	CCR2
RANTES	Monocytes,memory T Lymphocytes,basophils NKcells, Eosinophils, dendritic cells	TNF-α	CCR1,CCR3, CCR4,CCR5
MIP-1α	Monocytes,T lym.CD8+ Eosinophils,basophils	?	CCR1 CCR4, CCR5
MIP-1β	Monocytes,CD4+	?	CCR5
C10	T lymphoctes	IL-4	?
Eotaxin	Eosinophils	?	CCR3

chemoattracts eosinophils, monocytes, and certain T-lymphocyte subsets and code for by a gene cluster locate on human chromosome 17.[35,36] It is most potent C-C chemokine for CD8+ chemoattraction and it also attracts and activates N K cells. RANTES can play crucial role in allergic skin disorders as it is an important chemoattractant for eosinophils,[36] helper and memory-T cell subsets.[37] RANTES, like MCP- 1 induces histamine release from basophils.[38] Recent investigations have shown that skin cells can produce RANTES. It can bind to three chemokine receptors, CCRI, CCR2 and CCR3, and all three display overlapping specificity.[1] Another member of this subfamily which might emerge as important chemokine in skin diseases is Macrophage inflammatory proteins(MIP-α, β), which were purified from lipopolysaccharide (LPS) - treated monocytic cell lines. MIP -Iα has been reported to be a preferential and potent chemotactic factor for activated CD8+ T cells, whereas MIP-Iβ is found to be attractant for T lymphocytes of the $CD4^+$ type, although there is some overlap between subsets in response to both chemokines.[39] Recently it has been shown that MIP-Iα has selective activity on dendritic cells,[40] NK cells,[41] basophils,[42] and eosinophils, [43] whereas MIP -1β has no activity on dendritic cells and NK cells. One interesting function of MIP-α appears to be the inhibition of haematopoietic stem cell proliferation. MIP I-α binds with CCR4 and CCR5 receptors, and MIP-1β binds with CCR5 only . Recently another MIP called as MIP-1γ was isolated from murine macrophage cell line and been shown to be expressed by Langerhans cells.[44] It attracts both resting and activated CD4 and CD8 cells in vitro.

Eotaxin is also important chemokine with eosinophilic - specific activity.[45] Human eotaxin

has been shown to attract eosinophils when injected in primate skin and appears to use same receptor on eosinophils as MCP-3 and RANTES.

Recently many new C-C chemokines have been described mostly in the mouse (Table II). Among these C10 and MIP -3β might emerge as important members of chemokine network of skin.[46] MIP-3 B is expressed only in thymus, lymph nodes, dermal fibroblasts and appendix.[47]

C chemokine

So far, lymphotactin is the only member of this putative family. It was discovered in activated pro- T cells and lacks two of the four cysteine residues that are characteristic of the chemokines. It has chemotactic activity for T lymphocytes, but not for monocytes or neutrophils.[48] Lymphotactin is also expressed in CD8+T cells.

Chemokines and HIV

Chemokine receptors are emerging as new targets for anti-HIV therapy as the connection between chemokines and HIV-1 has been established . Cocchi et al showed that MIP-1α, MIP-1β, or RANTES could prevent infection by macrophage-tropic, nonsyncytium- inducing strains of HIV-1.[49] Soon after inhibitory effects of chemokines on HIV -1 replication were described, several different chemokine receptors were identified as coreceptor for T and M-tropic strains. Among the CXC chemokine receptors, CXCR4,[50] among the CC chemokine receptors, CCRI,CCR2b, CCR3, and CCR5 have been implicated as HIV -1 coreceptors.[51] Recently, CXCR4 has been identified as a receptor for the stomal cell - derived factor, SDF-1 and as a coreceptor for T-tropic viruses,[52] Furthermore it has been shown that multiple chemokine receptors could serve as coreceptors for HIV -1 entry into dendritic cell and CC chemokines RANTES, MIP-1α and MIP-1β has a profound effect on dendritc cell infection by M-tropic HIV-1.[53]

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