Chemotactic cytokines and inflammation. Biological properties of the lymphocyte and monocyte chemotactic factors ELCF, MCAF and IL-8.

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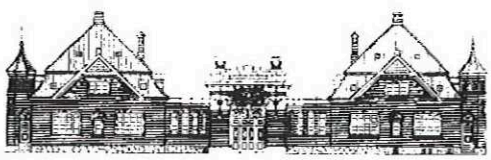

This thesis is based upon the following previously published papers, which will be referred to by their roman numericals:

I. Zachariae, C., Ternowitz, T., Larsen C.G., Nielsen, V., and Thestrup-Pedersen, K. (1988). Epidermal lymphocyte chemotactic factor specifically attracts OKT4 positive lymphocytes. *Arch. Dermatol. Res.* 280:354-357.

II. Zachariae, C.O.C., Kaltoft, K., and Thestrup-Pedersen, K. (1992). Human T lymphocytes and T-cell lines as target cells for lymphocyte chemotaxis. *Arch. Dermatol. Res.* 284:77-81.

III. Zachariae, C.O.C., Jinquan, T., Nielsen, V., Kaltoft, K., and Thestrup-Pedersen, K. (1992). Phenotypic determination of T-lymphocytes chemotactic towards fMLP, IL-8, IL-10, and epidermal lymphocyte chemotactic factor. *Arch. Dermatol. Res.* 284:333-338.

IV. Zachariae, C.O.C., Anderson, A.O., Thompson, H.L., Appella, E., Mantovani, A., Oppenheim, J.J., and Matsushima, K. (1990). Properties of monocyte chemotactic and activating factor (MCAF) purified from an human fibrosarcoma cell line. *J. Exp. Med.* 171:2177-2182.

V. Zachariae, C.O.C., Thestrup-Pedersen, K., and Matsushima, K. (1991). Expression and secretion of leucocyte chemotactic cytokines by normal human melanocytes and melanoma cells. *J. Invest. Dermatol.* 97:593-599.

VI. Smyth, M.J., Zachariae, C.O.C., Norihisa, Y., Ortaldo, J., Hishinuma, A., and Matsushima, K. (1991). Regulation of interleukin-8 gene expression in human peripheral blood lymphocyte subsets. *J. Immunol.* 146:3815-3823.

VII. Terkeltaub, R., Zachariae, C., Santoro, D., Martin, J., Peveri, P., and Matsushima, K. (1991). Monocyte-derived neutrophil chemotactic factor/interleukin-8 is a potential mediator of crystal-induced inflammation. *Arthritis and Rheumatism* 34:894-903.

LIST OF ABBREVIATIONS

ARDS:	Adult respiratory distress syndrome
CCF:	Crystal chemotactic factor
CD:	Cluster of differentiation
Con A:	Concavalin A
CSF:	Colony stimulating factor
CTAP-III:	Connective tissue activating peptide-III
C5a:	Complement split product 5a
ECGF:	Endothelial cell growth factor ß
ELAM:	Endothelial leukocyte adhesion molecule
ELCF:	Epidermal lymphocyte chemotactic factor
ETAF:	Epidermal thymocyte activating factor
ETH615:	4-(2-quinolylmethoxy)-N-(3-flurobenzyl)-phenylaminome-thyl-4-benzoic acid
FACS:	Fluorescence activated cell sorting
FCS:	Fetal calf serum
fMLP:	formyl-Methionyl-Leucyl-Phenylalanine
GCP:	Granulocyte chemotactic peptide
GM-CSF:	Granulocyte macrophage colony stimulating factor
GRO:	Growth related gene product
GSTM:	Gold sodium thio malate
HEV:	High endothelial venule
HUVEC:	Human umbilical vein endothelial cells
ICAM:	Intercellular adhesion molecule
IFN:	Interferon
IL:	Interleukin
γIP-10:	IFNγ-induced protein
JE:	Mouse "competence" gene
LAI:	Leukocyte adherence inhibitor
LAM-1:	Leukocyte adhesion molecule 1
LCF:	Lymphocyte chemotactic factor
LDL:	Low density lipoprotein
LECAM:	Lectin adhesion molecule
LFA:	Lymphocyte function associated antigen
LGL:	Large granular lymphocytes
LIF:	Leukocyte migration inhibitory factor
LPS:	Lipopolysaccharide
LTB_4:	Leukotriene B_4
MCAF:	Monocyte chemotactic and activating factor
MCP:	Monocyte chemoattractant peptide
MDNCF:	Monocyte derived neutrophil chemotactic factor
MGSA:	Melanoma growth stimulating activity
MIF:	Migrational inhibitory factor
MIP:	Macrophage inflammatory protein
mRNA:	Messenger RNA
NAF:	Neutrophil activating factor
NAP:	Neutrophil activating peptide
NCF:	Neutrophil chemotactic factor
NK:	Natural killer cell
NSAID:	Non-steroidal anti-inflammatory drug
PBMC:	Peripheral blood mononuclear cell
PBP:	Platelet basic protein
PDGF:	Platelet derived growth factor
PF4:	Platelet factor 4
PHA:	Phytohemagglutinin
PKA:	cAMP-dependent protein kinase
PKC:	Protein kinase C
PMA:	Phorbol 12-myristate 13-acetate
PMN:	Polymorphonuclear granulocyte
PPD:	Purified protein derivative
RANTES:	Regulated on activation, normal T expressed and secreted
CM-HPLC:	Carboxy methyl – high performance liquid chromatography
RP-HPLC:	Reverse phase – high performance liquid chromatography
SALT:	Skin-associated lymphoid tissues
SDS-PAGE:	SDS-polyacrylamide gel electrophoresis
SIS:	Skin immune system
TCF:	T lymphocyte chemotactic factor
TCR:	T cell receptor
ß-TG:	Beta thromboglobulin
TGF:	Transforming growth factor
TNF:	Tumor necrosis factor
TPA:	12-O-tetradecanoyl-phorbol-13-acetate
VCAM:	Vascular adhesion molecule
VLA:	Very late antigen

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1. INTRODUCTION

1.1. Introduction

One of the hallmarks of inflammation is the local tissue accumulation of leukocytes. The mechanisms by which leukocytes leave the vascular circulation and migrate to a specific site have fascinated biologists for over a century. In 1884, Pfeffer coined the term chemotaxis in his paper on the migration of plant sperm (Pfeffer 1884), but it was Leber who in 1888 first hypothesized that leukocytes migrate along a gradient in a directional manner (Leber 1888). During the same period Metchnikoff formulated the phagocytic theory of host defense and proposed that chemical signals emanating from sites of invading organisms attracted leukocytes, which play pivotal roles in host defense against microbial diseases. Rapid progress in the study of chemotaxis was not possible until 1962, when Boyden developed an *in vitro* method for the quantification of chemotaxis (Boyden 1962). The analysis involves leukocyte migration through a microporous filter across which a chemical gradient is established. It was then possible to define and characterize chemoattractants and examine the chemotactic behavior of cells.

The inflammation in many dermatological disorders such as psoriasis, atopic dermatitis, lichen planus, and discoid lupus erythematosus is characterized by an abundancy of T lymphocytes, but with very few B lymphocytes or null cells as compared to normal skin (Table 1). Similar changes are seen in the tuberculin skin reaction, but here monocytes are also present. Numerous monocytes/macrophages have been demonstrated in the stroma of malignant tumors originating from colon (Allen et al. 1985), breast (Gottlinger et al. 1985), skin (Brocker et al. 1988), and other tissues. In syphilis plasma cells are the predominant inflammatory cells.

The composition of the different infiltrates can not be explained by a random migration into the skin of all cell types, but must involve specific chemoattractants of which some are described here. It also involves specific migrational inhibitory factors such as (LIF, MIF) (Bendtzen 1980, Sorg et al. 1986) and induction of specific adhesion molecules (ELAM, ICAM), and factors which augment lymphocyte activation such as the proinflammatory cytokines like IL-1 and the autocrine lymphocyte cytokines (i.e. IL-2 and IFNγ).

During a lymphocyte mediated immunological reaction there is a secretion of a broad range of different hormone like peptide mediators called cytokines. Cytokines differ from classical hormones in that they are produced by a number of cell types rather than by specialized glands. Although some of the cytokines can be detected in serum during systemic inflammatory conditions, they generally act locally in a paracrine and/or autocrine way rather than in an endocrine manner. Cytokines modulate the host response to foreign antigens or injuries by regulating the growth, mobility and differentiation of leukocytes and other cell types.

Although some transformed cell lines of different origin spontaneously secrete cytokines, resting cells and most cell lines must be stimulated to produce cytokines. Cytokines are generally synthesized and secreted peptides or glycoproteins with a molecular size ranging from 6,000 to 60,000 daltons. They are extremely potent compounds which act in concentrations of 10^{-10} to 10^{-15} M to stimulate target cells through specific receptors (Oppenheim et al. 1990). Although some cytokines are produced by a few cell types and have a restricted number of target cells such as IL-2, others are produced by many cell types and have a broad spectrum of target cells. Cytokines may have multiple effects and thereby exhibit considerable overlap in their biological effects on lymphoid, myeloid, vascular, neural, and musculoskeletal cells. Furthermore certain cytokines initiates the production of cascades by releasing other cytokines. In addition cytokines interplay with neuroendocrine hormonal peptides such as endorphins and corticosteroids as well as products from the lipoxygenase and cyclooxygenase pathway exhibiting either agonistic or antagonistic effects. It should also be realized that cytokines can only induce their effect, if target cells express appropriate receptors thereby introducing a further dimension of regulation.

1.2. Purpose of the study

The purpose of the study was to investigate the chemotactic capacity of different leukocyte chemoattractants including ELCF, IL-8, MCAF and IL-10 (II, III). The origin of IL-8 from various lymphocyte subsets was established together with a description of different IL-8 inducers (VI). Monocyte chemotactic and activating factor (MCAF) was purified from a fibrosarcoma cell line and its *in vitro* and *in vivo* activities were described (IV). In addition, the production of IL-8 and MCAF in melanocytes and melanoma cell lines was compared. It is suggested that a difference in the ability to secrete various cytokines can determine inflammation and tumor growth control (V). The production of IL-8 by urate crystal stimulated monocytes is described and a possible significance of this molecule in urate arthritis is established (VII).

2. LYMPHOCYTE CHEMOTAXIS

2.1. Introduction

Chemotaxis is initiated by the binding of chemoattractants to membrane receptors. This binding alters transmembrane potential and activates ionic fluxes. The subsequent sequence of metabolic processes leads to changes in the cytoskeletal elements of the responding cells including orientation and migration of the cells towards the source of the chemotactic gradient. The cells must be capable of identifying a concentration gradient over the length of the cells for expressing directional migration (Zigmond and Hirsch 1973).

Leukocytes are capable of three types of movement: random migration, chemokinesis and chemotaxis. *Random migration* is a non-directional motility in the absence of chemical stimulation. *Chemokinesis* is chemically augmented, non-directional cellular motility occurring in the absence of a chemotactic gradient. *Chemotaxis* is directed, cellular migration along a concentration gradient of a chemoattractant. To distinguish chemokinesis from chemotaxis, Zigmond & Hirsch introduced the checkerboard procedure (Zigmond &

Hirsch 1973) employing a series of chambers with different concentrations and concentration gradients of attractant. In the original method the distances of the migrating cells were calculated and compared with the expected distance if the cells had only migrated in response to a chemokinetic force. When using a single filter system with a 10 μm thick polycarbonate membrane as done by Bacon and coworkers, Larsen and coworkers and by our group this is not possible. Rhodes has questioned the method used by Zigmond and coworkers for discriminating between chemotaxis and chemokinesis (Rhodes 1982), but despite this the "checkerboard assay" has been accepted as the preferred method for distinguishing between chemokinesis and chemotaxis. When measuring lymphocyte chemotaxis compared to monocyte or neutrophil chemotaxis there are different aspects to consider. First of all, lymphocytes migrate much slower than other leukocytes *in vitro*. It has been shown that lymphocytes migrate half as fast as neutrophils and monocytes (Wilkinson and Allan 1980), which explains the longer assay time for the optimal correlation between cells migrating towards medium alone or towards chemoattractant. T-lymphocytes show less adherence to different surfaces than neutrophils, monocytes and B lymphocytes. Further it has been estimated that only 10% of freshly isolated lymphocytes are capable of migrating in contrast to 90% of monocytes and neutrophils using an agarose technique. After cultivation of the lymphocytes for 48 h 44% of the lymphocytes could express a migrating morphology (Parrot and Wilkinson 1981). Lymphocytes activated *in vitro* by Con A and PHA have an increased migrational capacity (O'Neill and Parrot 1977). Also, lymphocytes stimulated with antigen, as in mixed lymphocyte cultures, migrate better than non-stimulated lymphocytes. It is therefore reasonable to suggest that small lymphocytes in blood or in unstimulated lymphoid tissues are not highly motile though some cells can migrate. Lymphoblasts on the other hand are highly motile cells and various procedures which induce blast transformation will also enhance their motility. These differences in locomotor behavior of lymphocytes at different stages of differentiation may be pertinent to the behavior of lymphocytes migrating into tissues. Furthermore the substrate in which T lymphocytes are migrating is also of importance. Thus, increasing amounts of serum albumin in the media will increase the migrational capacity of the cells. Albumin behaves as a chemokinetic factor for lymphocytes, probably because albumin-coated filters provide a better three-dimensional substratum that favors locomotion.

2.2. Lymphocyte chemotaxis – techniques

In the first method described by Boyden (1962) the leukocytes are separated from the chemotactic substance by a micropore filter, which has pores of a size that allows the leukocytes to squeeze through by active migration, but not to fall through passively. The chemotaxin in the lower compartment diffuses to the top chamber, and the cells move towards the higher concentration of the chemoattractant. Cells are incubated under the same conditions and for the same time period without chemoattractant as control. The migrating leukocytes are assessed by removing the filter, and after fixation, staining, dehydrating and clearing the filter, they are quantitated by morphological observation and counting.

The leading front technique measures the distance the cells have migrated. One can also count the number of cells on the lower surface and compare with control values. Alternatively radiolabelled leukocytes can be used and after a suitable time the radioactivity of cells that have penetrated one filter and accumulated on a second filter below can be quantitated (Ternowitz 1985).

The most commonly used filters are made of nitrocellulose and have a thickness of 145 μm with pore sizes of 0.45-12 μm. In the two-filter method the upper filter is a 10 μm thick polycarbonate filter. In 1980 Falk and coworkers developed a microwell assay system, which allows smaller cell numbers and smaller volumes of chemoattractants to be used (Falk et al. 1980). This system was first used for monocytes and neutrophils as lymphocytes do not adhere to the polycarbonate membranes. This problem was solved by coating the polycarbonate membranes with mouse type IV collagen, which allows T lymphocytes to adhere (Larsen et al. 1989c). By culturing T lymphocytes overnight in 10% FCS Bacon and coworkers observed T lymphocyte adherence to the membranes. However, FCS activates the lymphocytes and makes the results less comparable with freshly isolated T lymphocytes.

The migration assay under agarose is flexible, giving well reproducible results with a small amount of cells, but requires large amounts of chemotactic substances. The chemotactic factor and buffer are placed in reservoirs directly opposite each other in an agarose plate with cells in a third reservoir in aligned equidistance between the other two. The difference in the extent of migration between cells exposed to chemotaxin and to buffer is a measure of the extent of stimulated migration.

A long list of different factors have been described as lymphocyte chemoattractants during the last two decades. They are summarized in table 2. The true chemotactic activity of some of these chemoattractants is still disputed. Using the Boyden chamber assay and freshly prepared lymphocytes we have not been able to demonstrate chemotactic activity of IL-1α or IL-1ß (II, Ternowitz and Thestrup-Pedersen 1986, Larsen et al. 1989d). However using the 48-micro well assay we have been able to demonstrate a weak chemotactic activity of recombinant IL-1α towards T lymphocytes both freshly isolated and overnight cultured (unpublished results). The reason for the difference between the chemotactic activity of IL-1 in the Boyden assay system and the 48-micro well system may be explained by the fact that the micro-well assay detect another T lymphocyte subpopulation, where adherence to the lower surface of the polycarbonate membrane is the basis for measure in contrast to the Boyden chamber method, where the cells migrate through a polycarbonate membrane and drop on to a nitrocellulose filter. Differences has also been noted between freshly isolated T lymphocytes and cells which have been cultured overnight as done by Bacon et al. who demonstrated T lymphocyte chemotactic activity towards IL-1, RANTES, huMIP-1α and huMIP-1ß. IL-2

has only been demonstrated to have chemotactic activity for cultured T lymphocytes, which can be explained by the necessity of an upregulation of the IL-2 receptors for achieving a chemotactic response.

2.3. Human T-cell lines as target cells for lymphocyte chemotaxis

Using the modified Boyden chamber assay with ^{51}Cr-labelled T lymphocytes we observed an inter-individual variation in lymphocyte responsiveness towards different chemotactic stimuli (II). It could be due to variability in the amount of receptors for the different chemoattractants, or deficiency in the responding T lymphocytes at the time of investigation as only 10% of freshly isolated T lymphocytes will respond.

In order to achieve a standardized lymphocyte chemotactic assay, which could be used in screening for lymphocyte chemotaxis, we studied a number of human T-cell lines for their chemotactic reactivity towards different chemoattractants. Five different cell lines were tested. HuT78 responded to all of the chemoattractants tested, while K562, a granulocytic lineage cell line, responded to all except IL-1α and IL-1ß (II). The difference in response between the cell lines to IL-1 could be explained by the absence or presence of IL-1 receptors on the cells, as the JURKAT cell line which did not respond to IL-1 only have very few receptors. An interesting phenomenon is that HuT78 cells grow in clusters showing greater adhesiveness of cells. HuT78 correspond to normal human peripheral blood T lymphocytes with regard to pore size, optimal concentrations of chemoattractants and with a little shorter assay time. This cell line may be used in screening for lymphocyte chemotaxis, when a uniform responding cell is of importance.

3. EPIDERMAL LYMPHOCYTE CHEMOTACTIC FACTOR (ELCF)

3.1. Introduction

Trafficking of lymphocytes involves the migration of different subpopulations of lymphocytes at the proper time to proper sites of infection or antigenic challenge. This process contains two major components: homing and migration. Homing involves adhesion and migration involves chemotaxis.

In the late seventies and early eighties lymphocyte locomotion was observed in supernatants from Con A- and PHA-stimulated PBMC's and T lymphocytes, from mixed lymphocyte reactions, and following exposure to casein, C5a, and fMLP (Center and Cruikshank 1982, Cruikshank and Center 1982, El-Nagger et al. 1981, 1982, Parrott 1980, Van Epps 1982, Wilkinson et al. 1976). Chemoattractant substances responsible for the migration of subsets of lymphocytes to antigenic sites have until recently been largely unknown.

The first evidence of lymphocyte chemotactic activity in epidermal tissue came from experiments, where supernatants of homogenized epidermis overlying a positive tuberculin skin reaction contained chemotactic activity mostly for T lymphocytes, but also for non-T cells (Ternowitz and Thestrup-Pedersen 1986). When the homogenates were obtained from skin of patients with allergic contact dermatitis ELCF activity could be seen in all patients having a positive patch test at 48 hours (Larsen et al. 1988). A small, but significant ELCF activity was also found in normal looking skin of patients with eczema. This finding supports the well-known excited-skin-syndrome (Mitchell 1975). ELCF activity was seen in all patients with primary irritant dermatitis in the positive test area, and for some also in the normal looking skin after the irritant patch test (Larsen et al. 1989d). Even occlusion alone in patients with allergic contact dermatitis revealed a significant increase in ELCF activity in contrast to normal persons. Similar results were found using the tuberculin skin test (Ternowitz and Thestrup-Pedersen 1986). This suggests that T-lymphocyte chemotactic factors are important during the onset of a lymphocytic inflammation in the skin in allergic contact dermatitis and other delayed type hypersensitivity reactions as well as in primary irritant dermatitis.

3.2. ELCF is chemotactic for CD4+ cells

The predominant infiltrate of CD4+ T lymphocytes in the reactions mentioned above led us to investigate, which subset of T lymphocytes migrates towards ELCF. We separated OKT4+ (CD4+) and OKT8+ (CD8+) T lymphocytes by FACS and investigated their motility towards ELCF, LTB_4, C5a and fMLP at optimal concentrations. When ELCF was used we saw a significant migration of CD4+ T lymphocytes, but not of CD8+ T lymphocytes. The results were not due to an impaired chemotactic ability of the CD8+ T lymphocytes after FACS separation, because both CD4+ and CD8+ T lymphocytes showed similar, significant migration towards C5a, LTB_4 and fMLP.

3.3. Phenotypic determination of migrating T lymphocytes

T lymphocytes have in recent years been shown to form a large variety of subsets, where a major division is the helper and cytotoxic/suppressor cells. These subsets can be differentiated via their phenotype using monoclonal antibodies. Their function seems to reflect their maturity stage and ultimately their expression of adhesion molecules and cytoprofile (Akbar et al. 1991, Mosmann and Moore 1991). T lymphocytes can be determined as either naive or memory T cells, the latter associated with memory functions (Sanders et al. 1988). The memory T cells are considered to be long lived cells constantly recirculating between blood and lymphoid tissue and are more potent effectors than naive T lymphocytes due to their ability to produce different cytokines (Sanders et al. 1988).

CD4+ cells dominate many inflammatory skin diseases (table 1). We still lack information of the full cytokine profile of these cells even though IL-2 and IFNγ is known to be secreted. In recent years it has been shown that the neutrophil and T lymphocyte chemotactic cytokine IL-8 is strongly upregulated not only in the epidermis in psoriasis and during a tuberculin skin test, but also in other disorders such as atopic dermatitis (Paludan and Thestrup-Pedersen 1992a,

Schröder et al. 1992). It is most likely that the accumulation of T lymphocytes around vessels in stratum papillare and in epidermis is the end result of a delicate time-sequenced process, where the release of cytokines and expression of adhesion molecules determines the outcome of the cellular infiltrate. IL-10 has recently been described to inhibit the production of different inflammatory cytokines (de Waal Malefyt et al. 1991a) and to inhibit the proliferative response of CD4+ T lymphocytes to antigen stimulation (de Waal Malefyt et al. 1991b). IL-10 could therefore also be an important cytokine in the control of skin inflammatory mechanisms.

We wanted to study the phenotype of migrating T lymphocytes using the conventional Boyden chamber technique, where migrating T lymphocytes were collected from the lower chamber after passing through a filter. ELCF preferentially attracted the CD4+, CD45R0+ memory T lymphocytes, whereas fMLP and IL-8 did not selectively attract subpopulations as judged by comparison with cells "migrating" in the control chambers (III). hIL-10 selectively attracted the CD8+ subpopulation of the T lymphocytes (III). These results are in line with earlier results on IL-8 (Schall et al. 1990, Larsen et al. 1989c) and IL-10 (Jinquan et al. 1992). Antibodies towards IL-8 could only partly inhibit the chemotactic activity of ELCF (II). Further more only pg levels of IL-8 could be measured from suction blister fluid overlying a positive tuberculin skin reaction (II). IL-1 seems not to be involved as no ELCF activity can be detected in normal skin as opposed to IL-1 activity (Ternowitz and Thestrup-Pedersen 1986) and monoclonal antibodies towards IL-1 did not block ELCF activity (II). LTB_4 was removed by the dialysis and is an unstable factor. If C5a was responsible for the chemotactic activity both neutrophil and monocyte chemotactic activity should also be seen in the ELCF preparations.

We have not been able to purify ELCF due to the limitation of material. A newly discovered T lymphocyte chemotactic agent RANTES belongs to the same supergene family as IL-8 and attracts both monocytes and the CD45RO+ subpopulation of the CD4+ T lymphocytes (Schall et al. 1990). RANTES could account for some of the ELCF activity, even though RANTES has not yet been described in human skin. Another factor with molecular weight of 56,000 Da has been shown to be selective for CD4+ T lymphocytes, but it was only produced by CD8+ T lymphocytes when stimulated with Con A (Van Epps et al. 1983). This factor could also be responsible for some of the activity of ELCF even though the major part of the T lymphocyte chemotactic activity for ELCF was determined to be in the molecular weight range of 3,000 to 30,000 Da (unpublished results). In conclusion, ELCF is probably not a single factor but rather a mixture of chemotactic factors.

As already mentioned the number of lymphocyte chemotactic factors identified has increased rapidly within recent years. Some of them have selective chemotactic activity towards different subsets of T lymphocytes, while others like IL-8 attracts T lymphocytes as a whole. Table 2 gives an overview of the present status.

Table: 1: *Composition of leukocyte infiltrate in different inflammatory skin diseases.*

T lymphocytes	Mø	CD4+	CD8+	CD29+,4B4+,CD45R0+	Skin CD4+/CD8+	Blood CD4+/CD8+
Normal skin[1]						
+	–	+	+	+	1:1	7:3
Psoriasis[2]						
+	++ later	++		++	↑	
Allergic and irritative contact dermatitis[3]						
+	+ later	++	+	++		
Atopic dermatitis[4]						
+++	(+)	+++			7:1	
+++		+			4.8:1	↑
Positive patch test[5]						
++	+	++	(+)	++		
Delayed type hypersensitivity[6]						
++	++	++		++	2:1	7:3
Lichen Planus[7]						
++		+	+		↑	

Mø, macrophages

[1] (Bos et al. 1987)
[2] (Baker et al. 1984, Bos et al. 1983, Bjerke et al. 1978)
[3] (Gawkrodger et al. 1986, Sterry et al. 1990)
[4] (Lever et al. 1987, Leung et al. 1981, Zachary et al. 1985)
[5] (Ralfkiær et al. 1984, Sterry et al. 1991)
[6] (Kaplan et al. 1987, Platt et al. 1983, Poulter et al. 1982)
[7] (Gomes et al. 1982)

Table: 2: *T lymphocyte chemotactic factors.*

Factor	Responsive T lymphocyte	References
IL-1	T-lymph.$^{+*\circ}$	(Miossec et al. 1984, Bacon et al. 1989)
IL-2	T-lymph.*	(Potter and Van Epps 1987)
IL-3	T-lymph.$^{*\circ}$	(Bacon et al. 1990)
IL-4	T-lymph.*	(Bacon et al. 1990)
IL-6	T-lymph.$^{*\circ}$	(Bacon et al. 1990)
IL-8	CD4+, CD8+$^{*+\circ}$	(Larsen et al. 1989c)
IL-10	CD8+$^{+}$	(III, Jinquan et al. 1992)
ELCF	CD4+, CD45R0+$^{+\circ}$	(I, III)
LCF	CD4+, T-lymph.$^{+}$	(Berman et al. 1985, Center and Cruikshank 1982, Potter and Van Epps 1987)
C5a	T-lymph.$^{+\circ}$	(Hayashi et al. 1989)
fMLP	T-lymph.$^{+*}$	(El-Naggar et al. 1981, Bacon et al. 1988)
Casein	T-lymph.$^{+*}$	(El-Naggar et al. 1981)
LTB_4	CD4+, CD8+$^{+*\circ}$	(I, Bacon et al. 1989)
12(R)-HETE	T-lymph.$^{*\circ}$	(Bacon et al. 1988)
RANTES	CD4+, CD45R0+$^{+}$	(Schall et al. 1990)
huMIP-1α	CD8+, CD45RA+$^{+}$	(Schall 1991)
huMIP-1β	CD45RA+$^{+}$	(Schall 1991)

* Chemotactic activity was determined using cultured T lymphocytes.
\+ Chemotactic activity was determined using freshly isolated T lymphocytes.
° Can be detected in the skin.

3.4. Adhesion molecules and chemotaxis

A model has been proposed by Nickoloff for cutaneous lymphocyte trafficking divided into three phases: recruitment, retention, and the return to circulation (Nickoloff 1988). In the recruitment phase the essential cell is the endothelial cell, which produces both chemotactic factors and adhesion molecules in response to cytokines. The retention phase involves trapping of the lymphocytes in the skin and is based on adhesion molecules. Return of the lymphocytes to the circulation may be preceded by loss of expression of certain adhesion molecules by the keratinocytes. Thus it seems likely that the lymphocyte trafficking during an inflammatory reaction is controlled by cytokine-adhesion molecule cascades. The different adhesion molecules and their ligands, which are involved in the adhesion of T lymphocytes to endothelial cells and keratinocytes, have been listed in table 3.

The T cell derived cytokine IFNγ can rapidly induce expression of ICAM-1 mRNA and protein in keratinocytes. ICAM-1 is a cell surface ligand for the lymphocyte surface adhesion molecule, LFA-1. Immunohistochemical studies of several inflammatory disorders have demonstrated the presence of LFA-1 positive T lymphocytes -the presumed source of IFNγ within the epidermis (Griffith et al. 1989). Taken together lymphocytes in inflamed skin involve the secretion of IFNγ by the infiltrating T lymphocytes, which induce ICAM-1 expression on keratinocytes leading to binding of LFA-1 positive T lymphocytes in the epidermis. The adherence of T lymphocytes may permit both helper T-cell activation, production of inflammatory cytokines, and activation of cytotoxic T cells leading to keratinocyte lysis.

ICAM-1 is found on many cell types, and its expression on vascular endothelium is strongly up-regulated by IL-1, TNFα and IFNγ, both *in vivo* an *in vitro* (Pober et al. 1986, Dustin et al. 1988b, Cotran and Pober 1988). ELAM-1 is a cell surface protein rapidly synthesized by HUVEC's in response to IL-1 and TNFα, but not IFNγ (Bevilacque et al. 1987). ELAM-1 can mediate the adhesion of CD4+ memory T cells, but not naive T cells to endothelial cells (Shimizu et al. 1991). ELAM-1 may also mediate adhesion of PMN's and possibly monocytes (Bevilacque et al. 1987, 1989). The adhesion of memory T cells to ELAM-1 is independent of acute activation events unlike other integrin mediated adhesions. Thus, ELAM-1 may be of primary importance in the initial attachment of memory T cells to inflamed endothelium *in vivo* and to the preferential migration of memory T cells into tissue and inflammatory sites (Shimizu et al 1991).

It seems reasonable that memory cells are more responsive to migration than naive T cells, which express the RA isoform of CD45 and exhibit only low levels of adhesion molecules. The CD45R0+ memory T cell subpopulation expresses increased levels of adhesion molecules, including lymphocyte function-associated antigen 1 (LFA-1), VLA-4, CD44 and intercellular adhesion molecule 1 (ICAM-1) (Mackay 1991).

Table: 3: *Adhesion molecules involved in T lymphocyte – endothelial/keratinocyte adhesion.*

	T lymphocytes
Endothelial cells:	ICAM-1/LFA-1 VCAM-1/VLA-4 Hyaluronate/CD44 ? /LAM-1
	Memory lymphocytes
	ELAM-1/ ?
	T lymphocytes
Keratinocytes:	ICAM-1/LFA-1 LFA-3/CD2

There are three basic pathways for the extravasation of T lymphocytes into tissue. (1) across flat endothelium of tissues, (2) across endothelium of inflamed tissue, and (3) across HEV within a lymph node. Memory T cells selectively cross flat endothelium in inflamed tissues, and eventually drain to regional lymph nodes via the afferent lymph, while naive T cells selectively cross lymph node HEV, and drain into the efferent lymph and back into the circulation (Mackay 1991). These different recirculation pathways of naive and memory T cells can be explained by their expression of adhesion molecules (Mackay 1991).

T cell adhesion to normal flat endothelium or to inflamed endothelium involves VLA-4 binding to vascular cell adhesion molecule 1 (VCAM-1), CD44 binding to hyaluronate, and LFA-1 binding to ICAM-1. VLA-4, CD44 and LFA-1 are all upregulated on activated and memory T cells (Oppenheimer-Marks et al. 1990). Further endothelial-leukocyte adhesion molecule 1 (ELAM-1) is induced by cytokines especially IFNγ on inflamed endothelium. This molecule serves as a receptor for ligands expressed only on the memory subset of T cells (Mackay 1991). The initial migration of memory T lymphocytes into the skin (Sterry et al. 1990, 1991) may be influenced by ELCF and/or IL-8 and/or RANTES.

Although the ICAM-1-LFA-1 pathway does function in lymphocyte adhesion, MoAbs that block the LFA-1 pathway only weakly inhibit the binding of lymphocytic cells to activated endothelium (Dustin et al. 1988a). Similar results have been obtained *in vitro* for T lymphocytes, where lymphocyte chemotaxis towards IL-2 and LCF was inhibited by antibodies towards the ß-chain of LFA-1. Antibodies towards the α-chain only slightly inhibited the chemotaxis (Van Epps et al. 1989). Furthermore, in patients with a genetic defiency of CD18 the α-chain of LFA-1 shows normal lymphocyte recruitment into inflammatory sites (Anderson and Springer 1987).

We therefore wanted to evaluate the importance of adhesion molecules for the chemotactic response. Incubation of T cells with 300 U of IFNγ increased ICAM-1, LFA-1α and LFA-1ß expression by up to 50%. This did, however, not increase the migrational potential towards different chemotactic factors. In contrast, when the cells were incubated with antibodies towards LFA-1α and LFA-1ß we observed a reduction in the chemotactic response towards ELCF, C5a and fMLP, while LTB_4 remained unaltered (II). In addition we did not see any selective attraction of CD18+ T cells (III). Thus it seems that LFA-1 plays a role, but is not essential for the migration of T lymphocytes *in vitro*.

4. INTERLEUKIN-8

4.1. Introduction

In the early nineteen eighties interleukin 1 (IL-1) and tumor necrosis factor (TNF) were reported to have considerable leukocyte chemotactic activity using partially purified molecules (Luger et al. 1983, Ming et al. 1987). However, when purified natural IL-1α/β, recombinant IL-1α/β or recombinant TNFα became available, they did not show *in vitro* leukocyte chemotactic activity even though they could still induce an inflammatory response upon intracutaneous injection.

The neutrophil chemotactic activity was shown to be different from both IL-1 and TNF, and was purified from LPS-stimulated monocytes (Yoshimura et al. 1987). Consequently this monocyte derived neutrophil chemotactic factor (MDNCF) was cloned (Matsushima et al. 1988) and turned out to be identical to 3-10C, which was cloned as a gene inducible in enterotoxin stimulated human PBMC (Schmid and Weissmann 1987). Simultaneously an identical factor was purified by the groups of Baggiolini, Schröder and Van Damme and was termed granulocyte chemotactic peptide (GCP) (Van Damme et al. 1988), neutrophil activating peptide (NAP) (Schröder et al. 1987), or neutrophil activating factor (NAF) (Walz et al. 1987), respectively.

Larsen et al. showed that a T lymphocyte chemotactic factor (TCF) in PHA-stimulated human PBMC conditioned media was identical to MDNCF. Since this NCF had multiple target cells and was produced by a variety of cells, the molecule was renamed interleukin 8 (IL-8) (Westwick et al. 1989, Larsen et al. 1989c), and will be called so throughout the thesis.

IL-8 is synthesized in a pre-form of 99 amino acids and processed on excretion to an 8 KDa mature-form of 72 amino acids through proteolysis (Matsushima and Oppenheim 1989b). The isoelectric point of IL-8 is ≥ 8.5 and IL-8 binds to heparin. This property of IL-8 is due to the presence of its many basic amino acids (lysines and arginines). The mature IL-8 also contains 4 cysteine residues which form disulfide linkages as described (Clore et al. 1989).

The receptors for IL-8 are distinct from the receptors of other chemotactic factors such as fMLP, C5a, LTB_4, IL-1, TNF, monocyte chemotactic and activating factor (MCAF), and platelet activating factor, which all have been identified on human neutrophils (Samanta et al. 1989a). cDNA encoding for IL-8 receptors has been cloned (Holmes et al. 1991, Murphy and Tiffany 1991) The size has been estimated to 40 KDa. About 20,000 IL-8 binding sites have been detected per neutrophil, while T lymphocytes only have around 300 binding sites per cell (Samanta et al. 1989a). The dissociation constant (Kd) for IL-8 binding is 3.6 nM. IL-8 downregulates its own receptor expression. More than 90% of IL-8 receptors are internalized within 10 minutes of IL-8 binding (Samanta et al. 1989b).

The signals transduced through IL-8 receptors seem to be very similar to those of other chemoattractant receptors, like the MCAF signal transduction passway. In less than 2 seconds after IL-8 stimulation there is a rise in cytosolic Ca^{2+}, which can be inhibited by Bordetella pertussis toxin (IAP) (Thelen et al. 1988). The protein kinase C inhibitor, staurosporine blocks the superoxide generation by IL-8. These data suggest that protein kinase C activation may be essential for the effect of IL-8 on neutrophils and that a GTP binding protein is associated with the IL-8 receptor.

4.2. Cell sources and inducers of IL-8

Certain cytokines like IL-2, IL-4 and IFNγ are only produced by a specific cell type, T lymphocytes. In contrast

other cytokines like IL-1α, TNFα, IL-6, and IL-8 can be released by a variety of cells, including monocytes, fibroblasts, endothelial cells, synovial cells, chondrocytes etc. Table 4 describes the cell sources of IL-8. In this respect it is important to realize that cytokine expression varies for individual cell types. Thus, Kristensen and coworkers quantified and compared IL-8 mRNA amounts in cultures of keratinocytes, dermal fibroblasts, endothelial cells, and monocytes stimulated with IL-1α (Kristensen et al. 1991). The induction of IL-8 mRNA was in some cells found to be very rapid (< 30 min.) and reached maximum at different time points. Endothelial cells produced very high, fibroblasts and monocytes intermediate, and keratinocytes low amounts of IL-8 mRNA.

It has been suggested that IL-1 possesses an intermediate role in the induction of IL-8 by LPS in PBMC, because an IL-1 receptor antagonist inhibited IL-8 production by 30-40% (Porat et al. 1991). Also anti-TNF and anti-IL-1 antibodies reduced LPS-induced IL-8 mRNA induction and IL-8 production (Remick et al. 1991). We observed IL-8 mRNA as early as ½ hour after stimulation with LPS and TNFα, which makes it difficult for an intermediate system to be necessary. IL-1 and TNF could play a role in the second "wave" of IL-8 induction after stimulation with LPS.

IL-8 production can be induced by a broad range of stimuli (table 5). Different cell types respond variably when stimulated with different inducers (Van Damme 1991b).

Table: 4: *Cell sources of IL-8.*

Interleukin 8	Monocytes and macrophages (Yoshimura et al. 1987, Matsushima et al. 1988, VII) T-lymphocytes (Gregory et al. 1988, VI, Schröder et al. 1988) Large granular lymphocytes (VI) Neutrophils (Strieter et al. 1990, Bazzoni et al. 1991) Dermal fibroblasts (Schröder et al. 1990, Larsen et al. 1989b) Keratinocytes (Larsen et al. 1989b, Barker et al. 1990) Vascular endothelial cells (Sica et al. 1990a, Strieter et al. 1989b, Schröder and Christophers 1989a) Melanocytes (V) Hepatocytes (Thornton et al. 1990) Chondrocytes (Van Damme et al. 1990) Mesangial cells (Brown et al. 1991b) Various tumor cell lines (e.g. melanoma cell line, keratinocyte cell line, fibrosarcoma cell line, lung giant cell carcinoma, and osteosarcoma cell line) (Van Damme et al. 1989a, VI) Airway structural cells (Denburg et al. 1991) Synovial cells (Brennan et al. 1990) Retinal pigment epithelial cells (Elner et al. 1990)

4.3. IL-8 expression and secretion in human blood lymphocyte subsets

It has previously been reported that IL-8 can be produced by T lymphocytes upon stimulation with PHA or Con A alone (Gregory et al. 1988, Schröder et al. 1988), but in the studies it could not be excluded that the cells contained monocytes. A need for accessory cells has been shown for certain T lymphocyte functions (Unanue and Allen 1987). Whether the production of IL-8 is accessory-cell dependent remained to be determined.

We therefore studied whether highly purified T lymphocytes and LGL express IL-8 mRNA and secrete IL-8 protein, either alone or in the presence of accessory cells. IL-8 mRNA could only be induced by a combination of PMA and ionomycin or PHA and PMA, and to a minor extent with IL-2 and PHA, while none of the factors alone induced IL-8 mRNA (VI). Neither IL-2, ligation of the TCR/CD3 complex with OKT-3 mAb, IL-1α, TNFα nor LPS were able to induce the signal alone or in different combinations (VI). This is consistent with numerous reports of T lymphocytes requiring two signals for lymphokine expression (Wiskocil et al. 1985). The more specific T lymphocyte stimuli such as immobilized anti-CD3 mAb, IL-2 or their combination were not able to induce the signal. The reason for this is somewhat unclear.

IL-8 mRNA was induced in purified T lymphocytes after 3 hours and reached maximum at 6 hours followed by a decline to resting levels at later time points. This is similar to the induction of other T cell lymphokine genes (Lindstein et al. 1989). We further showed that IL-8 mRNA could only be induced in the CD4+ T lymphocytes (VI). This is in agreement with the concept that CD4+ T lymphocytes express a wider variety of lymphokines than CD8+ T lymphocytes (Janeway et al. 1988). We observed that purified T lymphocytes can express mRNA for IL-8, but not release any detectable level of IL-8 protein. This raised the possibility that IL-8 production by T lymphocytes was accessory cell dependent.

Antigen presenting cells such as monocytes, dendritic cells and macrophages contribute to T lymphocyte stimulation by their production of soluble monokines (Mizel 1982) or direct interaction. One of the possibilities was that stimulated monocytes produce an unknown factor that could induce IL-8 production by T lymphocytes. We observed that supernatant from LPS-stimulated monocytes did not induce any IL-8 secretion. An experiment with a mixture of T lymphocytes and monocytes stimulated with either PMA, or PMA in combination with ionomycin, makes it probable that T cells do not secrete IL-8 in response to PMA/ionomycin even in the presence of accessory monocytes. Immunoprecipitation analysis revealed that only minor levels of IL-8 were detectable in cell lysates from T lymphocytes stimulated with PMA/ionomycin (VI). This suggests that the production of IL-8 is inhibited at the posttranscriptional level, which can be responsible for the missing secretion of IL-8. Although PHA did stimulate PBMC IL-8 mRNA and PBMC IL-8 protein, PHA induced only minor production of IL-8 in monocytes and an insignificant amount in LGL. This raises the possibility that the effects of PHA on IL-8 production in PBMC is indirect or that PHA requires accessory cells to stimulate T lymphocytes. An explanation for the presence of the neutrophil stimulating peptide characterized by Schröder

Table: 5: *Inducers and suppressors.*

A. *Upregulation:* The production of the cytokine can be induced by the following diverse agents:	
Interleukin 8	PHA (Larsen et al. 1989c, Gregory et al. 1988) Con A (Gregory et al. 1988) LPS (Matsushima et al. 1988, Strieter et al. 1989b, Schröder et al. 1989) Zymosan (Rankin et al. 1990) IL-1α, IL-1β (V, Strieter et al. 1989b, Larsen et al. 1989b) TNFα (V, Strieter et al. 1989b, Larsen et al. 1989b) IL-2 (VI) IL-3 (Haslberger et al. 1990) IL-6 (Haslberger et al. 1990) IFNγ (Barker et al. 1991) Silica (VII) Phorbol esters (VI, Matsushima et al. 1988) TPA (Barker et al. 1991) PSK (Hirose et al. 1990) Ureic acids (VII) Paraquat (Bianchi et al. 1991) Double-stranded RNA poly(rI):poly(rC) (Van Damme et al. 1989a) Virus (Van Damme et al. 1989a) Bacteria (Unpublished observation) Substance P (Denburg et al. 1991) anti-CD16 mAb (VI)
B. *Downregulation:* The production of the cytokine is suppressed by the following agents:	
Interleukin 8	IL-4 (Standiford et al. 1990) IL-10 (de Wall Malefyt et al. 1991) Glucocorticoids (Dexamethason) (Mukaida et al. 1992) TGFß (Seitz et al. 1991) Antihistaminic compounds (Ketotifen) (Denburg et al. 1991) 5'-lipoxygenase inhibitor (ETH 615) (Kristensen et al. 1993) 1,25 $(OH)_2$-vitamin D_3 (Larsen et al. 1991) Tacalcitol (To be published) FK506 (Unpublished results) Cyclosporin A (Unpublished results) GSTM (Deleuran et al. 1992) D-Penicillamin (Deleuran et al. 1992) Salazopyrin (Deleuran et al. 1992)

and coworkers could be that accessory cells were responsible contaminating monocytes were the source.

LGL are CD3-CD56+ cells that exhibit NK activity (Phillips and Lanier 1986). The majority of LGL are also CD16+ cells, which are capable of mediating antibody-dependent cellular cytotoxicity (Ortaldo and Longo 1988). LGL – like monocytes are able to secrete neutrophil stimulating cytokines such as TNFα, IL-1ß, IFNγ and GM-CSF, suggesting that these two leukocyte subsets share some common functions in inflammation. Using the Northern blotting technique we determined that LGL stimulated with PHA in the presence of IL-2 or PMA, PMA alone, or in the combination with ionomycin induced IL-8 mRNA (VI). The induction of IL-8 mRNA was rapid and required only a single stimulus. In addition we established that anti-CD16 mAb induces IL-8 mRNA, which can be augmented by IL-2. This synergy has also previously been shown for the induction of IFNγ, TNFα, GM-CSF and CSF-1 genes in LGL (Cuturi et al. 1989, Anegon et al. 1988). IL-8 mRNA can be induced in LGL to the same extent as in monocytes, but the amounts of secreted IL-8 protein was 5- to 10-fold lower than for monocytes (VI). The LGL secretion of IL-8 is therefore regulated at the posttranslational level. The secreted peptide is fully biologically active (VI). Other monoclonal antibodies such as CD56 or CD2 did not stimulate LGL to IL-8 secretion.

4.4. Properties of IL-8

IL-8 was first purified and described as a neutrophil chemotaxin and as an inducer of the release of lysosomal enzymes from neutrophils (Yoshimura et al. 1987, Schröder et al. 1987). Since then ^{125}I-IL-8 has been shown to bind to a broad spectrum of cells (Matsushima and Oppenheim 1989b) such as neutrophils, monocytes, T lymphocytes, basophils, melanoma cells, and keratinocytes, and numerous investigations have documented its pleiotropic effects. However it seems to be primarily a chemotactic cytokine (Table 6).

In vitro IL-8 stimulates neutrophils to release superoxide anions and lysosomal enzymes in the presence of cytochalasin B. Furthermore IL-8 enhances candida albicans growth inhibitory effect of neutrophils (Djeu et al. 1990). IL-8 has also been shown to be chemotactic to human T lymphocytes (Larsen et al. 1989c) and human basophils (Leonard et al. 1990a), and to stimulate basophils to release leukotrienes (Dahinden et al. 1989). IL-8 increases the adhesiveness of monocytes to endothelial cells through an upregulation of CD11a,b, and CD18 (Brown et al. 1991a).

In vivo IL-8 shows several inflammatory actions (table 6). Intraperitoneal and subcutaneous injections of IL-8 can result in margination and emigration of neutrophils and lymphocytes from endothelial venules in a dose-dependent manner (Larsen et al. 1989c, Kudo et al. 1991). Lower amounts seem to induce a predominant lymphocyte emigration, whereas higher doses induce a predominant neutrophil infiltration, which corresponds with the chemotaxis data *in vitro* (Larsen et al. 1989c). The margination of neutrophils at the injection site can be explained by the rapid increase in expression of adhesion molecules like Mac-1, CD11abc and CD18 as well as the shedding of LECAM caused by IL-8. In rabbits intradermally injected IL-8 will induce a fast neutrophil accumulation of relatively short duration (t 1/2 60 -70 min) in the presence of a vasodilatator substance and this is associated with a plasma protein extravasation dependent on the presence of neutrophils (Colditz et al. 1989, Rampart et al. 1989). The short duration of the response may be explained by the results from a recent study, where erythrocytes were found to be able to bind IL-8 and MCAF in a reversible fashion (Darbonne et al, 1991). IL-8 bound to erythrocytes was incapable of stimulating neutrophils. Thus, erythrocyte absorption of IL-8 may serve to limit stimulation of leukocytes by IL-8 released into blood.

Intravenous injection of IL-8 in rabbits and mice leads to immediate and profound neutrophilia (Van Damme et al. 1988, Furuta et al. 1989). It is not clear whether the neutro-

Table: 6: *Biological effects of IL-8.*

Interleukin 8	
In vitro activities	
Neutrophils:	Chemotaxis (human, rat, mouse, guinea pig, rabbit, chicken, dog, goat, monkey and pig)(Rot 1991)
	Shape change (Thelen et al. 1988)
	↑ Aggregation (Thomsen et al. 1991)
	↑ Degranulation (Djeu et al. 1990, Peveri et al. 1988)
	↑ Respiratory burst (Schröder et al. 1987, Walz et al. 1987)
	↑ Lysosomal enzyme release (myeloperoxidase, elastase, gelatinase and β-glucoronidase) (Thelen et al. 1988, Djeu et al. 1990, Opdenakker et al. 1991)
	↑ Cytosolic free Ca^{2+} (Thelen et al. 1988)
	↓ Adherence to unstimulated endothelial monolayers, fibrinogen and subendothelial matrix proteins (Carveth et al. 1989)
	↑ Adherence to cytokin-activated endothelial monolayers (Gimbrone et al. 1989)
	↑ Binding of complement protein EC3bi and LPS (Detmers et al. 1990)
	↑ Complement receptor type 1 (CR1) (Detmers et al. 1990, Paccaud et al. 1990)
	↑ Granulocyte adhesion molecule (CD11 abc, CD18) (p150,95) Mac-1 (Detmers et al. 1990, Farina et al. 1989)
	Shedding of lectin adhesion molecule-1 (LECAM-1) (Huber et al. 1991)
	↑ Candida albicans growth inhibition (Djeu et al. 1990)
	Activates arichidonate-5-lipoxygenase and release LTB_4 and 5-HETE (in the presence of exogenous AA) (Schröder 1989b)
T lymphocytes:	Chemotaxis (Larsen et al. 1989c, Bacon et al. 1989)
Basophils:	Chemotaxis (Leonard et al. 1990a, Rot et al. 1991b)
	Histamin release (White et al. 1989, Dahinden et al. 1989)
	↑ Leukotriene release (Dahinden et al. 1989)
	↓ Histamin release (Kuna et al. 1991)
Keratinocytes:	↑ Proliferation (Krueger et al. 1990)
Monocytes:	↑ Adhesiveness to endothelial cells (Brown et al. 1991a)
Melanoma cells:	↑ Adhesiveness and haptotactic response (Wang et al. 1990)
Airway smooth muscle:	Contractions (Burrows et al. 1989)
In vivo activities	
	Induction of neutrophil infiltration (rat, mouse, human, dog and rabbit) (Rot 1991, Larsen et al. 1989c, Leonard et al. 1991)
	Induction of lymphocyte infiltration (rat) (Larsen et al. 1989c, Kudo et al. 1991)
	↑ vascular permeability (neutrophil dependent) (rabbit) (Colditz et al. 1989, Rampart et al. 1989)
	Neutrophilia (mouse and rabbit) (Furuta et al. 1989, Rot 1991a)
	Destruction of synovial membrane (rabbit) (Elford and Cooper 1991)
	Accumulation of neutrophils in the joint (rabbit) (Endo et al. 1991)
	Lung pathophysiological changes (rabbit) (Rot 1991a)
	Eosinophilia (guinea pig) (Smith et al. 1991)

philia is due to a release of granulocytes from the bone-marrow reservoir or whether the effect of IL-8 results from its interference with neutrophil adhesion to endothelial cells. IL-8 has not yet been shown to induce any adhesion molecules on lymphocytes and does not induce ELAM-1 and ICAM-1 expression on endothelial cells *in vitro* (Pober, personal communication). But IL-8 seems to play a role in the homing of lymphocytes, as injection of IL-8 causes accelerated emigration of lymphocytes into high endothelial venules of the draining lymph nodes (Larsen et al. 1989c).

In contrast to these pro-inflammatory activities of IL-8 Gimbrone and coworkers purified a leukocyte adherence inhibitory (LAI) activity, which inhibits the adherence of neutrophils to endothelial cells activated with either IL-1 or TNF (Gimbrone et al. 1989). Surprisingly this molecule had the same sequence as IL-8 except for additional five amino acids at the N-terminal end. The molecule can readily be cleaved by thrombin to the potent 72 amino acid form of IL-8 (Hebert et al. 1991). In addition, recent experiments have shown that IL-8 inhibits histamine release from basophils stimulated with histamine-releasing factors, CTAP III and IL-3 (Kuna et al. 1991). IL-8 related peptides seem thus to possess some overall anti-inflammatory capacities as supported by experiments, where intravenous administration of IL-8 inhibited an ensuing local inflammatory response to subsequent subcutaneous injections of IL-8, fMLP, C5a and TNF (Hechman et al. 1990). However, IL-8 primarily acts as an inducer of inflammation and this will be discussed in more detail below.

4.5. The IL-8 gene family

The IL-8 gene family consists of several 8 KDa polypeptide factors which are mapped to q12-21 of the human chromosome number 4 (Modi et al. 1990). These peptides which all have significant homology with IL-8 have been listed in table

Table: 7: *Relationships of cytokine family members.*

Cytokine α Subfamily		Cytokine β Subfamily	
Human Homologue	Murine Homologue	Human Homologue	Murine Homologue
IL 8	?	MCAF/MCP-1	JE
GRO/MGSA	MIP 2 and KC	LD78/pAT464/GOS19	MIP 1α
PF 4	?	Act-2/G-26/pAT744	MIP 1β
PBP/CTAPIII/βTG/NAPII	?	RANTES	?
γIP-10	CRG-2	I-309	TCA-3

IL-8: Interleukin 8 (Yoshimura et al. 1987)
GRO/MGSA: Growth regulated gene product/Melanoma growth stimulating activity (Anisowicz et al. 1987, Richmond et al. 1988)
PF4: Platelet factor 4 (Deuel et al. 1977)
PBP: Platelet basic protein (Holt et al. 1986)
CTAPIII: Connective tissue activating protein III (Castor et al. 1983)
βTG: β thromboglobulin (Begg et al. 1978)
NAP II: Neutrophil activating peptide II (Walz and Baggiollini 1990)
γIP-10: Gamma interferon induced protein (Luster et al. 1985)
9E3: Chicken v-src-inducible protein (Sugano et al. 1987)
MIP-2: Macrophage derived inflammatory protein-2 (Wolpe et al. 1988a)
KC: (Oquendo et al. 1989)
MCAF: Monocyte chemotactic and activating factor (Matsushima et al. 1989a)
LD78: (Obaru et al.1986)
Act-2: (Lipes et al. 1988)
RANTES: Regulated on activation, normal T expressed, secreted (Schall et al. 1988)
I-309: (Miller et al. 1990)
JE: (Cochran et al. 1983)
MIP-1: Macrophage derived inflammatory protein-1 (Sherry et al. 1988)
TCA-3: T cell activation gene (Burd et al. 1987)

7. PBP which is released from α-granules of platelets has been reported to be inactive. However, proteolytic removal of the first nine amino acids of PBP yields CTAP-III reported to stimulate the proliferation and glycosaminoglycan production by synovial cells (Castor et al. 1983). Loss of four more NH_2-terminal amino acids from CTAP-III yields ß-TG, which is a potent chemoattractant for fibroblasts with weak effects on neutrophils (Senior et al. 1983). Proteolytic removal of another 11 amino acids from ß-TG yields a biologically somewhat more potent 70-amino acid long "neutrophil activating peptide II" (NAP-II) (Walz and Baggiolini 1990). The NH_2-terminal processing of PBP seems important since only NAP-II has neutrophil activation *in vitro* (Walz et al. 1989, Brandt et al. 1990). In general NAP-II has a lower specific activity than IL-8 for both *in vitro* and *in vivo* effects on neutrophils.

PF-4 is derived from α-granules of platelets; and is reported to have weak chemoattractant activity for neutrophils and monocytes (Deuel et al. 1981), but more potent for fibroblasts (Senior et al. 1983). A tridecapeptide sequence from the carboxy-terminal end of PF-4 is a stronger neutrophil attractant than PF-4 itself (Osterman et al. 1982).

Treatment of human mononuclear cells, fibroblasts or endothelial cells with IFN-γ results in the production of a protein, γIP-10, which is expressed in delayed hypersensitivity reactions and in psoriatic plaques (Kaplan et al. 1987, Gottlieb et al. 1988). The functions of γIP-10 are as yet not determined.

We have investigated possible interactions between some members of the IL-8 supergene family by performing receptor competition studies. We observed that MIP-2 and MGSA/GRO could compete equally well with IL-8 for IL-8 receptors, while NAP-II did so at a 100 fold lower concentration (Oppenheim et al. 1991). LD 78, MIP-1, ACT-2, PF-4, CTAP-III, MCAF and C13 (PF-4) did not bind to IL-8 receptors. In agreement with this MIP-2 has been shown to be chemokinetic for both human neutrophils and monocytes (Wolpe et al. 1988b) and it induces a neutrophil infiltration after *in vivo* injection (Wolpe et al. 1988a). In addition to its proinflammatory effects MIP-2 enhances the hematopoietic colony forming activity of CAF-1 and GM-CSF (Broxmeyer et al. 1989).

There is yet no evidence of a murine homologue of IL-8. Even though MIP-2 shares some receptor competition with IL-8, it has recently been reported that MIP-2 is not the murine counterpart of IL-8, while the human counterpart of MIP-2 has been cloned (Tekamp-Olsen et al. 1990). Some forms of the human homologue of MIP-2 has been shown to be identical with human gro/melanoma growth stimulating activity (Haskill et al. 1990).

5. MONOCYTE CHEMOTACTIC AND ACTIVATING ACTIVITY

5.1. Introduction

In purifying IL-8, another monocyte chemoattractant activity distinct from IL 8 was detected in the same LPS stimulated PBMC conditioned media (Yoshimura et al. 1987). This other cytokine was purified as a 15 KDa moiety from a LPS-stimulated myelomonocytic cell line THP-1 (Matsushima et

al. 1989a, Furutani et al. 1989) and cloned using a cDNA library from an HL-60 cell line (Furutani et al. 1989). Concomitantly, the same "monocyte chemoattractant peptide-1" (MCP-1) was independently purified and cloned from culture supernatants of PHA-stimulated human PBMC (Yoshimura et al. 1989b, 1989c), fibroblasts (Yoshimura and Leonard 1990b), a human T cell hybridoma clone, D6-18 (Yoshizuka et al. 1989), and a human glioblastoma cell line, U-105MG (Yoshimura et al. 1989b). The factor was shown to specifically stimulate normal human monocytes to chemotaxis and to be growth inhibitory *in vitro* for several human tumor cell lines (Matsushima et al. 1989a). It was therefore called monocyte chemotactic and activating factor (MCAF).

5.2. Purification of MCAF

We purified MCAF from TNF-stimulated 8387 human fibrosarcoma cell line-conditioned media (IV). During the purification, MCAF was eluted at two distinct peaks on carboxymethyl (CM)-HPLC, which were both applied to RP-HPLC for the final purification. Monocyte chemotactic activity, ß-glucosaminidase-releasing activity and monocyte cytostatic augmenting activity coeluted at a discrete absorbance peak in both RP-HPLC eluates. SDS-PAGE analysis showed microheterogeneity in molecular mass at approximate 15 KDa. All bands detected on the SDS-PAGE showed immunological reactivity in Western-blotting experiments using a polyclonal rabbit antibody raised against synthetic MCAF (IV).

5.3. Properties of MCAF

The microheterogeneity, which we observed was also detected by Leonard and coworkers in purifying MCAF (MCP-1). The two fractions were shown to be identical in amino acid composition (Leonard et al. 1990b). Jiang and coworkers have found that the two predominant forms of MCAF (13 KDa and 9 KDa) can be distinguished based on lectin binding. There is a disaccharide galactose-beta 1-3D-N-acetyl galactosamine present on the 13 KDa form, but not on the 9 KDa form (Jiang et al. 1991). The glycosalation has not been shown to change the antigenic properties. Recently it has been shown that larger MCAF-like proteins are derived from a 9000 KDa precursor (Jiang et al. 1990). Post-translational modification involves the addition of O-linked carbohydrates and sialic acid residues. It is the differences in carbohydrate processing that account for the heterogeneity in MCAF (Jiang et al. 1990).

rMCAF has been shown to cause a rapid (< 5 sec) and transient increase of free cytosolic Ca^{2+} ions. The increase is dependent on the influx of extracellular Ca^{2+} through plasma membrane channels. Both the rise in intracellular free Ca^{2+} and the chemotaxis was blocked by Bordettella Pertussis toxin, suggesting involvement of a pertussis toxin sensitive G-protein. It was also found that PKC and PKA inhibitors caused a marked inhibition of rMCAF chemotaxis, suggesting that a serine/threonine kinase, possible PKC, is involved in the signalling by rMCAF (Sozzani et al. 1991). This has also been suggested for IL-8 (Thelen et al. 1988). MCAF and IL-8 belong to two different chemotactic families and have distinct cellular targets, but they share considerable similarities in their intracellular signalling pathways.

5.4. The MCAF gene family

The MCAF cDNA open reading frame codes for a 99 residue protein with the last 76 residues corresponding to MCAF (Furutani et al. 1989). The MCAF subfamily is characterized by having the first two cysteines in an adjacent position, and therefore has been called the "C-C" subfamily (Sager 1990) or the intercrine ß subfamily (Oppenheim et al. 1991), while the IL-8 subfamily has an amino acid between the two cysteines referred to as the "C-X-C" subfamily. The MCAF-subfamily includes human MCAF (Matsushima et al. 1989a), LD78 (Obaru et al. 1986), ACT 2 (Lipes et al. 1988) and RANTES (Schall et al. 1988) (table 7). These genes are located close to one another on the q 11-21 region of the human chromosome 17 (Irving et al. 1990). The genes for MCAF (Schyy et al. 1990), LD78α and β (Irving et al. 1990, Nakao et al. 1990), and ACT 2 (Napolitano et al. 1991) consist of 3 exons and 2 introns. The murine counterparts of the MCAF subfamily that have been identified are known as JE (Cochran et al. 1983, Rollins et al. 1988), TCA 3 (Burd et al. 1987), MIP 1α and MIP 1β (Sherry et al 1988) (table 7). They are clustered on mouse chromosome 11, which is the counterpart of human chromosome 17 (Wilson et al 1990). The *intra*species homology of the MCAF subfamily ranges from 28-45%, while the *inter*species homology ranges from 25-55%. About 20-40% homology is observed between members of the IL-8 and MCAF subsets.

5.5. The receptor-binding site for MCAF

Three-dimensional structural analysis has been performed of the human IL-8 by both nuclear magnetic resonance (Clore et al. 1989, 1990) and X-ray crystallography (Baldwin et al. 1990). At high concentration IL-8 exists as a hydrogen bonded dimer (Clore et al. 1989, 1990). The three-dimensional structural model of MCAF was predicted based on the structure of IL-8 and can be superimposed on the IL-8 structure (Gronenborn and Clore 1991) even though IL-8 and MCAF polypeptides show only 21% homology. The dimer reveals a structural motif with two symmetry-related antiparallel α-helices, lying on top of a six-stranded antiparallel ß-sheet platform, derived with three ß-sheets from each monomer unit (Clore et al. 1990). Due to the availability of the alpha-helices in the three-dimensional structure, it has been suggested that the C-terminal alpha-helices may determine the receptor binding specificity of these molecules. We therefore predicted that chimeric molecules consisting of the N-terminal 54 amino acids of IL-8 and the C-terminal 20 amino acids of MCAF should show MCAF activity. Unexpectedly, the purified recombinant IL 8-MCAF chimeric molecules, which were expressed in *E. coli,* showed potent neutrophil chemotactic activity and IL-8 receptor binding competing activity, but no significant monocyte chemoattractant activities (Oppenheim et al. 1991). Furthermore,

truncated variants of MCAFs (D-N 10 with truncation of N-terminal 10 amino acids of mature MCAF; D-C 8 and 17, with truncation of C-terminal 8 and 17 amino acids of mature MCAF) still showed significant monocyte chemotactic activity (Oppenheim et al. 1991). In contrast, truncated MCAF (D-C-20, with truncation of C-terminal 20 amino acids of mature MCAF) lost monocyte chemotactic activity. Studies with a synthetic MCAF (13-35) showed monocyte chemotactic activity and competition with native MCAF for binding sites (Valente et al. 1991). Taken collectively, these data suggest that the receptor binding sites of IL 8 and MCAF appear to be located between the four cysteines (residues 7-50), while the C-terminal alpha-helix region may be involved in stabilizing the molecules. It has further been determined that no MCAF-binding can be observed with either collagen, elastin-derived peptides or fibronectin (Valente et al. 1991).

5.6. The MCAF receptor

Using ^{125}I-labelled MCAF purified from a human myelomonocytic cell line, THP-1, we showed that MCAF binds to human PBMC at 37°C, but not at 4°C (IV). IL-8 at concentrations from 0.1 ng/ml to 10 μg/ml failed to inhibit the binding of ^{125}I-labeled MCAF PBMC's (IV). The binding of ^{125}I-MCAF starts rapidly with a maximum after 15 min. We further determined that the bound molecules were immediately internalized (IV). The receptors for MCAF (MCP-1) on human monocytes, which are distinct from the receptors for IL 8 (IV), have recently been characterized. The number of receptors for MCAF on human peripheral blood monocytes has been estimated to be 1,600 high affinity binding sites per cell with a Kd of 1.1 to 1.9 x 10^{-9}M (Yoshimura et al 1990a, Valente et al. 1991). No binding sites have been detected on neither lymphocytes nor polymorphonuclear leukocytes. Using ^{125}I-labelled recombinant MCAF Wang and coworkers estimated that peripheral blood monocytes and a human monocytic cell line THP-1 have 13,000 to 18,000 receptor-sites with Kd values of 25.5 nM and 25.7 nM respectively (Wang et al. 1991). The differences in the estimate might be explained by differences between recombinant material and human purified MCAF. As seen with IL-8, MCAF downregulates its own receptor expression (Samanta et al. 1990, Wang et al. 1991). Chemical cross linking of the MCAF ligand-receptor complex suggested a molecular mass of 40 KDa for the MCAF receptor (Wang et al. 1991), which is different from the 65,000 determined for the IL-8 receptor.

5.7. Cell sources of MCAF

Although the identification of cell sources is far from complete it has been established that MCAF is produced by a broad spectrum of nucleated cell types (Table 8). Among these are cells, which are thought to be of importance for the inflammatory reactions in the skin, the formation of atherosclerosis, and for host reactions towards tumors. Leukocytes as well as non-leukocytic cells express the gene for this cytokine and MCAF is produced in response to a wide variety of exogenous and or endogenous stimuli (Table 9). The fact that MCAF is a product of the "cytokine cascade" is illustrated by data showing that cytokines and growth factors such as IL-1, TNFα, IFNγ and PDGF are potent inducers of the cytokine. Conversely a suppressive agent such as dexamethasone downregulates IFNγ, IL-1, TNF and also the production of MCAF (Mukaida et al. 1991). The regulation of MCAF thus resembles that of other cytokines except that IL-4 inhibits the induction of IL-8 mRNA in stimulated human monocytes (Standiford et al. 1990), and stimulates MCAF mRNA expression in human endothelial cells (Rollins and Pober 1991b). This suggest that MCAF produced by endothelial cells may mediate some of the effects of IL-4 on monocyte physiology *in vivo*.

Table: 8: *Sources of MCAF.*

MCAF	Peripheral blood mononuclear cells (Yoshimura et al. 1989a, Matsushima et al 1989) B-lymphocytes (Strieter et al. 1990) Fibroblasts (Larsen et al.1989, Strieter et al. 1989a, Van Damme et al. 1989b) Vascular endothelial cells (Strieter et al. 1989a, Sica et al. 1990b) Keratinocytes (Barker et al. 1991) Melanocytes (V) Baboon smooth muscle cells (Graves et al. 1989) Myelomonocytic THP-1 cell line (Matsushima et al. 1989a), Osteosarcoma-, glioblastoma-, rhabdomyosarcoma-cell line (Graves et al. 1989, Yoshimura et al. 1989b, Van Damme et al. 1989b), fibrosarcoma cell line 8387 (IV), monocytic cell line HL 60 (Furutani et al. 1989). T cell hybridoma clone D6-18 (Yoshizuka et al. 1989)

Table: 9: *Inducers and suppressors of MCAF.*

A. *Upregulation:* The production of the cytokine can be induced by the following diverse agents:

MCAF	IL-1 (IV, Larsen et al. 1989a) TNF (IV, Larsen et al. 1989a) PDGF (Yoshimura et. 1990b) GM-CSF (Colotta et al. 1991) ECGF (Dixit et al. 1990) IFNγ (Barker et al. 1991) PHA, Con A (Yoshimura et al. 1989b) LPS (Matsushima et al. 1989a, Strieter et al. 1990) PSK (Hirose et al. 1990) TPA (Shyy et al. 1990) Hydroxyurea (Matsushima et al. 1989a) Silica (Matsushima et al. 1989a) Minimally modified low density lipoprotein (Cushing et al. 1990) Double-stranded RNA poly(rI):poly(rC) (Van Damme et al. 1989b) Virus (Van Damme et al. 1989b)

B. *Downregulation:* The production of the cytokine is suppressed by the following agents:

MCAF	Glucocorticoids (Mukaida et al. 1991)

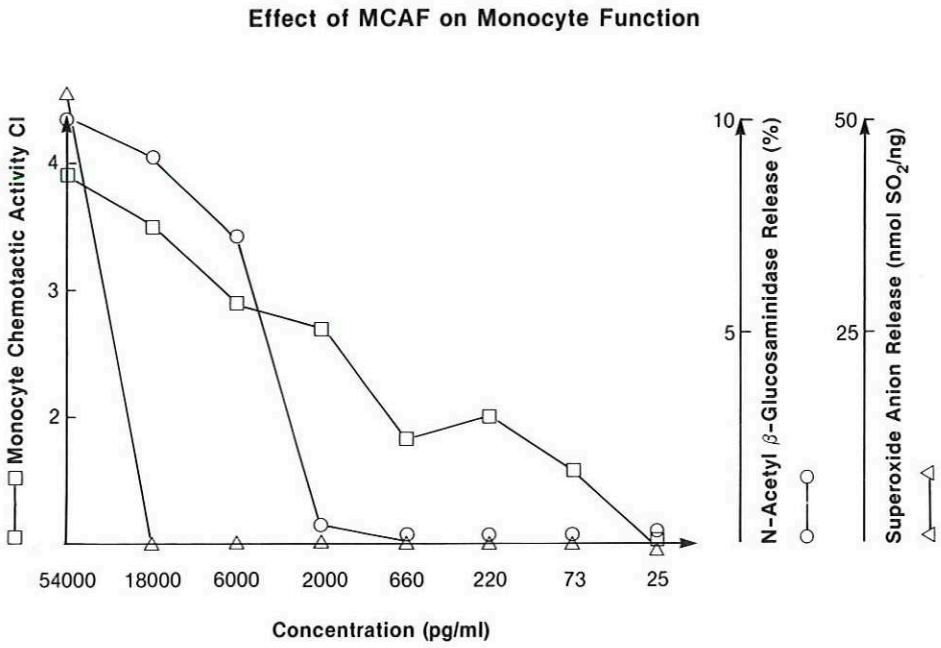

Fig. 1. Dose response curves for the monocyte chemotactic activity, N-acetyl β-Glucosaminidase release and superoxide anion release of MCAF. The data shown are representative of two independent experiments.

5.8. Biological activities of MCAF

To establish whether MCAF possesses other activities apart from being chemotactic for monocytes, we assayed the MCAF, purified from fibrosarcoma cell line 8387, in different enzyme release systems. We showed that MCAF stimulated superoxide anion and N-acetyl ß-glucosaminidase-releasing activity in monocytes (IV). As seen in fig. 1 the half maximal monocyte chemotactic response occurred at doses of ~ 1 ng/ml, while the N-acetyl glucosaminidase release showed an EC_{50} of ~ 3-5 ng/ml. In addition, although not directly inhibitory to tumor cell growth, MCAF over the course of several days activated cultured human monocytes to be cytostatic for several types of tumor cell lines (e.g., A 375 melanoma cells, HT 29 colon carcinoma cells, HTB82 rhabdomyosarcoma and MCF7 mammary carcinoma cells) (IV, Matsushima et al. 1989a).

To evaluate the *in vivo* effects of MCAF we injected purified human MCAF subcutaneously into the ears of Lewis rats at various times and concentrations. MCAF showed a significant selective monocytic infiltration beginning at 3 hours and being maximal at 18 hours without increasing neutrophil or lymphocyte infiltration (fig. 2) (IV). This corresponds with the observation by Dunn and coworkers, who found that intradermal slow release of IL-1 resulted in a late accumulation of activated monocytes (Dunn et al. 1988). Furthermore, intracutaneous injection of IL-1α and TNFα cause lymphocyte and neutrophil emigration at 3 hours and monocyte emigration later (Larsen et al. 1989b). The murine homologue of MCAF, termed JE, has also been shown to be a specific chemoattractant for monocytes (Yoshimura et al. 1989c, Cochran et al. 1983, Van Damme et al. 1991a). This has further been substantiated by transplanting MCAF-expressing cells into nude mice and demonstrating that this leads to a predominantly monocytic infiltrate at the site of injection (Rollins and Sunday 1991c). Recently it has been shown that MCAF is a potent histamine-releasing factor for human basophils (Kuna et al. 1992, Bischoff et al. 1992), which in atopics had a similar capacity to induce histamine release as anti-IgE and fMLP. The known biological activities of MCAF have been summarized in table 10.

5.9. The potential role of MCAF in tumor growth

A variety of murine and human tumor cell lines including our fibrosarcoma cell line 8387 and a baboon aortic medial smooth muscle cell line produce monocyte chemotactic activity *in vitro* (IV, Valente et al. 1988, Benomar et al 1987). The biological relevance hereof is indicated through the macrophage content of some tumors *in vivo* (Bottazzi et al. 1983, 1985). The large number of tumors infiltrated with

Table: 10: *Biological effects of MCAF.*

MCAF	
In vitro activities	
Monocytes:	Chemotaxis (human, guinea pig) (IV, Yoshizuka et al. 1989)
	↑ Superoxide anion release (IV)
	↑ N-Acetyl β-Glucoronaminidase release (IV)
	↑ Cytostatic augmenting activity (IV, Matsushima et al. 1989a)
	↑ Cytosolic free Ca^{2+} (Bischoff et al. 1992)
Basophils:	Histamine release (Kuna et al. 1992, Bischoff et al. 1992)
	↑ Cytosolic free Ca^{2+} (Bischoff et al. 1992)
	↑ Leukotriene release (Bischoff et al. 1992)
In vivo activities	
Monocytes:	Infiltration (rat) (IV)

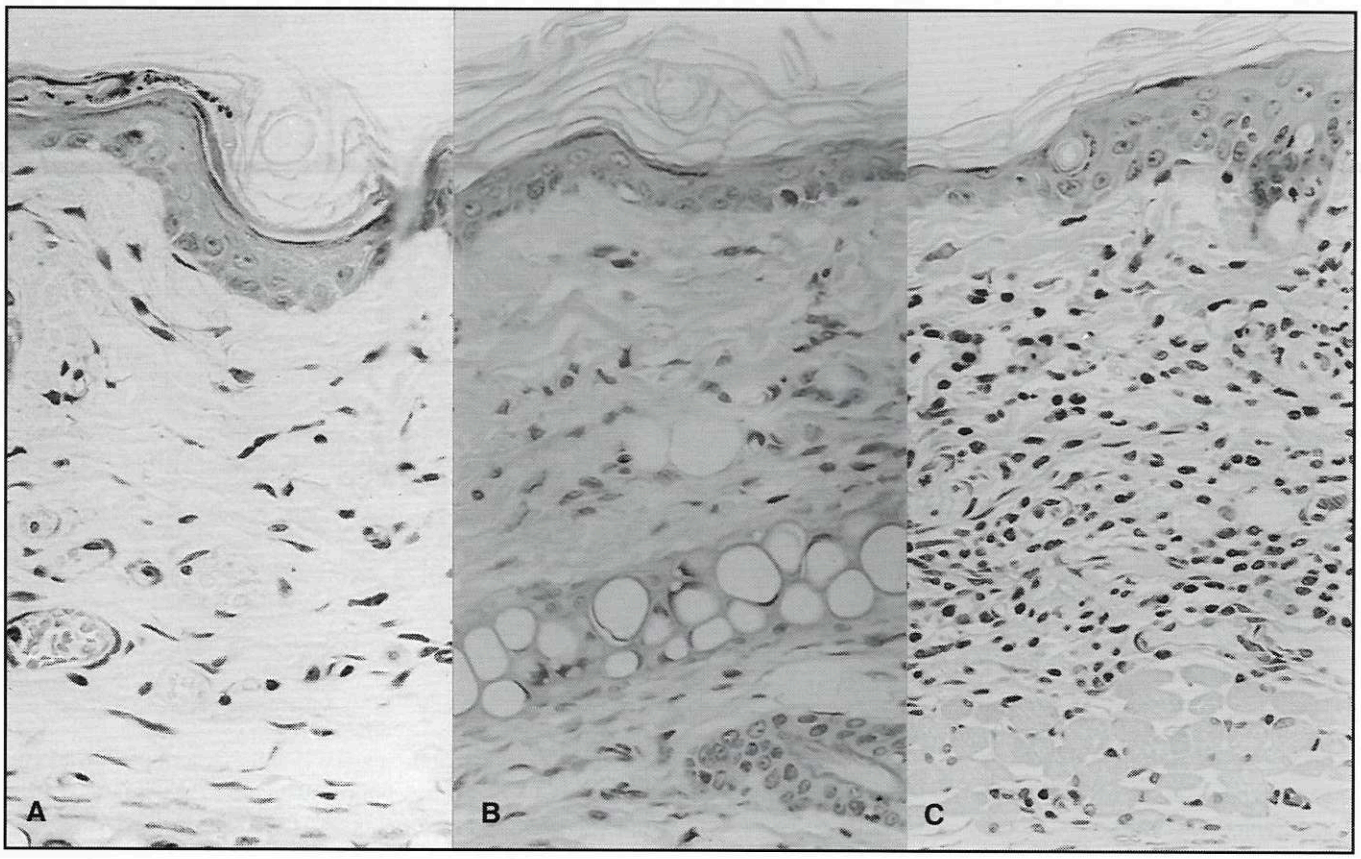

Fig. 2. The histological appearance (400x) of rat ear dermis 18 hours after injection of (A) PBS 20 μl and (C) MCAF 20 μl, 5.3 ng/ml. (B) represents the negative control. PBS inoculation (A) resulted in variable degrees of margination and infiltration of neutrophils, lymphocytes and monocytes as compared with control (B), where only a few recidental macrophages and no neutrophils or lymphocytes were seen. MCAF inoculation (C) produced a marked infiltration with monocytes/macrophages and few, if any, neutrophils or lymphocytes.

macrophages could suggest that tumor suppressive effects might be exerted through the release of "macrophage activating factors" such as MCAF, IFNß, TNF, or IL-1. Tumors infiltrated with macrophages *in vivo* are associated with a better prognosis (Wang et al. 1986).

After transfer of the MCAF gene into a murine melanoma cell line a marked increase in the percentage of tumor-associated macrophages was demonstrated and the tumor cells exhibited a slower growth rate *in vivo* with a prolonged survival time (Bottazzi et al. 1991). Rollins and coworkers found that CHO cells with an expressed MCAF gene loose their ability to form tumors in nude mice (Rollins et al. 1991c). Tumor-associated leukocytes *in vivo* are often ineffective of killing host tumors *in vitro*. This raises the possibility that interactions between tumor and leukocytes can somehow also be beneficial for the tumor. It is of interest that an inhibitor of monocyte chemotaxis has been identified and is coproduced with MCAF (Wang et al. 1986). This suggests a balance between chemotactic and inhibitory cytokines in the extravasation of monocytes into tumors, where IL-1 and TNF produced locally could be of importance for a cytokine cascade initiation.

In conclusion MCAF may contribute to tumor growth control by recruiting activated macrophages into the tumor site (IV). Further, MCAF can activate macrophage cytostatic activity, induce enzyme release and O_2^- release and induce the respiratory burst (IV, Rollins et al. 1991a). IL-6, IL-1 and TNFα can not be responsible for the cytostatic augmenting activity seen in our experiments, since MCAF does not induce mRNA for these cytokines in monocytes *in vitro*, and antibodies towards IL-1α, IL-1ß, TNF and IL-6 do not block the cytostatic effect of MCAF (Matsushima et al. 1989). In addition to the classical macrophage activators, endotoxin and IFNγ, various other cytokines than MCAF have been shown to induce or augment cytotoxic function of mononuclear phagocytes, including TNFα, IL-1 and M-CSF (Sampson-Johannes and Carlino 1988, Onozaki et al. 1985, Mace et al. 1988). Since these cytokines are produced by activated macrophages, they may be involved in an autocrine circle with enhancement of the macrophage activation processes.

Macrophage cytotoxicity involves a close relationship with the target tumor cells. LFA-1 antibodies (CD18/CD11a) inhibit human monocyte cytotoxicity (Peri et al. 1990). The tu-

mor associated macrophages are located in close relation to tumor cells and may in the future be targets for therapeutic interventions. Such possibilities include production of tumor-infiltrating lymphocytes expressing MCAF, which then might provide synergistic local tumor killing. Systemically infused MCAF would not permit formation of a chemotactic gradient to attract monocytes to residual tumor deposits, but might lead to a generalized increase in the activation state of an individuals monocytes. There has been some evidence of reduced monocyte function in patients with malignancies (Kleinerman et al. 1980). If the defect is based on a cytokine defect rather than a monocyte mediated defect, infusion of MCAF may correct the abnormality.

5.10. MCAF and immunological reactions

During a cell-mediated immune reaction monocyte accumulation can usually be seen after 24-48 hours. This is thought to be due to a local production of a monocyte chemoattractant after interaction between antigen and sensitized lymphocytes as shown when mononuclear cells from tuberculin positive persons were stimulated with PPD (Snyderman at al. 1972). Already in 1970 Ward and co-workers provided experimental evidence for the existence of specific lymphocyte-derived monocyte chemotactic factors (Ward et al. 1970). The factor was partially purified from supernatants of PHA-stimulated lymphocytes and shown to be a protein with a molecular mass of about 12.5 KDa (Altman et al. 1973). They also observed that the production of the monocyte chemotactic factor correlated well with *in vivo* delayed hypersensitivity. It is possible that the factor partially purified by Altman and coworkers in 1973 is identical with MCAF.

Soluble products of monocytes, including IL-1ß, TNFα and transforming growth factor-ß, affect resident cutaneous cell types such as epidermal keratinocytes, dermal fibroblasts, and vascular endothelium. IFNγ from activated T lymphocytes also seems important for MCAF production through stimulation of keratinocytes (Barker et al. 1991). IFNγ induces keratinocyte ICAM-1 expression allowing monocytes and T-lymphocytes to adhere via LFA-1 molecules to keratinocytes (Dustin et al. 1988a, Griffith et al. 1989) and inhibits monocyte random migration (Thurman et al. 1985).

5.11. MCAF and atherosclerosis

In atherosclerosis characteristic foam cells can be seen in the early lesions. These cells are thought to be macrophages and may have importance in the pathogenesis of the disease through their phagocytosis of lipids leading to the characteristic morphological changes such as fatty streak formation, endothelial cell damage, and exposure of the basement membrane. Cellular components of the arterial wall, endothelial cells, fibroblast and smooth muscle cells can secrete MCAF upon stimulation with oxidized low density lipoprotein (LDL) (Cushing et al. 1990). In addition MCAF can be produced by fibroblasts upon activation by PDGF released from platelets as a response to vascular damage (Yoshimura and Leonard 1990b). This could contribute to monocyte infiltration of the arterial wall (Valente et al. 1988, Sica et al. 1990b, Yoshimura et al. 1990b) observed in experimental atherosclerosis (Gerrity 1981). MCAF mRNA has been detected in atherosclerotic lesions by in situ hybridization (Ylä-Herttuala et al. 1991, Nelken et al. 1991), especially in macrophage rich regions of human and rabbit atherosclerotic lesions. These macrophages secrete a broad range of neutral hydrolases, which act against arterial matrix and blood coagulation proteins and produce potential toxic reactive oxygen radicals as well as growth factors, which may mediate intimal hyperplasia.

The induction of MCAF by TNF may also help explain an apparent paradox in the biological activity of TNF. While TNF is an inhibitor of endothelial proliferation *in vitro*, it is *in vivo* a potent inducer of angiogenesis (Frater-Schröder et al. 1987). Macrophages are able to induce angiogenesis (Polverini et al. 1977) and endothelial proliferation (Ooi et al. 1983), and it is possible that the *in vivo* angiogenic activity of TNF is mediated via MCAF-mediated monocyte accumulation.

6. INDUCTION OF IL-8 AND MCAF IN NORMAL HUMAN MELANOCYTES AND MELANOMA CELLS

6.1. Introduction

The predominant cell in the epidermis is the keratinocyte, which in recent years has been suggested to be an important immunological cell due to its immunoregulatory capacities. Keratinocytes produce and secrete cytokines such as IL-1α (Oxholm et al. 1988), IL-1ß (Kupper et al. 1986), IL-6 (Kirnbauer et al. 1989), IL-8 (Larsen et al. 1989b), TNFα (Oxholm et al. 1988), TGFα (Coffey et al. 1987), TGFß (Fisher et al. 1988), colony-stimulating factors (Kupper et al. 1987), interferons (Schnipper et al. 1984), and growth factors such as GM-CSF (Langdon et al. 1988). So far melanocytes had not been studied for a role in the epidermal cytokine cascade. We therefore wanted to investigate the possible ability of melanocytes to produce the chemotactic cytokines IL-8 and MCAF.

6.2. Production of IL-8 and MCAF by normal human melanocytes

We studied the ability of cultured normal human melanocytes to produce IL-8 and MCAF in response to rIL-1α (100 ng/ml), rTNFα (100 ng/ml), rIL-6 (100 U/ml), rIFNγ (10 ng/ml) or LPS (10 μg/ml) (V) using the Northern blotting technique (Lew et al. 1988). Dose-response experiments with rIL-1α (0 to 50 ng/ml) and rTNFα (0 to 100 ng/ml) showed that only these cytokines could induce IL-8 and MCAF in a dose-dependent fashion with rTNFα inducing the strongest signal for MCAF mRNA. As little as 0.1 ng/ml of rTNFα or 0.5 ng/ml of rIL-1α could induce significant amounts of mRNA for both chemotactic cytokines. Time course studies

showed that the induction happened within the first hour. IL-8 mRNA peaked around 2 hours and MCAF mRNA peaked around 4 hours (V).

In order to determine whether the induced mRNA for IL-8 was translated and secreted, we measured supernatants from overnight stimulated melanocyte cultures for IL-8 protein by a radioimmunoassay. As expected we could only detect IL-8 protein after rTNFα- and rIL-1α-stimulation (11.5 ng/ml and 4.1 ng/ml), whereas rIFNγ, rIL-6 or LPS did not increase the amount of IL-8 above background level. The IL-8 secretion corresponded in both a dose-dependent and a time-dependent manner to the increase in mRNA for IL-8. The amount of secreted IL-8 protein was comparable to the amount seen for keratinocytes, fibroblasts and LGL, but approximately 10-20 times less than the amount seen for endothelial cells and monocytes (VI, Kristensen et al. 1991).

The supernatants from stimulated melanocyte cultures were applied to a heparin-Sepharose column and IL-8 and MCAF were eluted and measured in a microwell chemotaxis assay. Both rTNFα- and rIL-1α-stimulated cultures generated significant neutrophil and monocyte chemotactic activity (V). Activity was detected in the time-dependent experiments at approximately 2 hrs.

Keratinocytes can not respond with IL-8 production upon stimulation with TNFα alone and only to a very small extent with MCAF production (Barker et al. 1990). This and the greater sensitivity of melanocytes found in our study indicate an importance of melanocytes for dermal inflammation. The location of melanocytes close to the basal membrane and their multiple dendritic elements may enable them to function as important effector cells in the early phases of the inflammatory process.

It has recently been shown that melanocytes increase their ICAM-1 expression upon stimulation with either IL-1α, TNFα or IFNγ (Yohn et al. 1990). In these investigations melanocytes were more sensitive to IFNγ than keratinocytes in their expression of ICAM-1. IL-1 did not induce ICAM-1 on keratinocytes, whereas melanocytes were able to respond to IL-1α. It has recently been shown that TNFα and IFNγ given in combination could increase the expression of mRNA for IL-8 and MCAF as well as mRNA for ICAM-1 in keratinocyte cultures (Barker et al. 1990). We repeated these experiments with melanocytes. 1 ng/ml of rTNFα stimulated melanocyte cultures to produce 2.6 ng/ml of IL-8, while 10 ng/ml of IFNγ was unable to do so. When given in combination the culture supernatants contained 5.1 ng/ml IL-8 suggesting a synergistic effect. This has also been shown for the production of fibronectin by melanocytes and melanoma cells (Varani et al. 1989). It will increase the possibility for circulating inflammatory cells to enter into the perivascular dermis, and further on towards the epidermis, provided they meet the proper chemotactic gradient. Melanocytes seem to be an active participants in these cascade-reactions. They also contain an IL-1ß convertase that can act on the keratinocyte-produced 33-KDa IL-1ß precursor to generate an active 17-KDa IL-1ß product, which then may act on melanocytes to produce chemotactic cytokines (Mizutani et al. 1990).

6.3. Expression of IL-8 mRNA – but not MCAF in melanoma cell lines

A member of the IL-8 gene family, MGSA/GRO, was previously isolated as a melanoma growth stimulating factor (Richmond et al. 1983, Richmond et al. 1988). This molecule exhibits structural similarities with IL-8 and has a 44% sequence homology with IL-8 protein (Matsushima and Oppenheim 1989b). MGSA stimulates the expression of MGSA mRNA in human melanoma cell lines (Richmond et al. 1988) and may therefore function as an autocrine growth factor for melanoma cells. As described earlier we have shown that MGSA and IL-8 competes equally well for the same receptor (Oppenheim et al. 1991). We also showed that MGSA possesses similar neutrophil chemotactic activity as IL-8 (Derynck et al. 1990). In addition we have in collaboration with Thomas Schwartz showed that IL-8 can be produced by and stimulate the growth of human melanoma cell lines (Förster et al. 1991).

An interesting feature of human melanomas is the predominance of a surrounding lymphocytic infiltrate, while monocytes or macrophages are practically never seen in the surrounding tissue (Clark et al. 1979, Lever and Schaumburg-Lever 1990). It was therefore important to show whether melanoma cells can produce IL-8 and MCAF.

Three different melanoma cell lines (A375 C6-14E, SK-MEL-2 and FMX-Met) were grown to confluency and stimulated with either TNFα or IL-1α in a dose- and time-dependent manner. Both IL-1α and TNFα very rapidly (30 min) induced IL-8 mRNA in the cell line A375 C6-14E with doses of IL-1α as low as 10 U/ml, and of TNFα down to 100 U/ml. Neither IL-1α or TNFα could induce mRNA for MCAF at any doses tested. Similar results were seen for all cell lines. Culture supernatants were collected for the three different cell lines stimulated with optimal doses of IL-1α, TNFα and LPS. The A375 C6-14E could be stimulated to give a 70-100 times increase in the IL-8 production, except after LPS stimulation, where production only increased two-fold. The other two cell lines produced less IL-8.

6.4. The correlation between MCAF and IL-8 in melanomas

These results have been confirmed by other groups using in situ hybridization (Reinhard Gillitzer personal communication). It made us speculate whether MCAF and IL-8 have an important role in the development of metastatic melanomas. Cancer cells need to undergo several changes to become metastatic. Some of the changes involve alterations in the methylation pattern of the DNA, which then causes genes to be turned of or on. This can then make cells produce growth factors like platelet derived growth factor (PDGF). It has recently been shown that IL-8 can induce migration of melanoma cells towards substrate-bound molecules (haptotactic migration) (Wang et al. 1990) and thereby increase motility of melanomas when they are in contact with IL-8. Additional evidence for the importance of cytokines in the development and spreading of melanomas is that *in vivo* inoculation of IL-1 augments metastasis of hu-

man melanoma cells in nude mice (Wang et al. 1990). It has further been shown that there is an augmented spreading of cancer at sites of inflammation (Murphy et al. 1988). This secondary enhancement of implantation could be contributed by locally produced IL-8 from melanoma cells, melanocytes, fibroblasts, keratinocytes and endothelial cells, when stimulated with IL-1. We have shown the inability of melanoma cell lines to produce MCAF. As discussed in chapter 5.9 there is a positive correlation between monocyte chemotactic activity of culture supernatants from solid human tumors and the percentage of tumor-associated macrophages. In conclusion the inability of melanoma cells to produce MCAF may account for a reduced number of infiltrating macrophages and thereby for reduced tumor growth control. In addition, production of IL-8 by the melanoma cells could act both by promoting tumor growth and melanoma cell motility.

7. INTERLEUKIN 8 AND DISEASE

7.1. Introduction

Because IL-8 is a specific chemotactic agent for neutrophils and T lymphocytes, but not for monocytes, it may play an important role in inflammatory conditions, where the cellular infiltrate is predominantly neutrophil-rich. This includes gout, rheumatoid arthritis, adult respiratory distress syndrome (ARDS), emphysema, glomerular nephritis, myocardial infarction, inflammatory bowel disease, and asthma. The deposition in the tissue of microcrystals of monosodium urate monohydrate (Levinson 1989), calcium pyrophosphate dihydrate (Doherty et al. 1988), and hydroxyapatite (Halverson and McCarty 1989) may be sequelae in articular inflammation. An occupational inhalation of microcrystals of silicon dioxide induces pulmonary inflammation and interstitial fibrosis (Adamson and Bowden 1984). Some common features of these conditions are physical ingestion of particles by the leukocytes and local neutrophil ingress and activation, which may promote tissue injury (Terkeltaub et al. 1989).

7.2. IL-8 is a possible mediator of crystal-induced inflammation

In acute gout the synovitis has both clinically and experimentally been shown to be dependent on neutrophil influx (Terkeltaub et al. 1989). Additionally the disease within hours responds to colchicine or corticosteroids, which have a broad suppressive action on neutrophil function (Famaey 1988) including an inhibition of the neutrophil stimulatory actions of IL-8 (Djeu et al. 1990). The neutrophils infiltrate the synovium and are seen in the synovial fluid, where they ingest urate crystals. The interaction between crystals and neutrophils result in a release of lysosomal proteases (Kozin et al. 1979), oxygen radicals (Abramson et al. 1982), and at least two distinct chemotactic factors, leukotriene B_4 (Serhan et la. 1984) and a low molecular weight polypeptide termed crystal chemotactic factor (Spilberg and Mandell 1982).

We investigated the effects on neutrophils from urate crystal – stimulated mononuclear phagocytes. This was done by stimulating normal human monocytes with 0.5 mg/ml urate crystals or medium alone. After collection at different time points we tested the conditioned media and found a neutrophil chemotactic activity. The release of chemotactic activity was dose-dependent. If the urate crystals were coated with low density lipoprotein 80% of the neutrophil chemotactic activity could be abolished. This procedure has been shown to inhibit the activation of cell membranes by the urate crystals (Terkeltaub et al. 1984). By pretreating the monocytes with cycloheximide we observed that the neutrophil chemotactic activity worked by a newly synthesized polypeptide.

Interleukin-8 and MGSA are heparin-binding polypeptides, we therefore fractionated the conditioned media by heparin-agarose chromatography and observed that a crystal-induced, heparin-binding polypeptide chemotactic factor(s) was responsible for a substantial proportion of urate-crystal induced, monocyte derived neutrophil chemotactic activity (VII). By radioimmunoassay the IL-8 amount increased over time while IL-1ß reached a plateau after 4 hours. Neutrophil chemotactic activity in supernatant from urate crystal stimulated monocytes could be inhibited by 89%, while activity from supernatant of either LPS- or Silica-stimulated monocytes were totally blocked. Similar results were obtained when the culture supernatants were tested for the release of the azurophil granule constituent α-mannosidase from neutrophils (VII). Our observations supported the role of IL-8 as a major functional neutrophil-activating factor released by monocytes that have been exposed to urate and silica crystals.

To evaluate the clinical significance of our observation we quantitated IL-8 in a group of synovial fluid samples from knee joints with acute gouty arthritis or from knee joint of patients with osteoarthritis in which no neutrophil inflammation was seen. We consistently found elevated levels of IL-8 (mean 8.4 ng/ml) in all samples of acute gouty arthritis, while only limited amounts were found in samples from patients with osteoarthritis (mean 1.5 ng/ml) ($P = 0.006$) (VII).

It is unlikely that the mononuclear phagocytes are the only cells in the synovial joints, which are responsible for the secreted IL-8. As described in table 4 other cell types such as synovial fibroblasts and vascular endothelial cells can upon stimulation with variable stimuli including IL-1α and TNFα produce IL-8. Additionally, urate crystal-stimulated monocytes and synovial lining cells release IL-1 (VII) and TNFα (Guerne et al. 1989).

As described earlier IL-8 can stimulate neutrophil 5-lipoxygenase activity (Schröder 1989b) and upregulate the expression of different neutrophil adhesion molecules (Detmers et al. 1990, Farina et al. 1989). Therefore IL-8 may promote the amplification of neutrophil ingress and activation in gout and contribute to the activation of neutrophils in synovia not in direct contact with urate crystals.

A chemotactic factor has previously been found following phagocytosis by neutrophils of urate crystals. This factor, termed crystal chemotactic factor (CCF), is of low molecular weight (Spilberg and Mandell 1982, Bhatt and Spilberg 1988) and the amino acid composition differs from IL-8. CCF is not rich in basic amino acids and totally lacks the cysteines, which are important for the structure of the IL-8 family.

Table: 11: *Diseases where IL-8 has been detected.*

Rheumatoid Arthritis:	Synovial Fluid (Seitz et al. 1991a, Brennan et al. 1990)
Gout:	(VII)
Psoriasis:	(Nickoloff et al. 1991, Sticherling et al. 1991, Schröder et al. 1992, Schröder and Christophers 1986)
Cutaneous T cell lymphoma:	(McLean et al. 1990)
Septic shock, endotoxemia:	(Van Zee et al. 1991, Martich et al. 1991)
Malaria:	(Friedland et al. 1991)
Jarisch-Herxheimer reaction:	(Negussie et al. 1992)
Acute bladder inflammation:	(Bettex-Galland et al. 1991)
Red blood cell incompatibility:	(Davenport et al. 1990)

The prominent leukocytosis seen in acute gout could be explained by release of IL-8 from inflamed joints and from IL-1α, TNFα, or IL-6 stimulated hepatocytes. It has been established that IL-6 can be produced by urate crystal stimulated synoviocytes and monocytes. IL-6 can stimulate acute-phase protein synthesis and immunoglobulin production, which are seen in acute gout (Guerne et al. 1989). Systemic TNFα can induce fever, acute phase responses, and metabolic and hemodynamic changes (Di Giovine et al. 1991).

7.3. IL-8 in other inflammatory disorders

Table 11 summarizes diseases, where IL-8 has been shown to play a potential role. Another inflammatory arthritic disease, rheumatoid arthritis, is also characterized by infiltration of polymorphonuclear granulocytes as well as lymphocytes in the synovium. The infiltrating cells are thought to contribute to the pathology of the disease through the release of degradative enzymes, prostaglandins, reactive oxygen species and proinflammatory cytokines such as IL-1 (Fontana et al. 1982) and TNFα (Di Giovine et al. 1991). We have shown that rheumatoid arthritis synovial cells spontaneously release IL-8 (Brennan et al. 1990). We further detected IL-8 in the synovial fluid from joints of rheumatoid arthritis patients (Brennan et al. 1990). The spontaneous release can be increased by stimulation with LPS, rheumatoid factor-containing complexes, zymosan, TNFα and IL-1 (Seitz et al. 1991). Lindley and coworkers detected similar amount of IL-8 in synovial fluid from RA patients and found a significant correlation between the IL-8 content, serum C-reactive protein and the number of cells in the joint (Lindley et al. 1991). In our studies we were unable to find a similar correlation between cell count and synovial fluid IL-8 content. Following injection of IL-8 into the knee joint space of rabbits has there been seen a rapid infiltration of neutrophils into the joint space and synovial membrane (Endo et al. 1991). To further support the importance of IL-8 in arthritis it has been shown that IL-8 can induce neutrophil-mediated cartilage damage (Elford and Cooper 1991). Human chondrocytes produce IL-8 upon stimulation with IL-1ß, TNFα, LPS, TGFß and chondrocyte growth factor (Lotz et al. 1992). IL-8 may therefore help to initiate and modulate inflammation in traumatic cartilage injury and in some of the inflammatory flares that can complicate osteoarthritis, furthermore neutrophils attracted to the synovial cavity and to the articular surface may release collagenase and initiate cartilage destruction (Jasin and Taurog 1991).

Rot has shown that i.v. injection of high doses of IL-8 into rabbits produced a strong blood neutrophilia, neutrophilia sequestration in the lungs and development of a lung pathology which very closely resembles human ARDS and its complications, thus suggesting the involvement of IL-8 in the pathomechanism of this disease (Rot 1991c).

As earlier described leukocyte infiltration is a predominant phenomenon in a long list of dermatological disorders so it is reasonable to speculate whether IL-8 can be an important mediator in dermatological diseases. IL-8 and MGSA/GRO have been purified in scales from psoriasis patients (Schröder et al. 1992). Using immunohistochemical localization Nickoloff and coworkers detected IL-8 expression in the suprabasal keratinocytes immediately above TNFα-positive dermal dendrocytes. mRNA for IL-8 could be detected in epidermis from psoriatic plaques in some patients, while no mRNA could be detected in normal skin (Nickoloff et al 1991). Thus IL-8 could be an important mediator of the inflammatory response in psoriasis. It was the only biological active cytokine, which could be detected in greater amounts in lesional skin (Camp et al. 1991, Paludan et al. 1992).

There has been one brief rapport suggesting a role of IL-8 in cutaneous T cell lymphoma as IL-8 could be detected in the skin by immunohistochemical staining although no mRNA for IL-8 was found (McLean et al. 1990). We observed increased T lymphocyte chemotactic activity in epidermal homogenates from patients with cutaneous T cell lymphoma (Zachariae et al. 1991a). Suction blister fluid contained only small amounts of IL-8, so the nature of the activity has yet not been established.

We have shown that IL-8 can be produced in considerable amounts by both endothelial cells and PBMC when stimulated with LPS or IL-1 (Kristensen et al. 1991). Thus one would expect that IL-8 could be detected in peripheral blood during inflammatory responses. Erythrocytes, however, may bind considerable amounts of IL-8 (Darbonne et al. 1991) and therefore diminish the concentration of IL-8 in peripheral blood. IL-8 absorbed to red blood cells did not stimulate neutrophil responses. Binding and neutralization of IL-8 by red blood cells may provide a line of defense against systemic activation and chemotactic disorientation of neutrophils. It has also been shown that red blood cells bind MCAF although they don't share the same receptor (IV).

This suggest that erythrocytes may act as a general intravascular sink for chemotactic factors. Thus the action of the chemotactic factors will be localized at the point of secretion and orientate the chemotactic factor concentration gradient across the vascular wall.

7.4. Possible clinical applications of IL-8

If IL-8 is essentially involved in the pathomechanism of several human diseases the development of antagonists to IL-8 at the receptor level, or inhibitors at post receptor level, blockers for the adhesion molecules on leukocytes and endothelial cells, and inhibitors of IL-8 production will be important for the treatment of these diseases.

IL-8's ability to cause neutrophilia and increase the function of neutrophils may also prove helpful in the treatment of patients suffering from granulocytopenia, and especially in severe infections against which antibiotics are not effective or in immunodeficiency caused by human immunodeficiency virus.

8. GENERAL DISCUSSIONS AND FUTURE ASPECTS

8.1. The skin immune system (SIS)

The skin is one of the largest organs of the human body. Its principal physical function is to form a barrier and secure the internal homeostasis. Major physiological functions include maintenance of body temperature, regulation of stable circulation, production of endocrine mediators, and carrying peripheral neural receptors and nerve endings. It is relatively late in medical history that the skin has become recognized as an independent and important organ. Within recent years, however the skin has been recognized as a main target for events leading to immunological responses.

Fichtelius and co-workers hypothesized that the skin might function as a primary lymphoid organ, placing it in the category of primary immunological organs such as bone marrow and thymus (Fichtelius et al. 1970). They suggested that immune non-competent lymphoid cells entered the skin and became competent by the interaction with differentiation and maturation factors. In 1978 Streilein described the concept of skin-associated lymphoid tissues (SALT) (Streilein 1978) including Langerhans cells with their antigen-presenting properties, epidermotropic recirculating T lymphocyte subpopulations (homing T lymphocytes), keratinocytes producing epidermal thymocyte-activation factor (ETAF), and the skin-draining peripheral lymph nodes including vascular high endothelial cells with the capacity to capture lymphocytes passing through the blood. In addition mast cells, macrophages, granulocytes, fibroblasts, indeterminate cells, vascular endothelial cells and finally melanocytes which in this thesis have been described to produce chemotactic mediators. All these cells form an intricate and complex system that was defined in 1986 by Bos and Kapsenberg as the cellular part of the "skin immune system" (SIS) (Bos and Kapsenberg 1986).

Another more recent major step in immunology was the discovery of adhesion molecules that not only play a central role in cell-to-cell contact within the immune system itself, but also have a role in the trafficking of immunocompetent cells between the skin and the rest of the immune system.

8.2. Cytokines

In addition to the cellular constituents of the SIS, a wide variety of inflammatory and immune mediators are present within the normal integument. Some reach the skin by the circulatory route, but a major part maybe produced by different immunocompetent cells in the skin it self. Keratinocytes are one important source of cytokines. Polypeptide hormone-like products that regulate growth and differentiation of cells in the immune system, and hematopoiesis were originally thought to be released only by lymphocytes and to interact selectively with cells of the immune system and were therefore named lymphokines. It is however, now clear that neither production nor effects of these factors are restricted to cells of the immune system. These factors are therefore to day called cytokines. An example has been given in this thesis where melanocytes have been shown to produce chemotactic cytokines such as MCAF and IL-8. As more of these factors have been isolated, characterized, and cloned, evidence has been given for a network of interacting cytokines which lead to activation, proliferation, and differentiation of immune as well as non-immune cells.

Some cytokines have been shown to have a selective ability to attract specific leukocytes and in combination with adherence molecules determine the inflammatory infiltrate in the dermis and epidermis. These include IL-8, MCAF and ELCF, which all attract specific cell types as described here. Apart from being chemoattractants they often possess general activating activities as shown for MCAF and IL-8. Some cytokines have been found to be produced in greater amounts in active stages of diseases such as rheumatoid arthritis, gout, psoriasis, atherosclerosis, and malaria. Although these cytokines do not seem to be the only responsible factors for the pathological events they seem to represent a link in the cytokine cascade which contributes to the clinical picture.

For almost half a century corticosteroids have been one of our most important antiinflammatory agents. They have been used topically as well as systemically. The mechanisms of action include a direct inhibitory effect on the transcriptional level of several cytokines as it has been shown for IL-1 (Lew et al. 1988) and now also for IL-8 and MCAF (Mukaida et al. 1991, 1992). Corticosteroids thereby inhibit activation of leukocytes. Due to the now well-known side-effects, there have been many efforts to develop compounds with similar antiinflammatory actions but without the side effects of the corticosteroids.

During the last decades a number of systemic non-steroidal anti-inflammatory drugs (NSAID) have been developed. Most of them inhibit arachidonic acid (AA) products. No NSAID has yet been able chemically to replace the corticosteroids in topical treatment. Recently we have identified a new compound ETH615, which has been found to be a po-

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