Regulation of the chemotactic cytokines IL-8 and MCAF and their induction in different cell types related to the skin.

Mette Søndergaard Deleuran

Department of Dermatology
University of Aarhus
Marselisborg Hospital
Aarhus, Denmark

Det Sundhedsvidenskabelige Fakultetsråd ved Aarhus Universitet
har på sit møde den 6. juni 1994 antaget denne afhandling til forsvar
for den medicinske doktorgrad.
Forsvaret finder sted fredag den 2. september 1994 kl. 13.00 præcis,
i Auditorium 424 på Anatomisk Institut, Aarhus Universitet,
Universitetsparken, indgang bygning 230.

Arvid B. Maunsbach,
dekan

Printed in Denmark by Werks Offset, Aarhus

PREFACE

This thesis is based on studies performed in the period from 1989 to 1993 during my appointment as a research fellow at the Department of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark. One of these years (1991) was spent as a guest researcher at the Charing Cross Sunley Research Centre and Kennedy Institute of Rheumatology in Hammersmith, London.

I am truely greatful to professor of dermatology Kristian Thestrup-Pedersen, who introduced me to the interesting world of dermatological science. He always encouraged me with helpful advice and discussions and supported me with his interest and friendship.

A very special thank to my friend Kirsten Paludan, who introduced me to the difficult field of molecular biology and thought me the basic principles of proper science.

I would also like to thank professor Hugh Zachariae and professor Knud Kragballe for their interest and support in my work.

I wish to express my sincere gratitude to professor Marc Feldmann and professor Tiny Maini who offered me excellent working conditions and laboratory facilities during my stay in London and consultant Stephen Breathnach for his valuable scientific support and friendsship. My thoughts go to all our dear friends and colleagues in England.

I also wish to thank my co-authors Christian Grønhøj Larsen, Claus Zachariae, Fionula Brennan, Peter K.A. Jensen, Poul Jørgensen, Mads K. Thomsen, Ian Ahnfelt-Rønne, Kouji Matsushima, Tan Jinquan and Autumn Chu for good collaboration and interesting discussions.

For expert technical assistance I want to thank Kirsten Stadel Pedersen and Birgith Pedersen.

Financial support was granted by the University of Aarhus, Institute of Experimental and Clinical Research, University of Aarhus, The Danish Psoriasis Foundation, Kgl. Hofbundtmager A.C. Bangs Fond and Christian and Ottilia Brorsons Travel Foundation.

Finally I wish to thank my dear husband and co-worker Bent Deleuran for his never failing support and for all his good ideas and love.

To Mads and Amalie

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

I. Kristensen M, Larsen CG, Jørgensen P, Paludan K. RNA purification from epidermal suction blisters. Acta Derm Venereol (Stockh) 1991: 71: 423-426.

II. Kristensen MS, Paludan K, Larsen CG, Zachariae COC, Deleuran BW, Jensen PKA, Jørgensen P, Thestrup-Pedersen K. Quantitative determination of IL-1α induced IL-8 mRNA levels in cultured human keratinocytes, dermal fibroblasts, endothelial cells, and monocytes. J Invest Dermatol 1991: 97: 506-510.

III. Larsen CG, Kristensen M, Paludan P, Deleuran B, Thomsen MK, Zachariae C, Kragballe K, Matsushima K, Thestrup-Pedersen K. 1,25(OH)2-D_3 is a potent regulator of interleukin-1 induced interleukin-8 expression and production. Biochem Biophys Res Commun 1991: 176: 1020-1026.

IV. Kristensen M, Deleuran B, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM. Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1α in normal and psoriatic skin; Decreased expression of IRAP in psoriatic lesional epidermis. Br J Dermatol 1992: 127: 305-311.

V. Kristensen M, Chu CQ, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM. Localization of tumour necrosis factor alpha (TNFα) and its receptors in normal and psoriatic skin: epidermal cells express the 55 kD but not the 75 kD-TNF receptor. Clin Exp Immunol 1993: 94: 354-362.

VI. Kristensen M, Deleuran BD, Jinquan T, Thomsen MK, Zachariae C, Paludan K, Ahnfelt-Rønne I, Matsushima K, Thestrup-Pedersen K, Larsen CG. ETH615, a synthetic inhibitor of leukotriene biosynthesis and function, also inhibits the production of and biological responses towards interleukin 8. Exp Dermatol 1993: 2: 165-170.

VII. Kristensen M, Deleuran BW, Larsen CG, Thestrup-Pedersen K, Paludan K. Expression of monocyte chemotactic and activating factor (MCAF) in skin related cells. A comparative study. Cytokine 1993: 5: 520-524.

LIST OF ABBREVIATIONS:

AA:	Arachidonic acid
ACD:	Allergic contact dermatitis
ANAP:	Anionic neutrophil activating peptide
APAAP:	Alkaline phosphatase anti-alkaline phosphatase
cDNA:	Complementary DNA
ELCF:	Epidermal lymphocyte chemotactic factor
ELAM-1:	Endothelial leukocyte adhesion molecule-1
ETAF:	Epidermal thymocyte activating factor
FCS:	Fetal calf serum
fMLP:	F-Met-Leu-Phe
GAPDH:	Glyceraldehyde-3-phosphate-dehydrogenase
GM-CSF:	Granulocyte macrophage-colony stimulating factor
GTC:	Guanidinium thiocyanate
HETE:	Hydroxy-eicosatetraenoic acid
ICAM-1:	Intercellular adhesion molecule-1
IFNγ:	Interferon γ
IL:	Interleukin
IL-1ra:	Interleukin 1 receptor antagonist
IL-1R1:	Type 1 interleukin 1 receptor
IL-1R2:	Type 2 interleukin 1 receptor
gIP 10:	IFNg inducible protein 10
IRAP:	Interleukin 1 receptor antagonist protein
kb:	Kilobase
kDa:	Kilodalton
LECAM-1:	Lectin adhesion molecule-1
LO:	Lipoxygenase
LPS:	Lipopolysaccharide
LT:	Leukotriene
Mac-1:	Granulocyte adhesion molecule-1
MCAF:	Monocyte chemotactic and activating factor
MCP-1:	Monocyte chemotactic protein 1
MDNCF:	Monocyte derived neutrophil chemotactic factor
MGSA:	Melanoma growth stimulatory activity
MIP:	Macrophage derived inflammatory protein
mRNA:	Messenger RNA
NAP:	Neutrophil activating peptide
NGS:	Normal goat serum
p55 TNF-R:	The 55 kD tumour necrosis factor receptor
p75 TNF-R:	The 75 kD tumour necrosis factor receptor
PBMC:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
PF4:	Platelet factor 4
PGE_2:	Prostaglandin E_2
PHA:	Phytohemagglutinin
PMN:	Polymorphonuclear granulocytes
PPD:	Purified protein derivative
rh:	Recombinant human
RIA:	Radioimmunoassay
ß-TG:	ß-tromboglobulin
TBS:	Tris buffered saline
TGFß:	Transforming growth factor ß
TNFα:	Tumour necrosis factor α
VCAM-1:	Vascular cell adhesion molecule-1
VDR:	Vitamin D_3 receptor

TABLE OF CONTENTS:

1. Introduction. 7

2. RNA extraction from epidermal suction blisters. 7

2.1. Introduction. 7
2.2. RNA purification and Northern blotting. 8

3. A quantitative method for studies of specific cytokine messenger RNA species. 8

3.1. An introduction to interleukin 8 (IL-8). 8
3.1.1. Biochemical properties and molecular aspects of IL-8. 8
3.1.2. Biological actions of IL-8. 9
3.1.3. IL-8 receptors. 10
3.2. Quantification of IL-8 mRNA in human keratinocytes, fibroblasts, endothelial cells, and monocytes. 10
3.2.1. Cell stimulation, RNA isolation and dot blot analysis. 11
3.2.2. IL-8 mRNA synthesis by four different cell types. 11
3.2.3. Time course studies of IL-8 mRNA synthesis. 12

4. The ability of different skin cells to express monocyte chemotactic and activating factor (MCAF). 13

4.1. An introduction to MCAF. 13
4.1.1. Biochemical properties and molecular aspects of MCAF. 13
4.1.2. Biological actions of MCAF. 13
4.1.3. MCAF receptors. 14
4.2. Quantification of IL-1α induced MCAF mRNA in human keratinocytes, fibroblasts, endothelial cells, and monocytes. 14
4.2.1. MCAF mRNA synthesis by four different cell types. 14
4.2.2. Time course studies of MCAF mRNA synthesis. 14

5. Distribution of IL-1α, interleukin 1 receptor antagonist protein (IRAP), and the type 1 IL-1 receptor (IL-1R1) in normal and psoriatic skin. 16

5.1. Introduction. 16
5.2. IL-1 in human skin. 16
5.3. The interleukin 1 receptors. 16
5.4. Biology, cellular sources and actions of IRAP. 17
5.5. Skin biopsies and immunohistological techniques. 18
5.6. Distribution of IL-1α, IRAP and IL-1R1 in normal skin. 18
5.7. Distribution of IL-1α, IRAP and IL-1R1 in psoriatic skin. 19

6. Tumor necrosis factor α (TNFα) and its receptors in normal human skin and skin from patients with psoriasis. 19

6.1. An introduction to TNFα. 19
6.2. The TNF receptors. 19
6.3. Immunohistochemical staining and specificity controls. 21
6.4. Localization of TNFα and its receptors in normal and psoriatic skin. 21

7. Regulation of interleukin 8 expression in the skin.. 21

7.1. Possibilities for therapeutical modulation of IL-8 levels in inflammatory and autoimmune diseases.. 21
7.2. Inhibition of IL-8 production and function by the synthetic leukotriene inhibitor ETH615. 22
7.3. 1,25(OH)2-D_3 can regulate IL-1α induced IL-8 expression and production in human keratinocytes, dermal fibroblasts, and peripheral blood mononuclear cells. 23

8. The use of different cytokine assays. 24

9. Conclusions. 25

10. Perspectives and future studies.. 25

11. Summary in Danish.. 26

12. References. 26

1. INTRODUCTION.

Cytokines are hormone-like polypeptides produced by a variety of cell types. This large group of related factors can be divided into several classes according to their biological activity as follows: interleukins, haematopoietic colony-stimulating factors, interferons, growth factors, and inhibitory factors. Cytokines seem to play an important role in the normal homeostasis of the body. Dysregulation of one or several of these mediators are believed to be involved in the development and/or maintenance of disease, especially in disorders characterized by chronic inflammation.

The purpose of the studies on which this thesis is based was to elucidate the regulation of the chemotactic cytokines interleukin 8 (IL-8) and monocyte chemotactic and activating factor (MCAF) in relation to inflammatory skin disease. IL-8 and MCAF which belong to the inflammatory peptide supergene family are involved in the recruitment and activation of cells from the blood into the skin. This can take place as part of the normal barrier function of the skin, but is believed to play a special role in inflammatory skin diseases such as allergic contact dermatitis and psoriasis.

A method for RNA extraction from epidermal suction blisters was established in order to investigate the expression of epidermal cytokines *in vivo* during the development of inflammatory skin diseases (I). As we were unable to detect specific messenger RNA for IL-1α and IL-8 in the epidermal suction blisters, we developed a technique that enabled us to quantitatively compare the amount of a specific messenger RNA in different cell types (II). By this method it was demonstrated that the keratinocyte, which is the major cell type in the epidermis, produces very low amounts of both IL-8 and monocyte chemotactic and activating factor (MCAF) *in vitro* when compared to other cell types in the skin (II,VII).

Interleukin 1 (IL-1) and tumor necrosis factor α (TNFα) are some of the main endogenous inducers of IL-8 and MCAF in many different cells. We therefore investigated the distribution of IL-1α, TNFα, their receptors, and the interleukin 1 antagonist protein (IRAP) in normal skin and compared their localization with the distribution in psoriasis (IV, V) in order to further illuminate the regulation of IL-8 and MCAF expression. By immunohistochemical techniques we found that the type 1 IL-1 receptor and the 55 kD TNF receptor are widely distributed in the skin together with expression of IL-1α and TNFα making both autocrine and paracrine stimulation of skin cells likely in order to express other cytokines such as IL-8 and MCAF. We additionally observed a down-regulation of IRAP in involved psoriatic skin indicating a possible role for this antagonist in the pathogenesis of psoriasis.

In the attempt to develop new drugs with a specific activity, low toxicity and few side-effects for the treatment of dermatological disorders, we investigated the effects of active vitamin D_3 (calcitriol) and the synthetic leukotriene inhibitor ETH615 on IL-8 expression in different cell types *in vitro*. We observed that calcitriol, which has proven useful in the treatment of psoriasis, is able to suppress IL-8 mRNA synthesis and protein release from keratinocytes, dermal fibroblasts, and peripheral blood mononuclear cells (PBMC) (III). Further, it was observed that ETH615, which has a local anti-inflammatory effect on zymosan-induced dermatitis in dogs, can inhibit both the expression and biological effect of IL-8 (VI).

These results from *in vitro* and *in vivo* studies indicate that expression of cytokines in a given organ will contribute to recruitment of inflammatory cells in disease.

2. RNA EXTRACTION FROM EPIDERMAL SUCTION BLISTERS.

2.1. Introduction.

Many inflammatory skin diseases are characterized by elevated concentrations of different cytokines in the epidermis. In order to understand the pathogenesis of these diseases it is important to establish whether the cytokines are produced locally or imported into the epidermis from the underlying tissues, either by simple diffusion or with infiltrating cells. Many investigations have measured specific cytokine messenger RNA (mRNA) in punch or shave biopsies from the skin, but these techniques do, however, not allow a clear distinction between cytokines produced in the epidermis or the dermis. Similarly, it is possible by immunohistochemical techniques to determine the in situ presence of cytokines, but this does not necessarily prove that the proteins are actually produced or released by the epidermal cells.

The presence of specific mRNA for a certain cytokine reveals an active and ongoing process in the production of the mediator, although this may not always lead to secretion of the protein (Schall et al. 1992). We therefore felt a need for a method that enabled us to extract total RNA from epidermal tissue only, isolated with the smallest trauma possible. The epidermal suction blister technique, which was first introduced by Kiistala and Mustakallio in 1964, satisfies these demands. The method separates the epidermis from the dermis at the dermo-epidermal junction, and the cells in the blister roof maintain a normal ultrastructural appearance (Kiisala and Mustakallio, 1967) and viability (Ingemansson-NordQuist et al. 1967). Further, this method is quite non-traumatic to the patient and it avoids the use of chemical substances, which may alter the expression levels of the mediators in question (Longley J et al. 1991).

It has previously been shown that a homogenate of epidermal suction blisters contains increased amounts of interleukin 1 (IL-1) and ELCF (epidermal lymphocyte chemotactic factor) activity if taken from an area where a cell-mediated immune response takes place (Larsen et al.1988 A, Larsen et al. 1988 B, Ternowitz and Thestrup-Pedersen 1986). The anionic neutrophil-activating peptide (ANAP) which was first isolated from psoriatic scales (Schröder and Christophers, 1986) has later been shown to be identical with the neutrophil and lymphocyte chemotactic factor IL-8. We therefore found it of interest to investigate the role of epidermal keratinocytes in the expression of IL-1 and IL-8 mRNA.

The preparation of RNA from epidermal suction blisters involves extraction from very small amounts of tissue, which in addition are very resilient and therefore difficult to homogenize. A modification of the method for RNA extraction

first described by Chomzynski and Sacchi in 1987 was found useful for this purpose.

2.2. RNA purification and Northern blotting.

Homogenization. We used suction cups with seven 5 mm holes which produced suction blisters with a total epidermal size of 1.4 cm^2 for each experiment. The blisters were immediately placed in guanidinium thiocyanate (GTC) buffer and frozen in liquid nitrogen. The samples were homogenized in a polytron while still partially frozen in order to liberate RNA into the protection buffer. The homogenate was cooled on ice between homogenization runs, and the polytron was rinsed with additional GTC buffer in order to save pieces of blisters, which could be stuck in the dispersing tool. We found that the homogenization step in the RNA purification procedure is very important as it liberates the RNA from the cells into the GTC buffer and that the use of a polytron is superior to grinding. Further, it is very important to homogenize the tissue when only partially thawed to avoid RNA degradation.

RNA purification and total RNA yield. Total RNA was extracted as described (I). Briefly, RNA in GTC was separated from protein and DNA in a mixture of sodium acetate, chloroform-isoamylalcohol and acid phenol followed by two precipitations with isopropanol and three washes in ethanol. The pellet was air-dried and redissolved in RNase-free water.

In 13 experiments we obtained from 12 to 38 μg RNA with an average of 22 μg. The 260/280 nm UV light absorption ratio was 1.8 which indicates a high degree of RNA purity (I).

Northern blotting and hybridization. The RNA integrity was confirmed by gel electrophoresis, and the samples transferred to nylon membranes as described (I). Northern blots were prepared of RNA taken at different timepoints from 1 hour up to 24 hours after tuberculin injection or application of patch tests on individuals who had previously shown positive reactions. We also isolated RNA from normal skin suction blisters. Radioactive probes were labelled by random priming (Feinberg and Vogelstein, 1983) and hybridization carried out overnight. We used cDNA probes for IL-1α, IL-8, and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), which is a household enzyme present in low amounts in all types of cells (Piechazyk et al. 1985).

A faint, but destinct hybridization signal was observed for GAPDH in all the samples tested. We therefore conclude that this method is useful for obtaining RNA from pure epidermal tissue of sufficient quality and quantity for Northern blot analysis.

By contrast no hybridization signal for IL-1 or IL-8 was observed. There may be several explanations to these findings:

1. The levels of IL-1 and IL-8 mRNA were below the detection limit of the assay. This is a likely explanation as cytokines are biologically active in concentrations down to 10^{-15} M (Larsen CG. personal communication).

2. Resent studies have shown that, by use of the polymerase chain (PCR) reaction, it is not possible to detect IL-8 mRNA transcripts in normal human epidermis from healthy volunteers. However, IL-8 mRNA can in some cases be found in normal looking skin from patients suffering from different dermatological disorders (Paludan and Thestrup-Pedersen, 1992). In addition Gillitzer and coworkers (1991) were unable to detect IL-8 mRNA in normal epidermis by in situ hybridization.

3. The timepoints for suction blister formation after application of antigen could be non-optimal. Time course studies on cultured keratinocytes (II) have ,however, revealed that IL-8 mRNA is induced and downregulated within 8-10 hours after lipopolysaccaride (LPS) or IL-1 stimulation *in vitro*.

4. The cytokines may be produced by other cell types present in the skin or imported from underlying tissues.

These results led to the development of a quantitative method which enabled us to compare the amount of specific mRNA of a given mediator in different cell types.

3. A QUANTITATIVE METHOD FOR STUDIES OF SPECIFIC CYTOKINE mRNA SPECIES.

3.1. An introduction to IL-8.

3.1.1. Biochemical properties and molecular aspects of IL-8. In 1987 a neutrophil chemotactic factor was purified to homogeneity from the supernatants of LPS stimulated human peripheral blood mononuclear cells (PBMC) (Yoshimura et al. 1987A, 1987B, Walz et al. 1987), and the following year cDNA for this factor was cloned and the complete amino acid sequence determined (Matsushima et al.1988). The factor was at that time named monocyte derived neutrophil chemotactic factor (MDNCF), but later renamed IL-8/neutrophil activating peptide-1 (NAP-1) (Larsen et al. 1989C). It was shown to be identical with a basic protein secreted from PBMC also purified by other groups (Peveri et al. 1988, Schröder at al. 1987, Van Damme et al. 1988). Molecular cloning of the genomic DNA of IL-8 revealed a gene which consists of 4 exons and 3 introns with a single "CAT"- and "TATA"-like structure (Mukaida et al. 1989). The 1.6 kb (kilobase) cDNA sequence encodes for a 99 aminoacid precursor of IL-8 including a signal peptide (Matsushima et al. 1988), and recognizes a 1.8 kb mRNA on Northern blots. The gene encoding for IL-8 is situated on the long arm of chromosome 4 (Modi et al.1990).

The mature form of IL-8 consists of 72 amino acids with an isoelectric point between 8 and 8.5. It contains 14 basic amino acids and four cysteine residues, where the first two are separated by one single amino acid. No N-glycosylation sites have been found. The estimated molecular weight is 8 kDa (Matsushima and Oppenheim 1989).

IL-8 belongs to a family of basic proteins including platelet factor 4 (PF4) and macrophage derived inflammatory protein-2 (MIP-2), which are also both chemotactic for neutrophils, interferon gamma (IFNγ) inducible protein 10 (γIP 10),

ß-thromboglobulin (ß-TG), and GRO/melanoma growth stimulating factor (MGSA).

Other forms of IL-8 have been described due to differences in the NH_2 terminus. Human vascular endothelial cells produce mainly a 77 amino acid form (Gimbrone et al. 1989), and the predominant IL-8 form found in psoriatic scales consists of 69 amino acids (Schröder et al. 1992). In addition, a 79 amino acid form has been described (Yoshimura et al. 1989, Schröder et al. 1990). Shortly after the initial characterization of IL-8, recombinant material cloned in Escherichia coli became available (Lindley et al. 1988, Furuta et al. 1989). It was shown that recombinant IL-8 had the same biological properties as the natural form and this has lead to a very thorough investigation of the actions of IL-8 both *in vivo* and *in vitro*.

3.1.2. Biological actions of IL-8.

Neutrophils. The best known action of IL-8 is probably the neutrophil chemotactic activity *in vitro* which was originally described by several different groups (Yoshimura et al. 1987 B, Schröder et al. 1987, van Damme et al. 1988). This effect has now been confirmed *in vivo* in humans (Swensson et al. 1991, Leonard et al. 1991), where intracutaneous injection of IL-8 resulted in infiltration with polymorphonuclear leukocytes (PMN), which were present within 1 hour and increased up to 5 hours. No macroscopic changes were observed. Other aspects of neutrophil activation by IL-8 include induction of exocytosis from cytochalasin B treated neutrophils, the formation of superoxide anion and hydrogenperoxide (Peveri et al. 1988, Djeu et al. 1990, Schröder et al. 1987, Thelen et al. 1988), and a rise in cytosolic free Ca^{2+} (Thelen et al. 1988, Peveri et al.1988) together with neutrophilic shape change (Thelen et al. 1988). Further, IL-8 is able to enhance PMN-mediated anti-candida activity (Djeu et al. 1990).

An enhanced expression of the complement receptor type 1 (CR1) (Paccaud et al. 1990, Detmers et al. 1991), the granulocyte adhesion molecule Mac-1 (CD11b/CD18)(Farina et al. 1989, Detmers et al. 1990) as well as CD11c/CD18 (Detmers et al 1990) on the surface of neutrophils has been observed in the presence of IL-8. These actions may promote leukocyte adhesion *in vivo* that could lead to recruitment of PMN to sites of tissue inflammation. A recent study has revealed that neutrophils are also able to synthesize IL-8 mRNA and produce IL-8 protein after stimulation with other cytokines (Strieter et al. 1992). This response may be important for the elicitation of additional neutrophils or to maintain the immune response at sites of inflammation.

Other neutrophil activating factors like FMLP and C5a are able to activate the neutrophil arachidonate-5 lipoxygenase with the release of leukotriene B_4 (LTB_4) from neutrophils, in the presence of arachidonic acid. As both LTB_4-like material and IL-8 have been found in elevated amounts in psoriasis (Brain et al. 1984, Schröder et al 1989), and probably are involved in the pathogenesis of this disease, Schröder tested the hypothesis that IL-8 could act in a similar way. He found that IL-8 was indeed able to activate PMN to release LTB_4 and 5-hydroxy-eicosatetraenoic acid (5-HETE) in the presence of exogenous arachidonic acid (Schröder 1989), and these studies have been extended by Fogh and coworkers (1990), who additionally showed that IL-8 was able to induce formation of 15-HETE via this pathway.

Basophils. Quite complex mechanisms are involved in the actions of IL-8 on basophils. IL-8 has been shown to be chemotactic to basophils *in vitro* (Leonard et al. 1990). This action was not confirmed *in vivo*, when IL-8 was injected intradermally in humans (Leonard et al. 1991). The difference, however, is probably due to the fact that lower concentrations of IL-8 were used for the *in vivo* studies. High doses of IL-8 can induce histamin release from unprimed human basophils (White et al. 1989), while an agonistic action of lower doses of IL-8 require priming of the basophils with IL-3, IL-5 or granulocyte/macrophage colony stimulating factor (GM-CSF) (Bischoff et al. 1990, Dahinden et al. 1989). By contrast, preincubation of basophils with IL-8 produces an inhibition of histamin release induced by different factors (Kuna et al. 1991).

Keratinocytes. IL-8 can act as a mitogen for keratinocytes in culture (Reusch et al. 1990, Tuschil et al. 1992), and these observations indicate that IL-8, apart from beeing pro-inflammatory, also has proliferative effects on keratinocytes. This feature may be of importance in diseases like psoriasis which are characterized by hyperproliferation of the epidermis.

Endothelial cells. The published data concerning biological actions of IL-8 on endothelium are closely linked to its actions on neutrophils. Several groups have reported that both exogenous IL-8 and IL-8 produced by cytokine or LPS stimulated endothelial cells induce an enhanced binding of neutrophils to endothelial monolayers and subendothelial matrix proteins via an induction of ß2 integrins and lectin adhesion molecule-1 (LECAM-1) on neutrophils (Carveth et al. 1989, Detmers et al. 1990, Huber et al. 1991). In a rat sponge model it has also been shown that IL-8 accelerates the sponge induced angiogenesis (Ahuja et al. 1992). Further, by the use of different vessel wall constructs *in vitro* it has been demonstrated that IL-8 promotes neutrophil transvenular migration and increases vascular permeability (Smith et al. 1991, Huber et al. 1991). Pre-incubation of neutrophils with IL-8 reduces their chemotactic transmigration response to an IL-8 gradient demonstrating desensitization and thereby IL-8 dependability of the response (Smith et al. 1993). Data published by another research group (Gimbrone et al. 1989, Hébert et al. 1990) contradict these results. However, in the model used by the latter, endothelial cell monolayers not only secrete IL-8 into the medium, but also deposit the molecule into the subendothelial matrix. Therefore, the apparent inhibitory effect on transendothelial migration, exerted under these conditions, may be caused by a collapse of the endogenous chemotactic gradient, which is necessary for the action of all chemoattractants.

As we have found that endothelial cells are one of the main producers of IL-8 (II), we believe that the endothelial cells play an important and central role in the recruitment of cells from the bloodstream and into the tissue during the development and maintainence of an inflammatory skin disease.

T-lymphocytes. Larsen and coworkers (1989 C) were the first to demonstrate that IL-8 can induce human T-lymphocyte chemotaxis. This observation has been confirmed by others (Bacon et al. 1989, Leonard et al. 1990). Only 13-15 % of an unactivated T-cell enriched cell suspension actually migrate (Zachariae et al. 1992), but this fraction of cells does not differ in composition from the input cell population. This observation has been confirmed *in vivo*, where intradermal injection into rat ears of low doses (0.001 μg/ml) of IL-8 resulted in lymphocyte accumulation in the tissue. However, this effect has not been shown *in vivo* in humans, where only neutrophil accumulation in the skin occured after intradermal injection of IL-8 (Leonard et al. 1991, Swensson et al 1991). The difference in reactivity in rats and humans could be due to the fact that about 75% of all circulating leukocytes in the rat are lymphocytes. It could also be speculated that stimulation of the T-cells would activate a larger percentage of the cells to migrate, but it has been demonstrated that pre-stimulation with PHA actually abolishes the chemotactic response (Larsen CG., personal communication).

B-lymphocytes. In 1992, Kimata and coworkers demonstrated that IL-8 can inhibit IL-4 induced IgE and IgG4 production in human B cells. The IgM, IgA and IgG1-3 production was not affected.

Monocytes. Very few data have been published concerning actions of IL-8 on human monocytes. In 1991, however, Brown and coworkers described that rh (recombinant human) IL-8 augments the adherence of monocytes to cultured endothelium. The induced attachment corresponded to an enhanced expression of the adhesion molecules CD11a, CD11b, and CD18. These activities could be important for the recruitment of circulating monocytes into inflammatory lesions.

3.1.3. IL-8 receptors. In order to induce its actions, IL-8 binds to specific cell surface receptors. Based on radiolabelling studies Samanta and coworkers (1989) estimated the receptor number on peripheral blood neutrophils to be approximately 20,000 high affinity binding sites per cell. They also found that IL-8 can down-regulate its own receptor more than 90% within 10 min at 37 °C and reappear on the cell surface 10 min after removal of free ligand. The reappearance is due to recycling of the receptors (Samanta et al. 1990). Using flow-cytometry another group have found approximately 7000 IL-8 receptors per cell on neutrophils (Leonard et al. 1990). By contrast the number of IL-8 receptors on resting T-lymphocytes is calculated to be only 300 per cell (Matsushima and Oppenheim 1991). Using binding and competition studies Krieger and coworkers (1992) found that normal human basophils have approximately 9600 specific IL-8 receptors per cell. Red blood cells also possess binding sites for IL-8 (Darbonne et al 1991). This is quite surprising, but as binding of IL-8 to this cell type inactivates the molecule and act as a sink it has been postulated that the binding may limit the stimulation of leukocytes by IL-8 released into the blood, and thereby prevent potentially harmful reactions.

In 1991 the cDNA cloning of two different IL-8 receptors on neutrophils was published. Holmes and co-workers found a high affinity IL-8 receptor with a size of 350 amino acids and a molecular weight around 40 kDa, also named the type 1 IL-8 receptor. It belongs to a superfamily of receptors that couple to guanosine triphosphate binding proteins also called G-proteins including the receptors for fMLP and C5a, which are also neutrophil chemoattractants. At the same time data on another G-protein coupled IL-8 receptor on HL-60 neutrophils were described (Murphy and Tiffany, 1991). This 355 amino acid receptor has 77 amino acid identity with the receptor mentioned above, is now named the type 2 IL-8 receptor and was shown to bind IL-8 with low affinity. They have additionally shown that this receptor can also be activated, but with much lesser potency by melanoma growth stimulatory activity (MGSA) and neutrophil activating peptide-2 (NAP-2), which are proteins very closely related to IL-8. This observation confirms the finding of Moser and coworkers (1991) who also reported that MGSA and NAP-2 can bind to IL-8 receptors on neutrophils. The amino terminus of the IL-8 receptors is of importance for the differential binding of MGSA and IL-8 (Gayle et al. 1993). Recently, Lee and coworkers (1992) expressed both IL-8 receptors in mammalian cells and found that both the high and "low" affinity IL-8 receptor bind IL-8 with high affinity (Kd approximately 2 nM) in their system. It can therefore be concluded that human neutrophils express at least two distinct IL-8 receptors, and that both receptors are active, as binding of IL-8 to either receptor can induce Ca^{2+} efflux from the cells.

3.2. Quantification of IL-8 mRNA in different cell types.

As described above IL-8 has a variety of effects on different kinds of cells. Psoriatic scales contain large amounts of IL-8 (Schröder et al. 1986, Schröder et al. 1992), and epidermal suction blisters from allergic patch test reactions possess lymphocyte chemotactic properties (Larsen et al. 1988 B), which can be partially blocked by the addition of antibodies against IL-8 (Zachariae C. Personal communication). These observations indicate that IL-8 is an important mediator in different skin diseases. It has previously been shown that a large number of cell types are able to express the IL-8 gene and also to produce IL-8 protein in response to different kinds of stimuli (for review, see Larsen CG. 1991). When concentrating on cell types relevant for the skin, IL-8 expression has been demonstrated in human keratinocytes (Larsen et al. 1989 A, Barker et al. 1990), dermal fibroblasts (Larsen et al 1989 A, Strieter et al. 1989 A, Mielke et al. 1990), melanocytes (Zachariae et al. 1991), endothelial cells (Schröder and Christophers 1989 C, Strieter et al. 1989 B, Sica et al. 1990), lymphocytes (Schröder et al. 1988, Gregory et al. 1988), neutrophil granulocytes (Strieter et al. 1990, Bazzoni et al. 1991), and monocytes (Walz et al. 1987, Matsushima et al. 1988).

Further, immuno-reactivity for IL-8 has been demonstrated in both normal and diseased skin (Sticherling et al. 1991, 1992, Anttila et al. 1992, Elder et al. 1991).

In order to clarify the role that some of these cell types play in the expression of IL-8 in skin, we wanted to quantitatively compare IL-8 mRNA levels.

Normally a quantification of the amount of a specific mRNA in cultures of the same kind of cells is done by relating to an internal standard (e.g. actin or GAPDH mRNA) in Northern blot analysis, as the amount of specific mRNA for these standards is quite stable within the same cell type.

By use of this method, however, a comparison between different cell types is not possible due to the fact that these standard mRNA species can vary considerably from one cell type to the other. We therefore chose to develop a method which allows such a comparison by relating the amount of a specific messenger to the total amount of RNA in a given sample. The total amount of RNA is a quite stable parameter, and as it consists predominantly of ribosomal RNA only striking differences between cell types in the proportion of mRNA to ribosomal RNA could bias the results. With this technique we were able to compare the relative amount of specific IL-8 mRNA at different time points after stimulation with IL-1α or LPS in cultures of human keratinocytes, dermal fibroblasts, endothelial cells, and monocytes.

3.2.1. Cell Stimulation, RNA Isolation and Dot Blot Analysis. The different cell types were cultured as described (II) and stimulated with recombinant human (rh) IL-1α (1000 U/ml for keratinocytes, 100 U/ml for the other cell types) or LPS (100 mg/ml for keratinocytes, 10 mg/ml for the other cell types) as keratinocytes need a strong stimulation to upregulate their IL-8 expression in vitro (Larsen et al. 1989 A).

At different time points up to 24 hours, cells were lysed and total cellular RNA isolated by the method of Chomczynski and Sacchi (1987). We confirmed that the RNA preparations were pure and undegraded by UV light spectrum analysis and gel electrophoresis. Samples of total RNA were spotted onto nylon membranes, using a minifold apparatus. The wells were allowed to drain and washed in buffer. The nylon membranes were rinsed, baked, and stained with methylene blue. Methylene blue stains DNA and RNA, spotted onto Zeta-probe nylon membranes, with equal intensity, while no staining of proteins can be observed. It is therefore important that RNA used for this quantitative method is free of DNA contamination, as judged by gel electrophoresis, to avoid bias when measuring the total amount of RNA on the membrane. Staining intensities of the spots were measured by scanning the still wet membrane in an ELISA reader at 540 nm.

Test samples were spotted on membranes together with a standard series of known amounts of RNA applied in triplicates. We have demonstrated that light absorption at 540 nm accurately reflects the RNA amount bound in each spot (Fig. 1). By referring to this standard curve it is possible to calculate the precise amount of total RNA in a given spot.

The probe was IL-8 cDNA cloned in the *Eco*RI site of pBR322 (Matsushima et al. 1988). The cDNA was labeled by random priming, first described by Feinberg and Vogelstein (1983). By Northern blotting we confirmed that our probe hybridized to one band of the correct size. The membranes were destained and prehybridized followed by hybridization overnight. After autoradiography, the hybridization intensities of spots were measured by scanning the film in an ELISA reader at 405 nm. Specific IL-8 hybridization could then be calculated for each spot as hybridization intensity per μg of total RNA.

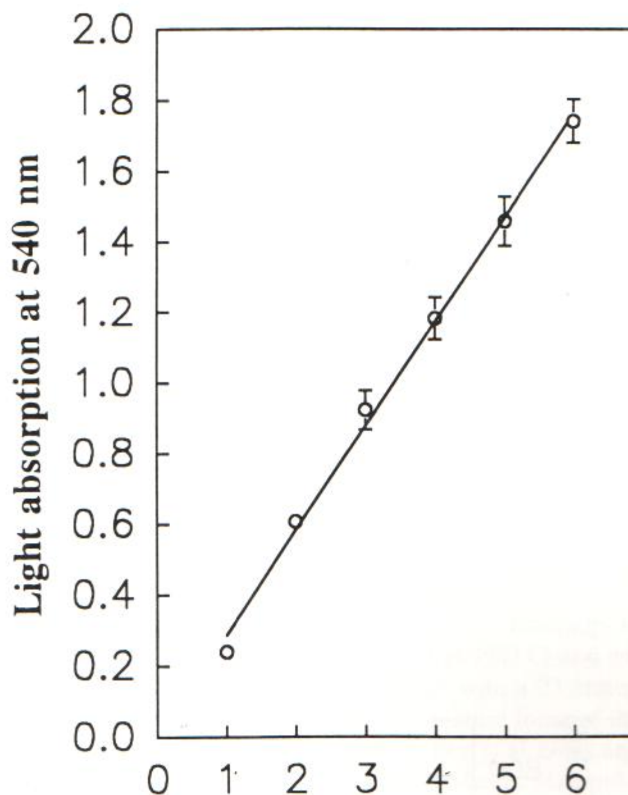


Fig. 1. Light absorption by methylene blue stained RNA. The RNA concentration of a standard sample was determined, and the indicated amounts spotted onto a membrane in triplicates. The membrane was then stained and scanned in an ELISA reader at 540 nm. *Error bars,* SEM.

3.2.2. IL-8 mRNA synthesis by four different cell types. We used our method to obtain quantitative information of the relative IL-8 mRNA amounts synthesized by keratinocytes, dermal fibroblasts, endothelial cells, and monocytes, as they all play a role in inflammatory skin reactions. As stimulant we chose rhIL-1α since this compound is known to induce IL-8 in all the cell types we wanted to analyze, and because IL-1a is believed to be involved in the pathogenesis of many different skin disorders.

As expected, all cells responded to stimulation with an increase in IL-8 mRNA, but there were marked differences (Fig. 2). Endothelial cells produced on the average more than ten times the IL-8 mRNA amount produced by keratinocytes, and fibroblasts and monocytes expressed intermediate amounts. The small amount of IL-8 mRNA synthesized by keratinocytes required stimulation with ten times the IL-1α amount used for the other cell types.

IL-8 accumulation over a 24 hour period in the medium of keratinocyte and endothelial cell cultures was proportional to IL-8 mRNA levels, from 25 to 100 ng for keratinocyte cultures and from 555 to 750 ng for endothelial cell cultures, as determined by a specific radio-immuno assay for IL-8.

In order to see whether this picture was unique for IL-1α-

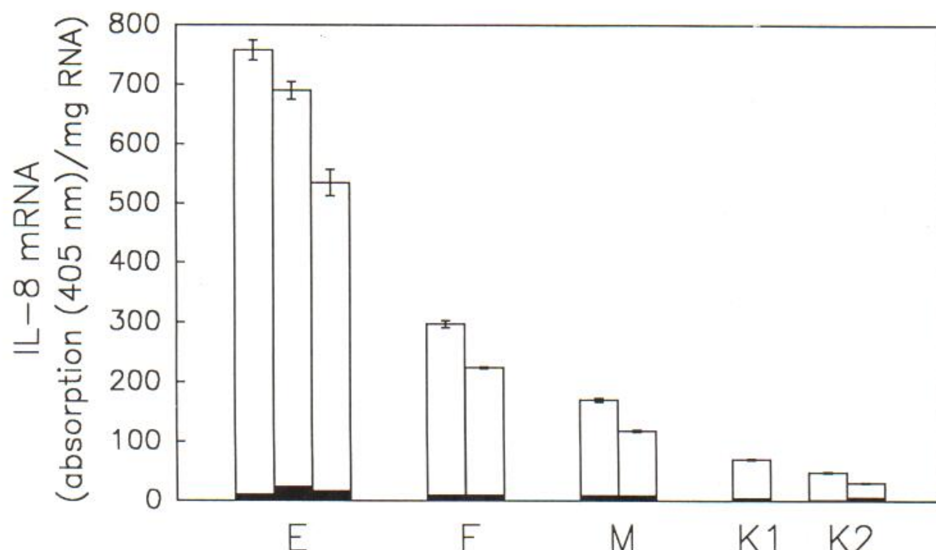


Fig. 2. IL-8 mRNA in different cell types with and without IL-1α stimulation. Values were determined by the quantitative dot blot method. Each value is the average of triplicate determination for one culture. *Open bars*, culture stimulated for 6h with 100 U/ml (endothelial cells (E), fibroblasts (F), Monocytes (M)) or 1000 U/ml (keratinocytes: SVK14 (K1), primary keratinocyte cultures (K2)). *Solid areas*, values for the corresponding unstimulated cultures. *Error bars*, SEM.

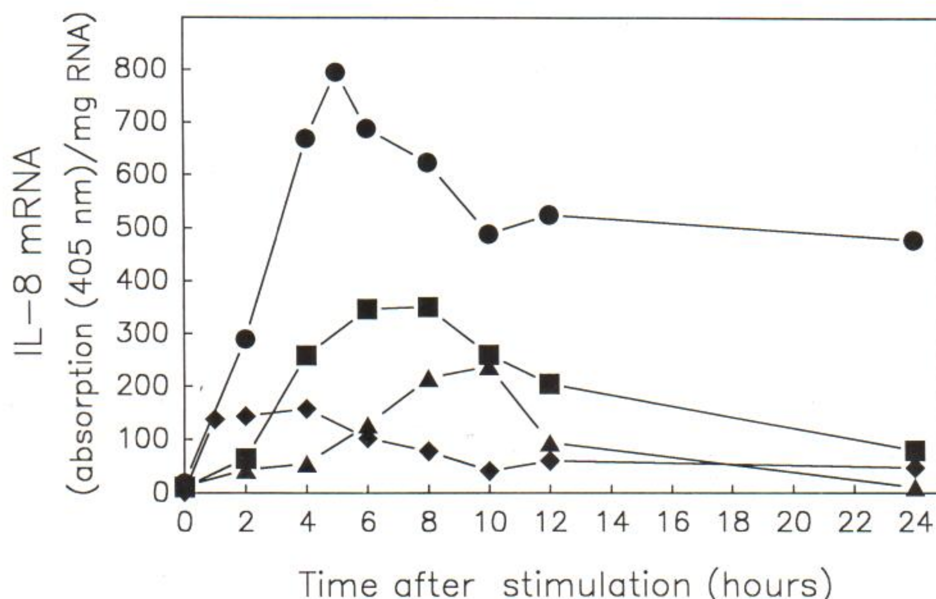


Fig. 3. Time course of IL-8 mRNA in different cell types after IL-1α stimulation. The time course for two cultures of each cell type was determined and each plot represents the average results for the two cultures. (●) endothelial cells; (■) dermal fibroblasts; (▲) monocytes; (◆) normal human keratinocytes.

stimulation, we stimulated one culture of each cell type with LPS. For keratinocytes, monocytes, and endothelial cells the responses were similar to the IL-1α responses, but the fibroblast response was considerably lower than with IL-1α.

3.2.3. Time course studies. It is well known that maximal mRNA synthesis can be found at different time points for different cell types *in vitro*. Since these values may also be relevant for the *in vivo* situation, we wanted to compare maximal amounts of IL-8 mRNA synthesis. We therefore analyzed the time course of IL-8 mRNA accumulation during 24 hours after IL-la-stimulation for the same four cell types. Two cultures of each were analyzed. The time course of IL-8 expression varied considerably (Fig 3). Keratinocytes showed a rapid, but transient response; fibroblasts and monocytes a transient response, slow for monocytes and more rapid for fibroblasts, while endothelial cells responded rapidly and stably over a 24 hour period. After 48 hours, the value for endothelial cells was still high. At most time points the order of relative IL-8 mRNA levels was the same as described above; the endothelial cell response being high, fibroblast and monocyte responses intermediate, and the keratinocyte response low even with ten times stronger stimulation.

These results may reflect specific responses to specific stimuli; for a more general picture further analyses are needed using a whole panel of known stimuli of IL-8 expression. Still

we feel that the information presented here may provide a basis for speculations about the role of these four cell types as producers of skin-derived IL-8.

The demonstration that keratinocytes, the first cells to be encountered by external stimuli, can express IL-8 (Larsen et al. 1989 A, Barker et al. 1990), pointed to this cell type as a candidate for a leading part in IL-8-mediated skin reactions. In our experiments, cultured keratinocytes synthesized very low amounts relative to other cell types. The keratinocyte mRNA response was, however, remarkably rapid and transient, with a decrease before other cell types reached maximum expression, and the very high number of keratinocytes, compared to the other cell types present, could also add to their importance concerning IL-8 expression in the skin. Finally it must be born in mind that in spite of the small IL-8 mRNA expression in and protein secretion from the keratinocytes this cell type may store larger amounts of IL-8 protein intracellularly. The results presented here may suggest a role for the keratinocyte as a weak, but rapidly acting initiator of a reaction later sustained by other cells.

The observation that keratinocytes are less sensitive to IL-1 with respect to IL-8 induction may relate to the fact that IL-1 is found in high amounts in normal epidermis as well as in epidermis in certain skin diseases (Larsen et al. 1988 A+B). However, LPS stimulation also gave a very low keratinocyte response. By contrast, keratinocytes seem to express higher amounts of IL-8 in response to a combination of tumour necrosis factor α (TNFα) and IFNγ (Barker et al. 1990). This could be important in diseases such as allergic contact dermatitis where activated T-cells release IFNγ and the epidermis also contains TNFα.

The responses of fibroblasts and monocytes to IL-1α were stronger than that of keratinocytes, but slower, reaching maximal values only after about 7 and 9 hours, respectively. These slower responses were, however, also transient. After 24 hours, IL-8 mRNA had almost disappeared from monocytes and fibroblasts showed a marked reduction. The transient nature of the responses of keratinocytes, dermal fibroblasts, and monocytes is consistent with a rapid down-regulation of IL-8 mRNA which might be desirable in a potentially damaging reaction.

The most striking response was that of endothelial cells, which was very high after stimulation with both IL-1α and LPS and, in agreement with the findings of others (Sica et al. 1990 A, Strieter et al. 1989 B), rapid and stable over a considerable period. This unique behaviour may suggest a key role of this cell type in IL-8-mediated reactions. Constituting a barrier between circulating cells and dermal and epidermal tissue, vascular endothelial cells occupy an important position in deciding the admittance of inflammatory blood cells to the skin.

The results reported here must be interpreted with the caution always to be exerted, when extrapolating from *in vitro* experiments to the *in vivo* situation. Still, the marked differences we observe in patterns of IL-8 expression between cell types may suggest an elaborate and dynamic cooperativity between involved cells in initiation, maintenance, and down-regulation of IL-8 mediated events.

4. THE ABILITY OF DIFFERENT CELL TYPES TO EXPRESS MONOCYTE CHEMOTACTIC AND ACTIVATING FACTOR (MCAF).

4.1. An introduction to MCAF.

4.1.1. Biochemical properties and molecular aspects of MCAF. The monocyte chemotactic activating factor (MCAF) or monocyte chemotactic protein 1 (MCP-1) was originally purified from the human myelomonocytic cell line THP-1 (Matsushima et al. 1989) cultured in the presence of LPS, silica and hydroxyurea, and from phytohemagglutinin (PHA) stimulated PBMC (Yoshimura et al. 1989 B). MCAF is a potent monocyte chemoattractant and its existence was probably first described by Snyderman et al. (1972), who observed that PBMC stimulated with purified protein derivative (PPD) or PHA produce a monocyte chemotactic factor. Molecular cloning of the genomic DNA for MCAF revealed a gene with 3 exons and 2 introns (Shyy et al. 1990), which is localized to the long arm of chromosome 17 (Rollins et al. 1991 A, Mehrabian et al. 1991) The cDNA for MCAF is 672 basepairs in length (Furutani et al. 1989, Yoshimura et al. 1989 C) and encodes a 99 amino acid precursor MCAF with a 23 amino acid hydrophobic signal sequence. The mature form of the protein consists of 76 amino acids (Robinson et al. 1989), and its basic properties is due to 13 basic aminó acids. SDS-polyacrylamide-gel electrophoresis has revealed peptides with apparent molecular masses of 15 and 13 KDa (Yoshimura et al. 1989 B). They do, however, represent a single gene product, and the difference is probably due to as yet unidentified post-translational modifications. Like IL-8, MCAF contains 4 half-cysteines, but in MCAF the first two of these are adjacent to each other and not separated by a single amino acid. This conserved position of the two first cysteines groups the MCAF protein with other peptides as MIP-1, RANTES, JE, and LD78 which share 25 to 55% homology with MCAF (Leonard and Yoshimura 1990).

4.1.2. Biological actions of MCAF.

Monocytes. MCAF is thought to be responsible for monocyte recruitment in acute inflammatory conditions and may also be an important mediator in chronic inflammation. Its monocyte chemotactic activity *in vitro* (Matsushima et al. 1989, Yoshimura et al. 1989 B) has been confirmed *in vivo* where intracutaneous injection of MCAF into the ears of Lewis rats resulted in monocyte/macrophage recruitment, which started 3 hours after injection and became maximal after 18 hours (Zachariae et al. 1990).

Other aspects of monocyte activation include a rise in the cytosolic free calcium concentration due to influx of extracellular calcium (Sozzani et al. 1991, Bischoff et al. 1992, Rollins et al. 1991), induction of the respiratory burst (Zachariae et al 1990, Rollins et al. 1991 B) in monocytes, and an enhancement of the cytostatic activity of human monocytes against several types of human tumour cell lines *in vitro* (Matsushima et al. 1989, Zachariae et al. 1990).

Similar to the actions of IL-8 on neutrophils, MCAF can upregulate expression of the ß-2 integrins CD11b (Mac-1 sub-

unit) and CD11c on monocytes. Further, MCAF is able to induce IL-1 and IL-6, but not TNF production in monocytes (Jiang et al. 1992). These interesting observations suggest that MCAF may play a role in both cellular immune reactions and responses to acute tissue injury, as a pro-inflammatory mediator, via induction of adhesion molecules and cytokines. As described in the following, we and others have shown that IL-1 is a potent inducer of MCAF expression in monocytes, and this observation implicates a cytokine amplification pathway which might serve to sustain an inflammatory response.

Basophils. MCAF has been shown to be chemotactic to human basophils (Leonard and Yoshimura 1990), and recombinant MCAF can induce histamine release from the same cell type in a concentration range between 10^{-9} and 10^{-6} M (Kuna et al. 1992, Alam et al. 1992, Bishoff et al 1992). Suboptimal doses of MCAF, however, require IL-3 or IL-5 priming of the basophils in order to induce histamin release (Kuna et al. 1992). No difference has been observed in MCAF induced histamine release between allergic subjects and normal persons (Alam et al. 1992). Other aspects of basophil activation via MCAF include a rise in the cytosolic free calcium level and the formation of leukotriene C_4 (LTC_4) in IL-3, IL-5 or GM-CSF primed cells. This last observation draws another parallel to the actions of IL-8 on neutrophils, where Schröder (1989) showed that IL-8 can potentiate the 5-lipoxygenase pathway. A similar action of MCAF on basophils further supports the close relationship between IL-8 and MCAF.

4.1.3. MCAF receptors. So far only monocytes have been reported to express receptors for MCAF. Studies with radioiodinated MCAF indicate that monocytes have approximately 1600 binding sites per cell with a Kd of 1.1 nM (Yoshimura and Leonard 1990 B), and these findings have been confirmed by others (Valente et al. 1991). No binding to neutrophils or lymphocytes was observed. MCAF can down-regulate its own receptor due to internalization, and chemical cross-linking of the MCAF ligand-receptor complex suggests a molecular mass of 40 kDa for one MCAF receptor (Wang et al. 1991). Resent data, however, suggest the presence of at least two different MCAF receptors on human monocytes. Radiolabeling studies have revealed that one unique receptor for MCAF is present on monocytes while another receptor is shared between MCAF and MIP-1 peptides. (Wang JM et al. 1993). As it has recently been shown that MCAF has certain actions on basophils, further studies are needed to investigate whether also this cell type has receptors for MCAF.

4.2. Quantification of IL-1-induced MCAF mRNA in human keratinocytes, fibroblasts, endothelial cells, and monocytes.

Several studies have shown that a significant proportion of the early infiltrating cells in skin diseases such as psoriasis and allergic contact dermatitis are cells of monocytic origin (Gawkrodger et al. 1986, Platt et al. 1983, Bjerke et al. 1978). Although it remains to be shown that MCAF is excreted in response to antigenic stimulation, its secretion by stimulated skin cells and its actions on monocytes suggest that this agonist is a key cell mediator of delayed cutaneous hypersensitivity and other cellular immune reactions. MCAF may therefore play an important role in immunological and inflammatory processes through attraction of monocytes into the skin.

So far, MCAF expression in the skin has not been studied *in vivo*. MCAF is expressed *in vitro* by a number of cell types, either constitutively or upon stimulation including keratinocytes (Barker et al. 1990 and 1991), dermal fibroblasts (Strieter et al. 1989 C, Van Damme et al. 1989, Larsen et al. 1989 B), endothelial cells (Schyy et al. 1990, Strieter et al. 1989 C, Sica et al. 1990 B, Rollins et al. 1990, 1991 C, Dixit et al. 1990), monocytes (Sica et al. 1990 B, Decock et al. 1990), and melanocytes (Zachariae et al. 1991). As their relative potential for MCAF mRNA synthesis is an important factor in understanding the dynamics and interactions between the different cell types involved in inflammatory skin diseases we used our method for quantitating mRNA, described in chapter 3. Monocytes, neutrophils, and lymphocytes are often present together in inflammatory skin diseases. We therefore found it of interest to investigate if there are similarities in the ability of cultures of human keratinocytes, dermal fibroblasts, monocytes, and endothelial cells to synthesize MCAF- and IL-8 mRNA (II,VII). As stimulant we chose rIL-1α, since this compound is known as a potent MCAF inducer in monocytes, fibroblasts and endothelial cells, and because IL-1α and the type 1 IL-1 receptor (IL-1R1) is present in both normal and diseased skin (IV).

4.2.1. MCAF mRNA synthesis by four different cell types. All cultures were established from different individuals. Monocytes were used immediately after purification, the other cell types when they were about 90% confluent. From our experience with IL-8 mRNA synthesis we chose to compare the expression 6 hours after stimulation (Fig. 4). Monocytes, fibroblasts, and especially endothelial cells showed appreciable levels of MCAF mRNA in unstimulated cultures, while keratinocytes did not. All four cell types responded to stimulation with a 2-8 fold increase in MCAF mRNA, but keratinocytes required stimulation with ten times the IL-1α amount used for the other cell types to produce a measurable hybridization signal. Endothelial cells reached levels more than ten times those of keratinocytes, while monocytes and fibroblasts showed intermediate and almost equal amounts (VII).

4.2.2. Time Course studies. The kinetics and maximal ability for MCAF mRNA synthesis in different skin cells may be of importance for the recruitment of monocytes during an immuno-inflammatory reaction. We therefore also analyzed the time course of MCAF mRNA accumulation during 24 hours after IL-1α stimulation of cultures of human keratinocytes, dermal fibroblasts, monocytes, and endothelial cells (Fig. 5). Two cultures of each cell type were analyzed. Keratinocytes and fibroblasts showed a rapid, but transient response, monocytes a slower and more stable response, while endothelial cells responded rapidly and strongly over a 24 hour period. At most time points, the order of relative MCAF mRNA levels was the same: The endothelial cell response being high,

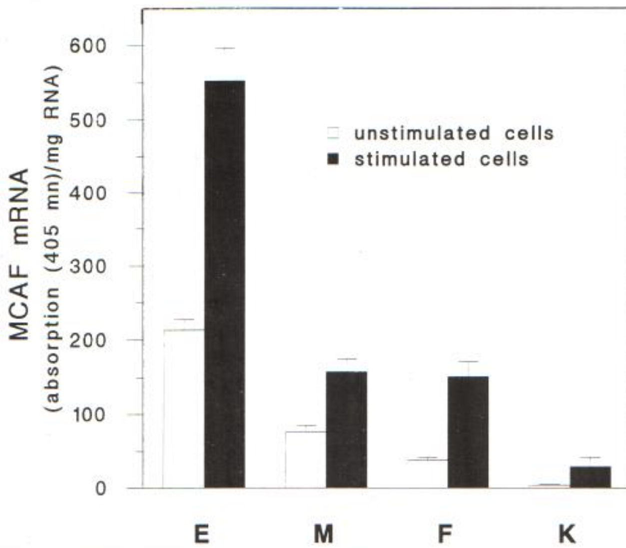


Fig. 4. MCAF mRNA expression in different cell types with and without IL-1α stimulation. Values were determined by the quantitative dot blot method. Each value is the average of triplicate determination for two or three cultures. Black bars, cultures stimulated for 6h with 100 U/ml IL-1α (endothelial cells (E); dermal fibroblasts (F); monocytes (M)) or 1000 U/ml IL-1α primary keratinocytes (K)). Open bars, values for the corresponding unstimulated cultures.

fibroblast and monocyte responses intermediate, and the keratinocytes low (Fig. 5). The fact that keratinocytes can express MCAF mRNA upon IL-la stimulation is a novel observation, and others have failed to demonstrate this (Barker et al. 1991 A). The discrepancy could be due to different cell culture conditions. Another explanation could be that our dot blot technique is possibly more sensitive than the traditional Northern blot method.

We also examined the ability of the virus-transformed human keratinocyte cell line SVK-14 to synthesize MCAF mRNA in time course studies. Interestingly, and in contrast to the normal human keratinocytes, this cell line failed to produce MCAF mRNA under identical conditions, while we have previously shown that SVK-14 cells are capable of IL-8 mRNA synthesis. A possible explanation could be that virus-transformation of cells induce changes on the gene transcriptional level. Therefore results obtained with transformed cell-lines must always be interpreted with caution.

So far, limited data have been published on the kinetics of MCAF mRNA expression in human monocytes. It has been shown that purified, unstimulated monocytes express MCAF mRNA and that LPS can augment this synthesis (Sica et al. 1990). Further, LPS, PHA, and IL-1ß can induce MCAF mRNA synthesis in peripheral blood mononuclear leucocytes (Yoshimura et al. 1989). We found that there was a basal expression of MCAF mRNA in monocytes and that IL-1α stimulation led to an increased synthesis with maximum after 8 hours. Of interest was the observation that after 24 hours of IL-1α stimulation the mRNA level was still quite high. These results place the monocytes in an important position using MCAF as a potent autocrine stimulator, because monocytes have specific receptors for MCAF as described above. To exclude that the constitutive MCAF mRNA synthesis was due to stimulatory factors present in the fetal calf serum or LPS contamination of the growth medium, we examined the IL-8 mRNA synthesis under identical conditions and found very low amounts synthesized. We conclude that unstimulated MCAF mRNA synthesis is not caused by the presence of any compound capable of inducing IL-8 expression such as LPS, IL-1 or TNF. It is, however, well known that monocytes can be activated by adhesion to a plastic surface.

IL-1 and TNF are potent inducers of MCAF mRNA synthesis in human dermal fibroblasts (Larsen et al. 1989, Strieter et al. 1989). The response of fibroblasts upon IL-1α stimulation was rapid, reaching a maximum after 4 hours and was down-regulated to the pre-stimulatory level after 10 hours.

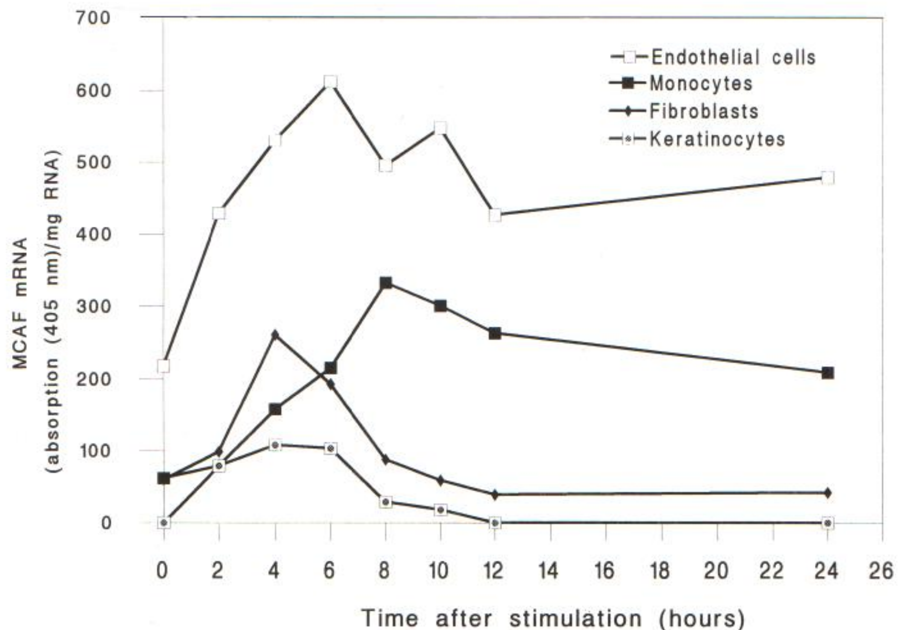


Fig. 5. Time course of MCAF mRNA expression in different cell types after IL-1α stimulation. 1000 U/ml IL-1α for keratinocytes, 100 U/ml IL-1α for the other cell types. Each plot represents the average result for two cultures. The value for each culture is a result of triplicate determination.

There was a fast and strong induction of MCAF mRNA synthesis in endothelial cells, and the expression remained stable in a period up to at least 24 hours. These results suggest an important role for this cell type in MCAF mediated reactions. When comparing the time course patterns for the responses of MCAF and IL-8 mRNA synthesis upon IL-1α stimulation for all the cell types examined striking similarities become clear (Fig. 2-5). Both MCAF and IL-8 mRNA's are synthesized in high amounts by endothelial cells, when compared to other cell types present in the skin. It must however be born in mind that adult dermal microvascular cells may react differently from the umbilical cord vein endothelial cells. Such a possible difference remains to be demonstrated.

These findings support our emerging view that the endothelial cell has a central role in the regulation of recruitment and activation of leucocytes from the blood into the skin, and indicate that endothelial cells take part in the regulation of actions carried out by mediators belonging to the chemotactic cytokine supergene family.

5. DISTRIBUTION OF IL-1α, INTERLEUKIN 1 RECEPTOR ANTAGONIST PROTEIN (IRAP) AND THE TYPE 1 IL-1 RECEPTOR (IL-1R1) IN NORMAL AND PSORIATIC SKIN.

5.1. Introduction.

IL-1 is one of the main endogenous inducers of IL-8 and MCAF in many different cell types, and it is well known that large amounts of especially IL-1α is present in both normal and diseased skin. The actions of IL-1 and its ability to induce expression of other cytokines like IL-8 and MCAF, however, depends on the presence of specific IL-1 receptors on target cells and also on the presence of possible specific IL-1 regulators such as interleukin 1 receptor antagonist protein (IRAP).

In order to clarify the balance and distribution between receptor, agonist and antagonist in the skin we investigated the presence and distribution of IL-1α, the type 1 IL-1 receptor (IL-1R1), and IRAP in normal skin, and compared their distribution and intensity between uninvolved and involved lesional skin obtained from psoriasis patients. Specific monoclonal antibodies and immunohistochemical techniques were used (IV).

5.2. IL-1 in human skin.

The term IL-1 covers two different polypeptides – IL-1α and IL-1ß that bind to the same receptors and have identical biological properties (for review see Dinarello and Schindler 1990). Il-1ß was originally cloned by Auron et al. (1984) and IL-1α by Lomedico et al. (1984). The genes for both forms are localized on chromosome 2 (D'Eustachio et al. 1987) and share 45% homology. Both proteins are synthesized as 31-kDa precursors which are processed into 17 kDa mature proteins with 26% amino acid homology, and both lack a signal or cleavage sequence usually associated with secreted proteins.

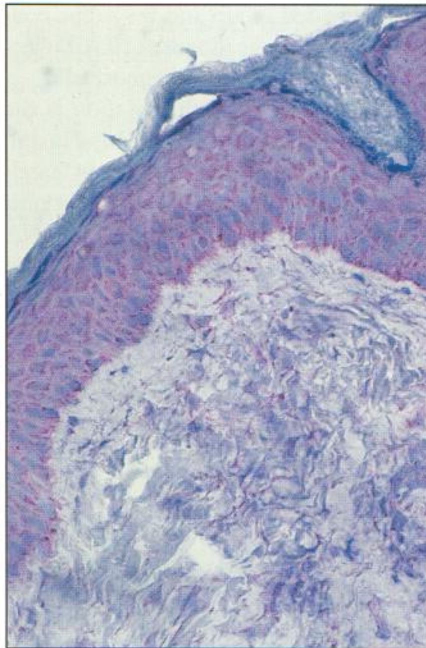
IL-1α remains primarily cell associated in its precursor form, while IL-1ß is released into the supernatant both in its precursor and mature 17-kDa form.

The presence of bioactive IL-1 in normal human epidermis that cross-reacts immunohistochemically with both the IL-1α and IL-1ß forms is now well established (Luger et al. 1981, Sauder et al. 1982, Gahring et al. 1986, Kupper et al. 1986, Larsen et al. 1988A+B, Didierjean et al. 1989). The reported localization of intra-epidermal IL-1 using immunostaining has varied between different studies, probably as a result of technical factors. A recent study involving a range of fixatives and a panel of antibodies has revealed that keratinocytes at various stages of maturation display both membrane-associated and cytosolic IL-1α and ß immunoreactivity (Anttila et al. 1990). In addition, both IL-1α and ß have been detected in the human eccrine sweat-gland apparatus (Reitamo et al. 1990). IL-1α appears to be the major IL-1 species in terms both of immunohistochemical staining (Romero et al. 1989) and of the amount of bioactive material recovered from the epidermis (Camp et al. 1990), although IL-1ß immunoreactivity without corresponding IL-1ß bioactivity has been identified in extracts of normal human heel stratum corneum (Gearing et al. 1990). This is in contrast to cells of monocytic origin, which preferentially produce IL-1ß. The steady state rate of epidermal IL-1α mRNA synthesis appears very low, and the large amounts of epidermal IL-1α may therefore be the result of storage (Didierjean et al 1991). The expression of IL-1 mRNA and IL-1 protein secretion by keratinocytes is enhanced by UV-B irradiation (Oxholm et al. 1988, Kupper et al 1987) and IL-1 may in general play a central role in the immunopathogenesis of skin inflammation (Barker et al.1991). Recombinant IL-1 is pro-inflammatory *in vivo*; IL-1 enhances epidermal Langerhans cell-dependent T cell activation in the skin (Heufler et al. 1988) and induces expression of IL-8 and MCAF mRNA (II, VII) similar to tumour necrosis factor α (TNFα). In addition, IL-1 can stimulate mouse keratinocyte proliferation (Ristow H-J, 1987). Others (Morhenn et al. 1989) have ,however, been unable to demonstrate this effect of IL-1 on human keratinocytes in culture.

There has been a single report of increased IL-1α expression in psoriatic epidermis (Romero et al. 1989), whereas the majority of studies have described a reduction in IL-1 bioactivity (Cooper et al. 1990), although an increase in immunoreactive, but non-functional IL-1ß has been observed (Prens et al. 1990, Cooper et al. 1990). In addition, increased levels of bioactive IL-1 have been demonstrated in epidermal extracts from positive allergic patch test reactions, when compared to normal epidermis (Larsen et al. 1988 B). For these reasons interest has been focused on the potential role of IL-1 and other cytokines such as IL-8 and MCAF in the pathogenesis of psoriasis and other inflammatory skin diseases such as allergic contact dermatitis.

5.3. The interleukin 1 receptors.

Cross-linking studies of radiolabelled IL-1 have shown that several proteins are able to bind IL-1. Of these, however, two IL-1 binding proteins have been prominent. The IL-1R1 is a



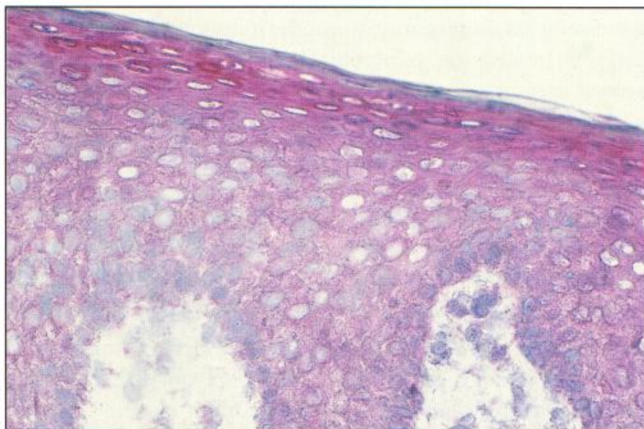
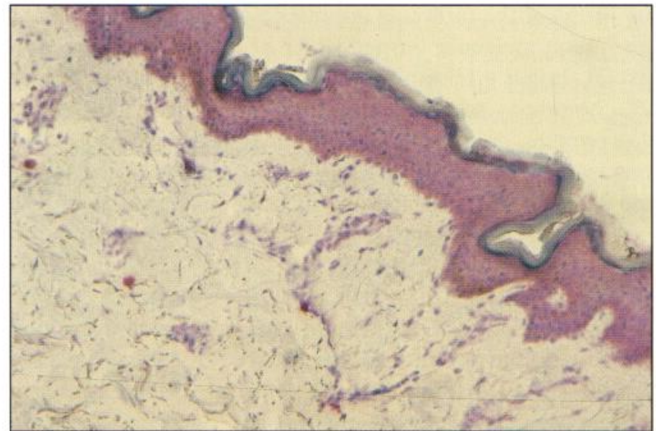


Fig. 6.
(a) Immunoreactive IL-1R in normal human skin (x 250).
(b) IL-1R1 immunoreactivity is distributed throughout the epidermis in involved psoriatis skin (x 250).



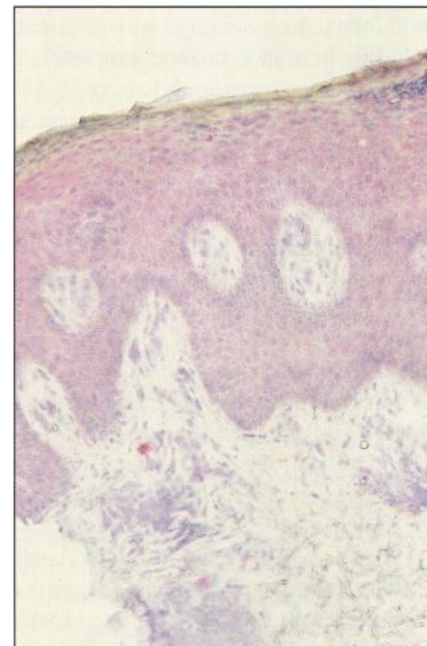


Fig. 7.
(a) Marked staining with anti-IRAP of all the layes of the epidermis in uninvolved psoriatic skin (x 100). (b) Decreased staining intensity with anti-IRAP of the epidermis in involved psoriasis skin (x 100).

522 amino acid protein with a molecular weight of 80 kDa. This receptor, which has recently been cloned in human cells (Sims et al. 1989), is found on T-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes and hepatocytes. The type 2 IL-1 receptor (IL-1R2) is a 68 kDa protein found on B-cell lineages, neutrophils and bone marrow cells (Dinarello CA. 1991).

Both receptors belong to the Ig superfamily with three Ig-like domains in the extracellular segment, a single transmembrane region and a cytosolic region (Dower et al 1990 A, Dower et al. 1990 B, Sims et al. 1988). N-linked glycosylation appears to be essential for optimal binding and activity of IL-1 to the IL-1R1 (Mancilla et al. 1992). The lower molecular weight of the IL-1R2 is due to a shorter cytoplasmatic domain. Although it is generally accepted that both receptors can recognize both IL-α and ß (Kilian et al.1986, Kupper et al. 1988, Matsushima et al. 1986 A), several studies have shown that IL-1α binds best to the IL-1R1 and Il-1ß to the IL-1R2 (Dinarello 1991).

IL-1 bound to the IL-1R1 is internalized and only a small amount is degraded (Matsushima et al. 1986 B). Additionally it has been shown that IL-1 can down-regulate the IL-1R1 expression (Ye et al. 1991). By contrast, IL-1 bound to the IL-1R2 is found in the extracellular fluid in a degraded form and the binding of IL-1 to human neutrophils or bone marrow cells is enhanced when IL-1 is administered (Shieh et al. 1990). These observations indicate that the regulation of surface expression and signal transduction differs for the two IL-1 receptors, and a recent study actually suggests that the IL-1R2 is not able to mediate IL-1 biological responses (Sims et al. 1993).

5.4. Biology, cellular sources and actions of IRAP.

Recent work from several laboratories has led to the purification and cloning of an IL-1 receptor binding protein which inhibits IL-1 activity from human monocytes and the myelomonocytic cell line U937 (Carter et al. 1990, Eisenberg et al. 1990, 1991, Hannum et al. 1990). The cDNA sequence codes for a polypeptide of approximately 17 kDa, whereas lar-

ger forms of the molecule are due to glycosylation. This recombinant molecule, termed IRAP or IL-1 receptor antagonist (IL-1ra), is a 177 amino acid protein which shows 19 and 26% homology to IL-1α and IL-1ß respectively. Unlike the IL-1 proteins, monocyte derived IRAP has a classical secretory leader peptide. The IRAP gene is similar to the IL-1α and ß genes in intron-exon organization indicating that they belong to the same gene family. IRAP is a true IL-1 antagonist, as it is able to bind to both the IL-1R1 (Dripps et al. 1991 A, Carter et al. 1990), and the IL-1R2 (Dripps et al. 1991 B, Granowitz et al. 1991 A) without inducing signal transduction. In addition an intracellular form of IRAP has recently been described. It differs from the IRAP form found in monocytes in the sense that it lacks the leader sequence necessary for secretion and therefore stays intracellularly. This special form of IRAP has been shown to be associated with different kinds of epithelium including human keratinocytes (Haskill et al. 1991). Apart from the cell types mentioned above IRAP has been reported to be expressed by B- and T-lymphocytes (Bajetto et al. 1991) human synovial- and dermal fibroblasts (Krzesicki et al. 1993) and by neutrophil granulocytes (Haskill et al. 1991).

Different signals can induce expression of IRAP. In PBMC and monocytes addition of serum, IgG, LPS, IL-4, transforming growth factor ß (TGFß), or GM-CSF to cells in culture have this effect (Turner et al. 1991, Poutsiaka et al. 1991, Arend et al. 1989, 1991, Shields et al. 1990, Vannier et al. 1992, Wahl et al. 1993, Wong et al. 1993) In human polymorphonuclear cells LPS, IL-4 GM-CSF and TNF induce the expression of IRAP (Re et al. 1993).

IRAP has been shown to inhibit many of the actions carried out by IL-1. In vitro rIRAP is able to block IL-1 augmentation of thymocyte proliferation, IL-1-induced synovial cell prostaglandine E_2 (PGE_2) synthesis and collagenase synthesis from chondrocytes (Arend WP, 1990). IRAP also blocks the production of IL-1 induced IL-1, TNF, and IL-6 from PBMC as well as from purified monocytes (Granowitz et al. 1991 B).

Of special relevance for this thesis is the recent study by DeForge and coworkers (1992) who showed that rhIRAP can inhibit LPS induced IL-8 expression in human whole blood. No effects on LPS induced IL-1α or IL-1ß was observed. In general a large excess of IRAP is required to inhibit the actions of IL-1. This could be due to the fact that only a small percentage of IL-1 receptors (5 %) on target cells has to be activated in order to induce an IL-1 signal transduction (Arend et al. 1990).

Here follows some examples of the actions of IRAP *in vivo*: Injection of rIRAP reduces the mortality from endotoxin induced shock in rabbits and mice (Ohlsson et al. 1990, Alexander et al. 1991), inhibits IL-1 induced fever and sleep in rabbits, prevents pulmonary fibroses elicited in mice by bleomycin or silica (Piguet et al. 1993) and reduces the severity of IL-1 induced synovitis and articular cartilage proteoglycan loss in the rabbit knee (Henderson et al. 1991). It should also be mentioned that intravenous injection of IRAP in humans have been carried out. No changes in the blood chemistry profiles, physical examination, symptoms or mononuclear cell phenotype were observed between the treated group and the saline control group (Granowitz et al. 1992), indicating that IRAP injection is safe and does not significantly affect homeostasis.

Of special interest in terms of skin inflammation is the IRAP form found in keratinocytes. The findings by Haskill and co-workers (1991) have been extended by Gruaz-Chatellard et al. (1991) and Bigler et al. (1992), who showed that normal human keratinocytes in culture synthesize IRAP mRNA and produce large amounts of the intracellular variant of IRAP. This expression is enhanced during differentiation of the cells. In addition, extracts of normal skin also contained biologically active IRAP (Gruaz-Chatellard et al. 1991). Hammerberg et al. (1992) reported that IRAP is present in both normal and psoriatic epidermal cytosol, and that IRAP mRNA is present in both normal and psoriatic skin demonstrated by use of the PCR technique. These observations suggest that mechanisms exist for the regulation of IL-1 bioactivity in the skin.

5.5. Skin biopsies and immunohistological techniques.

Samples of normal perilesional skin (n = 10) were obtained from subjects undergoing minor dermatological surgery procedures. In addition, paired biopsies from involved and uninvolved areas were obtained from individual patients (n = 7) with stable plaque psoriasis. Psoriasis patients were on no systemic therapy, and had been off all local therapy on the biopsy sites for at least seven days.

For immuno-staining we used an enhanced alkaline phosphatase-anti alkaline phosphatase (APAAP) technique based on a method first described by Cordell et al. (1984). All tissue sections were counterstained with haematoxylin and positive staining was visualised by bright pink colouration.

The specificity of the monoclonal antibodies was confirmed by ELISA (IV).

To confirm the specificity of the staining the diluted antibody was preincubated overnight at 4°C, either alone or in the presence of 10 mg/ml of the relevant ligand, i.e. monoclonal anti IRAP antibody with recombinant IRAP, monoclonal anti IL-1α with rIL1α, and monoclonal anti IL-1 receptor with rIL-1α (the binding of this antibody is blocked by ligand, Dr Steven Dower, personal communication). It was possible to totally block the staining for IL-1α and IRAP, whilst the staining for IL-1R1 was strongly inhibited. Mouse IgG_1 (same isotype as monoclonals) was included as a specificity control to exclude Fc binding to the tissue.

5.6. Distribution of IL-1α, IL-1R1, and IRAP in normal human skin.

Staining with anti-IL-1α antibody was predominantly seen in the basal layers of the epidermis in 7 of the 10 biopsies. In the remaining three biopsies staining was distributed throughout the epidermis with the exception of the stratum corneum. The secretory coils of eccrine sweat glands, the sebaceous gland lobules, and upper dermal blood vessels were also stained.

In contrast to anti-IL-1α, epidermal staining with anti-IL-1R1 antibody was detectable within both the basal and upper

layers of the epidermis in 9 of the 10 biopsies. In addition, scattered upper dermal dendritic cells also stained positive in all biopsies. Furthermore IL-1R1 staining was detected in eccrine sweat glands and sebaceous glands as described above for IL-1α. Staining was also found associated with upper dermal blood vessels, and with arrector pili muscle fibres.

The finding that staining for both IL-1α and the IL-1R1 was present on the the same cells provides evidence for the possibility of autocrine stimulation.

Of particular interest was the observation that antibodies directed against IRAP also stained the epidermis and in a similar distribution to that detected with the anti IL-1R1 antibody. Eccrine ducts, sebaceous glands, and an area of arrector pili muscle were also stained. Hammerberg and coworkers (1992) have performed immunoflourescence studies on normal and psoriatic skin and found IRAP protein concentrated in the stratum granulosum of normal epidermis and a more basal-midbasal distribution in psoriatic epidermis. The differences could be due to differences in staining techniques.

Our finding that the inhibitor protein IRAP is present in areas in which the pro-inflammatory cytokine IL-1α is also found, provides evidence in favour of a cytokine regulatory system in the skin. As described above, an intracellular form of IRAP, which lacks the leader sequence, has recently been characterized in cultured epithelial cells including keratinocytes. It has been proposed that it may therefore function as an inhibitor/regulator of intracellular IL-1 (Bigler et al. 1991).

5.7. Distribution of IL-1α, IL-1R1, and IRAP in psoriatic skin.

The overall distribution of staining in both uninvolved and lesional skin from psoriasis patients was not different from normal human skin regarding the antibodies directed against IL-1α, IL-1R and IRAP. However, in psoriatic skin staining with anti-IL1α was seen throughout the living layers of the epidermis in all specimens rather than being predominantly basal; there was prominent staining of papillary blood vessels with the anti-IL-1α antibody. In addition, staining with all three antibodies was detectable in the parakeratotic stratum corneum in lesional psoriatic skin. However, there was considerable inter-patient variation in the intensity of staining with anti-IL-1α. No consistent difference in the degree of expression of IL-1α between uninvolved and lesional skin of psoriasis patients could be determined. Similarly there was no consistent difference in upper and basal epidermal staining for IL-1R1 between uninvolved and psoriasis skin. Fig. 6 shows the distribution of IL-1R1 in normal skin and involved psoriasis skin.

The finding that IL-1α and IL-1R1 is present on the same cell types in the skin provides strong evidence for the possibility of IL-1 induced actions, including induction of IL-8 and MCAF expression, and thereby activating the influx of different inflammatory cell types from the blood and into the skin, which is observed in psoriasis.

In contrast to IL-1α and IL-1R1, there was a consistent trend towards reduced expression of IRAP in the basal and upper layers of lesional skin as compared to uninvolved skin in 6 out of 7 patients with psoriasis (Fig. 7). The biological significance of this finding is at present difficult to interpret, in view of the documented reduction in bioactive IL-1α in psoriatic skin. Reduced expression of IRAP in lesional psoriasis might reflect decreased synthesis and consequent intracellular storage of IRAP.

Another important aspect of these results is that inflammation may not only be induced by upregulation of stimulatory factors, but also by downregulation of specific inhibitors.

6. TUMOUR NECROSIS FACTOR α (TNFα) AND ITS RECEPTORS IN NORMAL HUMAN SKIN AND SKIN FROM PATIENTS WITH PSORIASIS

6.1. An introduction to TNFα.

TNFα is a cytokine with widespread actions in inflammatory and autoimmune skin diseases as recently reviewed (Wakefield et al. 1991). The human TNFα cDNA gene codes for a mature polypeptide of 157 amino acids, preceded by a 76 amino acid long sequence (Pennica et al. 1985). The active form of TNFα is a trimer with a total molecular mass of app. 55 kDa (Smith and Baglioni 1987). TNFα is produced by keratinocytes (Köck et al. 1990), dermal dendritic cell (Nickoloff et al. 1991), macrophages, and lymphocytes (Tracey et al. 1989) and acts on these and other cell types present in skin after binding to specific receptors.

TNFα inhibits keratinocyte and endothelial cell proliferation (Pillai et al. 1989, Detmar et al. 1989 A+B), and stimulates the growth of fibroblasts (Vilcek et al. 1986). It also induces neutrophil mediated injury of cultured human endothelial cells (von Asmuth et al. 1991), and expression of intercellular adhesion molecule-1 (ICAM-1) on keratinocytes and endothelial cells (Detmar et al. 1989 B, Detmar and Orfanos 1989). This induction can be synergized in the presence of IFN-γ, and ICAM-1 expression has been shown to correlate with the lymphocytic infiltration in psoriasis and other skin diseases (Griffith et al. 1989). As described for IL-1, TNFα is one of the main endogenous inducers of IL-8 expression in human keratinocytes, dermal fibroblasts, endothelial cells and monocytes (Larsen et al. 1989 A, Strieter et al. 1989 B, Matsushima et al. 1988). These findings indicate that TNFα plays an important role in inflammatory skin diseases, such as psoriasis, where lymphocytes and neutrophils are involved.

6.2. The TNF receptors.

TNFα and TNFß or lymphotoxin bind to the same two receptors: The 55 kD and the 75 kD TNF receptor (p55 TNF-R and p75 TNF-R respectively) which have recently been cloned and expressed by a number of groups (Nophar et al. 1990, Smith et al. 1990, Dembic et al. 1990, Himmler et al. 1990, Loetscher et al. 1990, Schall et al. 1990). They both belong to the same family of receptors including the nerve growth factor receptor and the B-cell surface antigen CDw40 due to similarities of their extracellular domains (Dembic et al. 1990).

TNF receptors are present on nearly all cell types with a

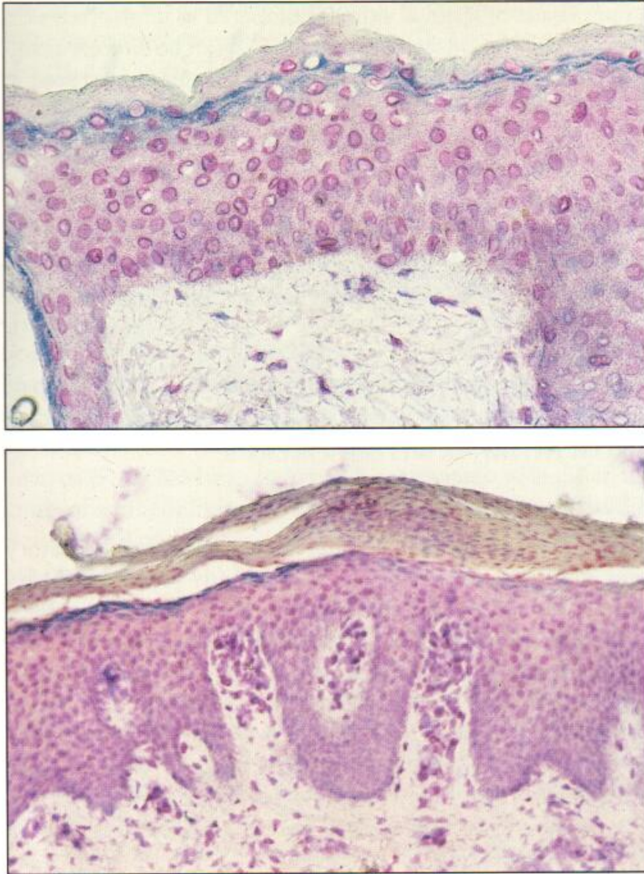


Fig. 8. (a) Distribution of immunoreactive p55 TNF-R in normal human skin detected by the htr-9 antibody. Staining is present throughout the viable epidermis and in association with upper dermal dendritic cells (x 350). (b) p55 TNF-R immunoreactivity is distributed to all the layers of the epidermis, including the parakeratotic stratum corneum, in involved psoriasis skin. Note perivascular cell-associated staining within the papillary dermis (x 100).

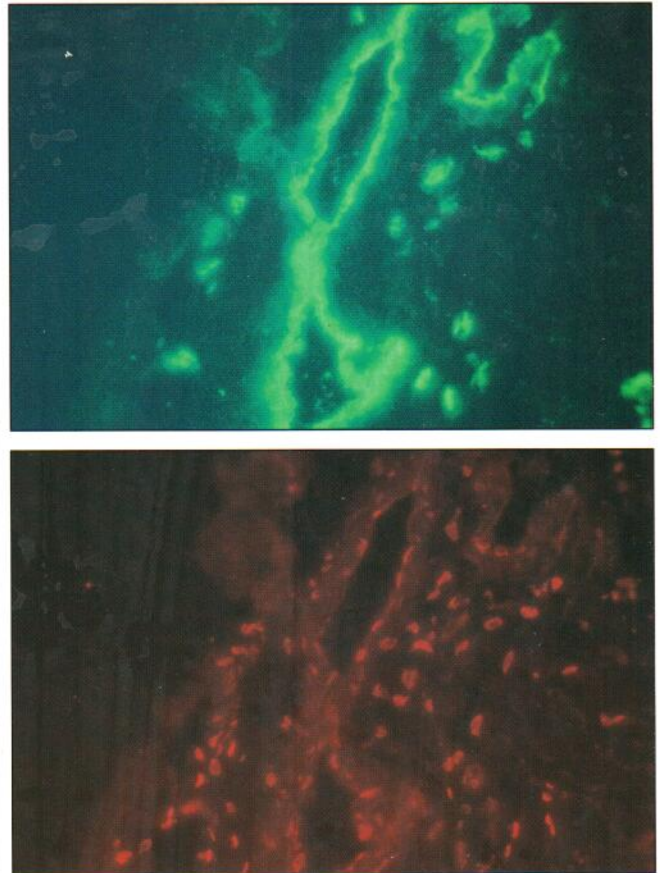


Fig. 9. Colocalization of staining on dermal blood vessels from lesional psoriasis skin, with antibodies to (a) the endothelial cell marker von Willebrand factor, and (b) p55 TNF-R (htr-9) (x 385).

few exeptions such as erythrocytes. Resting T-cells express only very few receptors. The p55 TNF-R seems to be ubiquitous and occurs, amongst others, on peripheral blood monocytes (Scheurich et al. 1989), keratinocytes (Trefzer et al. 1991), fibroblasts (Espevik et al. 1990), endothelial cells (Espevik et al. 1990, Shalaby et al. 1990), NK-cells (Naume et al. 1991), and several adenocarcinoma cell lines (Brockhaus et al. 1990). By contrast, the p75 TNF-R seems more restricted to cells of hematopoietic origin and is strongly and preferentially expressed upon induction of NK-cells (Naume et al.1991), and T- and B-cells (Fiers W. 1991, Erikstein et al. 1991, Heilig et al. 1991). Resent data suggest that different actions can be signalled through the two TNF receptors. Wong et al. (1992) showed that the antiviral activity of TNF is only mediated via the p55 TNF-R and biological active anti-p55 TNF-R antiserum induced proliferation of MRC-5 fibro-

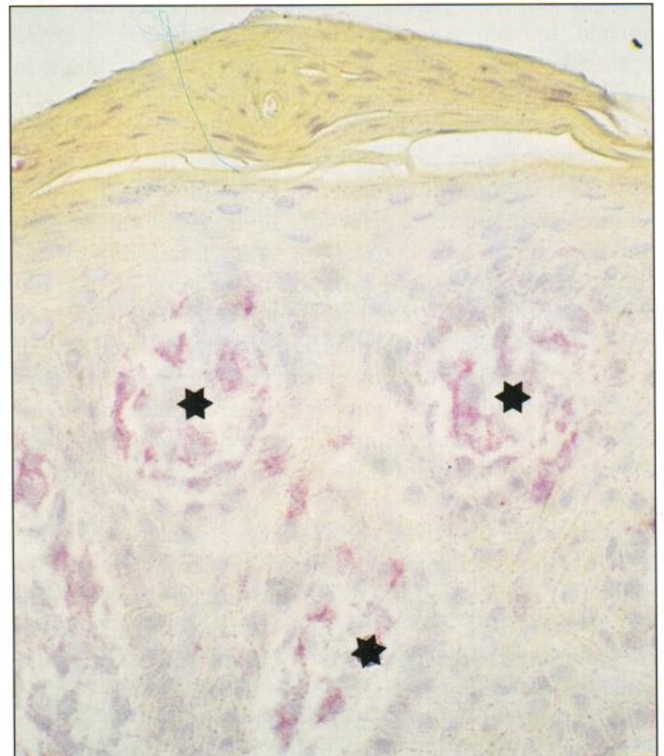


Fig. 10. Immunoreactive p75 TNF-R is localized to the perivascular region in the dermal papillae of lesional psoriatis skin (utr-1 antibody) (x 350).

blasts while anti-p75 TNF-R antiserum did not have this effect (Abe et al. 1993).

In addition to the membrane bound TNF receptors a group of soluble TNF binding proteins have been described. Some of the TNF binding proteins are probably soluble forms of the extracellular cytokine binding domains of the membrane bound TNF receptors (Lantz et al. 1990, Nophar et al. 1990). They may be important agents that can block harmful effects and functions of TNF by competing with TNF for the membrane bound TNF receptors (Engelmann et al. 1989, Lantz et al. 1990, Olsson et al. 1989). These actions draw a parallel to the actions of IRAP on IL-1 activity described earlier.

Previous studies have indicated that the skin contains a very large number of TNF receptors (Beutler et al. 1985). Recent data show that normal human keratinocytes in culture only express the p55 TNF-R (Trefzer et al. 1991). The exact cellular localization of the TNF receptors in the skin in situ has, however, not been described before and is of importance in understanding the pathogenesis of inflammatory skin diseases, where TNFα and mediators induced by TNFα such as IL-8 and MCAF are involved.

To address this question we investigated the presence and distribution of TNFα and its two receptors, the p55 TNF-R and the p75 TNF-R, in normal skin, and compared their distribution and intensity between uninvolved and lesional skin obtained from patients with psoriasis, using specific mono- and polyclonal antibodies (V).

6.3. Immunohistochemical staining and specificity controls.

A specific polyclonal rabbit anti-TNFα antibody was isolated from serum of rabbits immunized with recombinant TNFα, as previously described (Chu et al. 1991). We also used mouse monoclonal antibodies directed against p55 TNF-R (htr-9) and p75 TNF-R (utr-1) The utr-1 and htr-9 antibodies both compete with TNFα and Lymphotoxin for binding to the p75 TNF-R and p55 TNF-R respectively. They therefore only bind to TNF receptors which are not occupied by TNF. Additionally, two mouse MoAbs against the p55 TNF-R (TBP-1 and TBP-2) were used. The immunohistochemical staining was carried out as previously described (V) by use of the enhanced APAAP technique.

The following controls were included for the specificity of the immune reaction: Substitution of the primary TNF receptor antibodies with mouse IgG1 (same isotype as the monoclonals) resulted in negative staining. The immunoreactivities of the TNFα and p55 TNF-R antibodies were totally blocked when preincubated with 10 μg/ml of rhTNFα and recombinant soluble p55 TNF-R respectively. Further, the TNF receptor antibodies are very well characterized, and in particular, the staining intensity obtained with the p75 TNF-R (utr-1) and the p55 TNF-R antibody is reduced to background level, when tissue sections are pre-incubated with recombinant TNFα (Brockhaus et al. 1990).

6.4. Localization of TNFα and its receptors in normal and psoriatic skin.

We have documented for the first time (V) that the p55 TNF-R is present in all the living layers of the normal epidermis and is also associated with eccrine sweat glands, sebaceous glands, hair follicles, errector pili muscles and upper dermal dendritic cell (Fig. 8). Uninvolved and lesional skin from psoriatics stained in a similar fashion (Fig 8). In lesional psoriasis skin there was an upregulation of expression of the p55 TNF-R in association with upper dermal blood vessels, confirmed by double label immunofluorescence staining with anti-p55 TNF-R and anti-von Willebrand factor antibodies (Fig. 9). The importance of this finding is further underlined by resent data indicating that the TNFα-induced cell adhesion to human endothelial cells *in vitro* is under dominant control of the p55 TNF-R. (Mackay et al. 1993).

In contrast, the p75 TNF-R was not expressed by either normal or psoriatic epidermis. Staining for the p75 TNF-R was only present in eccrine sweat glands and in dermal dendritic cells in normal and uninvolved psoriasis skin. Of interest was the novel observation that the upper dermal blood vessels in the dermal papillae of lesional psoriasis skin also expressed the p75 TNF-R (Fig. 10).

TNFα was predominantly localized to the basal cell layers of the epidermis in normal and uninvolved skin from psoriatics whereas in affected skin a mostly basal, but more widely distributed staining of the epidermis occurred. No marked difference in the level of epidermal TNFα expression could, however, be observed. TNFα was also found in eccrine sweat glands, dermal blood vessels, upper dermal dendritic cells, sebaceous glands and errector pili muscles. Of interest was the observation that in 4 out of 7 lesional skin biopsies, there was also prominent staining for TNFα associated with upper dermal blood vessels.

In conclusion: Our findings that the p55 TNF-R is present in epidermis, where TNFα is also distributed, provides evidence that the actions of TNFα on epidermal cells *in vivo* is carried out via the p55 TNF-R. Further, as TNFα can induce the expression of IL-8, MCAF, and adhesion molecules on vascular endothelium, an upregulation of both TNFα and its two receptors in association with upper dermal blood vessels and perivascular inflammatory cells indicates an important role for TNFα in promoting recruitment of inflammatory cells into the inflamed psoriasis skin.

7. REGULATION OF INTERLEUKIN 8 EXPRESSION IN THE SKIN.

7.1. Possibilities for therapeutical modulation of IL-8 levels in inflammatory and autoimmune diseases.

As described in the previous chapters IL-8 works primarily as a pro-inflammatory mediator taking active part in different inflammatory diseases. The expression of IL-8 is therefore an obvious target for therapeutical intervention, emphasized by the fact that the acute neutrophil granulocyte accumulation in LPS induced skin inflammation can be strongly inhibited

by simultaneous intravenous administration of IL-8 antibodies (K. Matsushima, personal communication).

Some cytokines possess the ability to inhibit IL-8. Standiford and co-workers (1990 A+B) showed that IL-8 expression from stimulated human monocytes was inhibited by IL-4, whereas no effect could be observed on endothelial cells or fibroblasts. In addition, the recently discovered IL-10 has also the ability to inhibit IL-8 mRNA synthesis (de Waal Malefyt et al. 1991).

Glucocorticoids have for many years been used as some of the most potent anti-inflammatory and immuno-suppressive drugs, and several studies have investigated the *in vitro* effects of these drugs on IL-8 expression. Dexamethasone have been shown to suppress the IL-8 production in PBMC (Mukaida et al. 1989, Seitz et al. 1991), in human lung fibroblasts (Tobler et al. 1992), and in the human fibrosarcoma cell line 8387 (Mukaida et al. 1992). Further, the actions of glucocorticoids *in vitro* have been confirmed *in vivo* where intraarticular injection of betamethasone results in an almost total inhibition of spontaneous IL-8 production in synovial fluid mononuclear cells (Seitz et al. 1991). Another immuno-suppressive drug, cyclosporin A, can inhibit IL-8 mRNA expression in activated T-cells (Zipfel et al. 1991), and in human keratinocytes (Kristensen et al. 1991). Finally, we have described an inhibitory effect on IL-8 mRNA synthesis and protein release in endothelial cells *in vitro* by the anti-inflammatory drugs D-penicillamine, gold sodium thiomalate, and sulphasalazine (Deleuran et al. 1992).

As, however, side-effects accompany treatment with both steroids and cyclosporin A, development of drugs with equal anti-inflammatory potency, but less toxicity is wanted. In the following I describe our studies of the potent inhibitory effect of a new synthetic leukotriene inhibitor ETH615 (Kirstein et al. 1991) and active vitamin D (calcitriol) on IL-8 expression in different cell types relevant for inflammatory skin diseases.

7.2. Inhibition of IL-8 production and function by the synthetic leukotriene inhibitor ETH615.

Both IL-8 and leukotrienes have been suggested to act as inflammatory mediators during the development of skin diseases (Fogh K. 1990, Thomsen MK. 1991, Gillitzer et al. 1991). Although this system was previously regarded as a separate biochemical pathway of cell activation during inflammation, recent research suggests that important interactions exist between IL-8 and arachidonic acid metabolites. In 1989 (A) Schröder reported the stimulating capacity of IL-8 on leukotriene B_4 production by human neutrophils, and enhanced production of 15-HETE under identical conditions has also been described (Fogh et al. 1992). It has been demonstrated that LTB_4 can stimulate production of IL-1 (Rola-Plezczynsky and Lemaire 1985) and that lipoxygenase products are critical in the activation of murine thymocytes by IL-1 (Dinarello et al. 1983). These observations suggest the existence of regulatory circuits involving 5-LO products as well as cytokines, indicating that both groups of mediators may enhance and possibly even substitute for some of each other's functions such as the promotion of leukocyte chemotaxis. Therefore, an inhibition of the function and/or production of IL-8 and the 5-LO product LTB_4, is a desirable feature of anti-inflammatory drugs.

Recently, Kirstein et al. (1991) described the inhibitory effect of a group of synthetic substances, quinolyl-methoxyphenylamines, on leukotriene biosynthesis and LTB_4-directed canine neutrophil chemotactic activity. We tested one of these compounds, ETH615 (4-(2-quinolylmethoxy)-N-(3-fluoro-

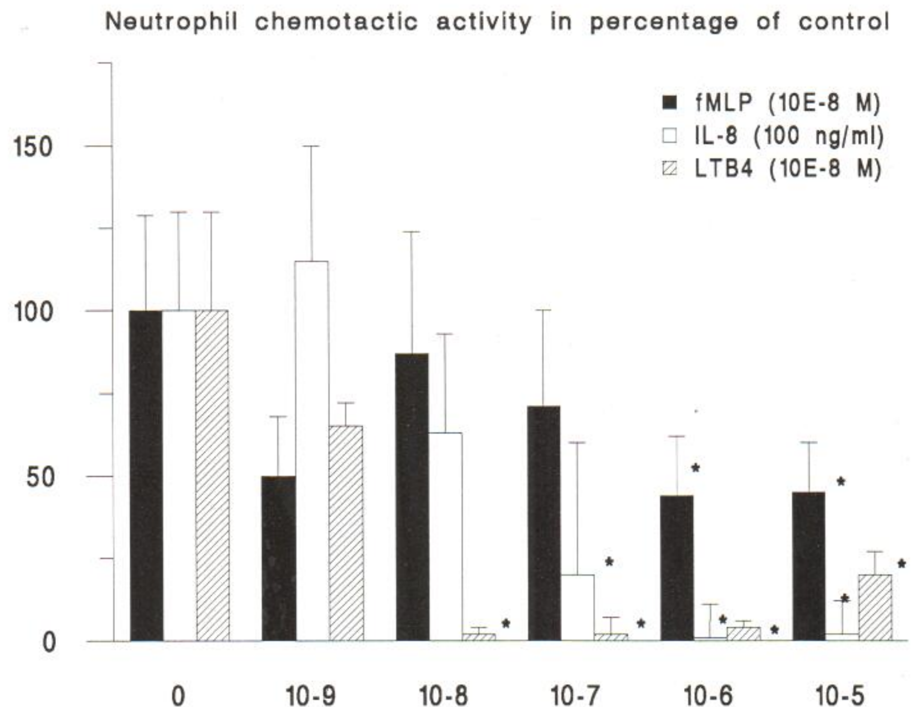


Fig.11. Relative inhibitory effect of ETH615 when added to neutrophils 30 min before chemotactic migration towards IL-8 (100 ng/ml), LTB_4 (10^{-8} M) or fMLP (10^{-8} M). Migratory response was calculated as chemotactic index and the data are brought as percentage of control. Thus, 100% equals no use of inhibitor (ETH615), i.e. maximum chemotactic activity of either IL-8, LTB_4 or fMLP. (* $p < 0.01$).

benzyl)-phenyl-aminomethyl-4-benzoic acid) for its effect on the IL-8 mRNA synthesis and protein release from human PBMC, as well as its actions on neutrophil and T-cell chemotaxis against IL-8, LTB_4 and f-Met-Leu-Phe (fMLP) (VI).

Normal human PBMC (1 x 10^6 cells/ml) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 2% FCS. ETH615 dissolved in DMSO was added to the cultures in varying concentrations 30 min before adding 10 μg/ml LPS. After 3 hours of incubation cells were isolated and fixed for IL-8 mRNA detection as described before (I). For determination of IL-8 protein levels the cells were cultured overnight before the supernatants were isolated and analyzed by a specific ELISA.

ETH615 inhibited in a dose-dependent manner the expression of IL-8 mRNA in LPS-stimulated normal human PBMC without decreasing the cell viability at any concentration between 10^{-5}M and 10^{-8}M judged by the trypan blue exclusion test. (VI).

Using a specific ELISA-technique (Ko et al. 1992) it was established that the inhibition of IL-8 mRNA expression by ETH615 was accompanied by a moderate inhibition of the secretion of IL-8 from LPS-stimulated PBMC cultured overnight (IV).

Normal human PMN and T-cell chemotaxis was carried out using the 48 well Microchamber chemotaxis assay (VI). ETH615 also reduced the migratory response of neutrophils towards IL-8 (100 ng/ml) as well as LTB_4 (10^{-8}M), when neutrophils were pre-incubated with ETH615 for 30 min before the chemotaxis assay was carried out. The inhibitory effect of ETH615 was dose-dependent when using either of the chemoattractants. Concentrations of ETH615 between 10^{-7} and 10^{-5}M gave almost complete inhibition of the chemotactic response towards either of the chemoattractants (Fig. 11). The inhibitory effect on fMLP directed neutrophil chemotaxis was not as pronounced and higher concentrations of ETH615 were needed.

By contrast, no effect of ETH615 could be detected on the migratory response of T-cells against IL-8, LTB_4 or fMLP.

The observation that a 5-LO inhibitor also inhibits the production of a cytokine is not unique, since Marshall et al (1991) recently reported the potent IL-1 inhibitory effect of the synthetic 5-LO inhibitor SK&F 105561, and corticosteroids can also inhibit the production of both cytokines and metabolites of arachidonic acid.

In conclusion, we have demonstrated the dual inhibitory effect of ETH615 on IL-8 expression and biological activity as well as LTB_4 (Kirstein et al 1991). These results support the concept of a cytokine-leukotriene regulatory circuit.

Recent observations additionally have demonstrated the local anti-inflammatory effect of ETH615 on zymosan-induced dermatitis in dogs (Thomsen et al. 1991). ETH615 may therefore be a potential therapeutic agent with an effect in inflammatory skin diseases, and preliminary studies have revealed a small, but significant beneficial effect on eczema caused by nickel allergy when ETH615 was applied topically (K. Thestrup-Pedersen, personal communication).

7.3. $1{,}25(OH)2\text{-}D_3$ can regulate IL-1α induced IL-8 expression and production in human keratinocytes, dermal fibroblasts, and peripheral blood mononuclear cells.

For many years it has been known that the skin takes part in the synthesis of vitamin D_3 (Holick MF. 1983). In the epidermis cholecalciferol is formed from previtamin D_3 (7-dehydrocholesterol) on exposure to sunlight, but in addition, keratinocytes possess the capacity to convert 25 hydroxyvitamin D_3 into the active vitamin D_3 form (1,25-dihydroxyvitamin D_3 or calcitriol) (Bikle et al. 1986). Calcitriol is present in plasma and acts via a specific cytosol/nuclear receptor for calcitriol, which belongs to the steriod receptor family (Baker et al. 1988). This receptor has been found in almost all tissues examined (DeLuca and Schnoes 1983), indicating that the physiological role of calcitriol is not limited to calcium homeostasis. Several studies have shown important interactions with the immune system. Activated human macrophages can produce calcitriol in the absence of 1-α hydroxylase activity (Rigby and Waugh, 1992). It has been de-

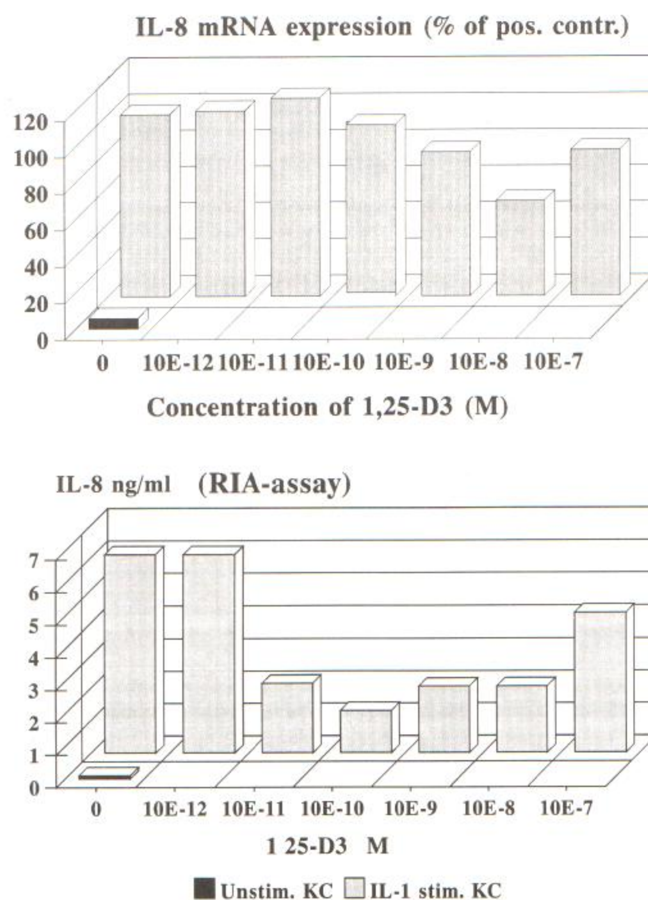


Fig 12. (a) Expression of IL-8 mRNA in cultured normal human keratinocytes stimulated with 1000 U/ml rIL-1α and inhibited by varying concentrations of $1{,}25(OH)2\text{-}D_3$. Data are brought as percentage of the positive control, i.e. cells stimulated with rIL-1α without addition of $1{,}25(OH)_2\text{-}D_3$. The cells were harvested after 3 hrs. (b) Concentrations of IL-8 protein in the media of normal human keratinocytes treated similarly as described under (a).The supernatants were harvested after 16 hrs. Data are representative of three similar experiments in both (a) and (b). .

monstrated that calcitriol can stimulate phenotypic and functional changes on monocytes (Lemire et al. 1984) and increase cellular adherence (Amento EP. 1987). Other actions on monocytes include decreased expression of class II HLA-DR and CD4 antigen expression, and a marked reduction in the capacity to present antigen (Rigby et al. 1990).

It has also been shown that calcitriol can inhibit IL-2 production in CD4+ cells from rheumatoid arthritis patients and controls, and it also inhibits the proliferation of CD4+ cells after stimulation with phytohemagglutinin (Gepner et al 1989). A suppression of IL-2 and IFNγ mRNA synthesis by activated human T-lymphocytes is observed in the presence of calcitriol (Rigby et al. 1987). Further, calcitriol is able to inhibit the effect of IL-1 on thymocyte proliferation, but not the production of IL-1 by LPS-stimulated PBMC (Muller et al.1988) or monocytes from patients suffering from rheumatoid arthritis (Gepner et al. 1989).

Concerning specific actions of calcitriol in the skin a recent study demonstrated that the vitamin D_3 receptor (VDR) is expressed in all the layers of the epidermis in normal skin, and upregulated in psoriatic skin. VDR expression was also found on Langerhans cells and melanocytes, and on macrophages, T-cells and endothelial cells in the dermal compartment (Milde et al. 1991). Calcitriol is known to inhibit proliferation and induce differentiation of keratinocytes in culture (Smith et al. 1986, Kragballe and Wildfang 1990), and these effects have also been demonstrated for the synthetic vitamin D_3 analogue calcipotriol (Kragballe and Wildfang 1990). Further, the proliferation of dermal fibroblasts is inhibited in the presence of calcitriol (Maclaughlin et al. 1985). Both topical and oral treatment with calcitriol (Morimoto et al. 1986, Smith et al. 1988) and synthetic calcitriol analogues (Kragballe K. 1989) have proved useful in the treatment of psoriasis. This beneficial action could mainly be due to the effects on epidermal cell proliferation. We speculated, however, that a possible inhibitory effect on IL-8 expression and thereby on the strong neutrophil accumulation in psoriatic skin and joints would add to the therapeutical effect of calcitriol in psoriasis. We therefore investigated the actions of calcitriol on IL-8 mRNA synthesis in and protein secretion from cultured keratinocytes, dermal fibroblasts, PBMC, and endothelial cells stimulated with rhIL-1α (III).

All the cell types were cultured in DMEM in the presence of 2% FCS. Calcitriol dissolved in ethanol was added to the cultures in varying concentrations 30 min before adding 100 U/ml rhIL-1α. Keratinocytes were stimulated with 1000 U/ml rhIL-1α, due to the reduced sensitivity of keratinocytes towards IL-1α stimulation with respect to IL-8 induction (II, Larsen et al. 1989 A). For RNA studies the cells were isolated and fixed after 3 hours of incubation, and for IL-8 protein determination the supernatants were harvested after incubation overnight. The amount of specific IL-8 mRNA was measured by use of our quantitative Dot Blot technique (II).

A maximal inhibition of calcitriol was observed on the IL-8 mRNA level when calcitriol was added to the culture medium in concentrations of 10^{-8} M for keratinocytes (Fig. 12), 10^{-10} M for PBMC, and 10^{-11} M for fibroblasts (III). By contrast, no effect of calcitriol was observed on endothelial cells, which, as described above, are very potent producers of IL-8.

Concerning IL-8 protein secretion from the same cell types, measured by a specific IL-8 RIA (Smyth et al. 1991) a similar pattern was seen. Maximal inhibitory concentrations of calcitriol was 10^{-10} M for keratinocytes (Fig. 12) and PBMC, and 10^{-11} M for fibroblasts. Again no effect was observed on endothelial cells (III). The lower concentration of calcitriol required to induce maximal inhibition of IL-8 release in keratinocytes, when compared to the mRNA synthesis, suggests that the translational process is also affected. The missing effect of calcitriol on IL-8 expression in endothelial cell is at the moment difficult to interpret, but could be due to a difference from the other cell types in terms of vitamin D receptor expression. This, however, remains to be investigated.

In conclusion we have demonstrated that calcitriol, apart from regulating mineral homeostasis as well as the growth and differentiation of cells, also interacts with and regulate a cytokine pathway responsible for the accumulation of leukocytes during inflammation. Our results encourage the development of new synthetic calcitriol analogues with potent effects on the immune system and less effect on the calcium metabolism if possible.

8. THE USE OF DIFFERENT CYTOKINE ASSAYS.

The role of cytokines in the pathogenesis of skin inflammation can be demonstrated by the use of different cytokine assays. It is, however, important to bear in mind the advantages and limitations of a certain assay when results are interpreted. In the present thesis different techniques have been used in order to seek information concerning the importance and interactions of different cytokines in inflammatory skin diseases. The following contains a general view on the different methods used.

The presence of specific mRNA for a mediator in cells indicates an active ongoing gene transcriptional process at the time when the cells are studied. In most cases mRNA synthesis is followed by detectable protein production (II), and therefore provides useful information on the kinetics of a given cytokine response. It is, however, important to remember that this is not always so (Schall et al. 1992). Possible mechanisms for the lack of detectable cytokine protein following mRNA synthesis include absorption to receptors (Symons et al. 1988), degradation by enzymes, inhibition by antagonists or translational regulation by other cytokines (Feldmann et al. 1991).

The presence of cytokine protein can be measured in different ways: By bioassays, by immuno-chemical techniques, or by immunohistochemistry. The bioassays have the advantage that they ensure the cytokine is functional and not degraded or neutralized. Their disadvantage is that they are often not molecule-specific, but detect the activity of several cytokines. It is therefore important to use neutralizing antibodies raised against recombinant cytokines to demonstrate the specificity of the assay. Proper RIA and ELISA assays are molecule-specific and therefore give accurate measurements of

the presence of a given cytokine. They do, however, not distinguish between biologically active and inactive substances and reflect the net outcome of produced, absorbed and degraded cytokine protein.

Immunohistochemistry differs from the above mentioned methods in the way that it is not directly quantitative. It does, however, allow localisation of cytokine producing cells in tissues or cytospins, providing information about the complex dynamics of cytokine interactions in inflammatory sites. When immunohistochemical methods are used it is crucial to include proper controls to avoid the presence of unspecific staining. Other problems include diffusion artifacts, destruction of epitopes by the used fixative and masking of epitopes by cross-linking fixatives (Anttila et al. 1990). These problems can often be solved by experimenting with a range of fixatives at different temperatures and fixation times, by enzyme treatment of the tissues and by the use of a number of specific antibodies.

In future studies it will therefore be valuable to use different techniques and approaches for cytokine measurement as every method has its advantage and limitation. Taken together the methods can provide useful information on the complex cytokine interactions involved in inflammation.

9. CONCLUSIONS.

This thesis demonstrates the ability of different cell types in human skin to express the chemotactic cytokines IL-8 and MCAF. Further it describes the presence of IL-1 and TNF, the potent endogenous inducers of IL-8 and MCAF, and their receptors in normal and diseased skin. The possibilities for autocrine and paracrine stimulation are discussed. Finally, the regulatory effect on IL-8 expression and biological function of different drugs and endogenous mediators is presented.

On the basis of the obtained data the following conclusions can be made:

1. It is possible to isolate sufficient amounts of pure and undegraded RNA from epidermal suction blisters to look for the presence of specific mRNA species. This method can provide useful information on the *in vivo* expression of epidermal mediators, and avoids the presence of a possible contamination with dermal components.

2. By use of a new quantitative dot blot technique it is demonstrated that IL-1α stimulated endothelial cells *in vitro* synthesize large amounts of IL-8 mRNA compared to keratinocytes. IL-1α stimulated monocytes and dermal fibroblasts express intermediate amounts.

3. When studying MCAF we observed a similar pattern of changes as for IL-8 when the above mentioned four cell types were tested for their ability to express MCAF mRNA upon IL-1α stimulation. These results indicate a common pathway of activation for IL-8 and MCAF, which both belong to the inflammatory peptide supergene family.

4. By immunohistochemical techniques it is demonstrated that IL-1α, the IL-1R1, and IRAP is present in the same cell types in both normal and psoriatic skin *in vivo*. These observations create the possibility of both stimulation and inhibition of IL-1 mediated actions. A down-regulation of IRAP expression was observed in psoriatic lesional epidermis.

5. TNFα and the p55 TNF-R are expressed in both normal epidermis and epidermis from involved psoriasis skin *in vivo*. By contrast, the p75 TNF-R is not expressed in the epidermis. These results indicate that the actions of TNFα in the human epidermis are carried out via the p55 TNF-R.

6. A known 5-LO inhibitor, ETH-615, is shown to inhibit IL-8 expression and biological function *in vitro*. These results support the concept of a cytokine-leukotriene regulatory circuit and may add to the explanation why ETH-615 inhibits zymosan-induced dermatitis in dogs.

7. The active compound of vitamin D_3, calcitriol, is demonstrated to inhibit IL-8 mRNA expression and protein release in human keratinocytes, dermal fibroblasts, and PBMC *in vitro*. These effects could add to the beneficial effect of this compound due to its regulatory effects on cell proliferation and differentiation, observed in the treatment of psoriasis.

10. PERSPECTIVES AND FUTURE STUDIES.

Chronic skin inflammation may occur due to a dysregulation of the cytokine cascade in cells. As described in this thesis large variations exist in the ability of different cell types to express the chemotactic cytokines IL-8 and MCAF. Whether the ability to regulate IL-8 or MCAF expression will influence the risk for an individual to aquire a chronic inflammatory skin disease remains to be shown.

Future studies should also focus on the characterization of specific cytokine regulators and possible targets for therapeutical intervention. We are at present examining the effects of IL-10, also known as cytokine synthesis inhibitory factor (CSIF). We have shown that IL-4 and IL-10 can down-regulate IL-8 production in chronic activated synovial joint fluid cells from patients suffering from rheumatoid and psoriatic arthritis (Deleuran et al. 1993). In addition, IL-10 can selectively inhibit the chemotactic response of purified CD4+ T cells towards IL-8 (Jinquan et al. 1993) and it inhibits mRNA synthesis and protein production of IL-8 and several other cytokines in human monocytes (de Waal Malefyt et al. 1991). IL-10 is therefore an endogenous mediator, which might prove useful in the treatment of inflammatory skin disease.

The observations presented in this thesis, combined with the results of others, indicate that MCAF and IL-8 play a significant role in the recruitment of cells and maintenance of inflammation. Another therapeutical approach in the future would therefore be development of specific humanised anti-

bodies, which could block the activity of these cytokines. Although these studies are still at an early stage, animal models point towards an effect of monoclonal anti-IL-8 antibodies in preventing skin inflammation. In preliminary studies we have shown that intravenous injection of the neutralizing IL-8 antibody WS-4 in rabbits dramatically reduce the size of tuberculin reactions and that the leukocyte accumulation in skin is significantly suppressed when compared to control animals treated with an isotype control antibody.

11. SUMMARY IN DANISH

Afhandlingen diskuterer induktionen og regulationen af de pro-inflammatoriske peptider interleukin-8 (IL-8) og monocyt kemotaktisk og aktiverende faktor (MCAF) under normale forhold og ved inflammatoriske hudsygdomme. Undersøgelserne er udført *in vitro* på dyrkede celler i kultur. IL-8 er en kendt neutrofil og T-lymfocyt kemotaktisk faktor, mens MCAF aktiverer og inducerer vandring af monocytter. Disse cytokiner er af betydning for tiltrækningen og vandringen af de nævnte celletyper fra blodbanen og ind i væv, herunder huden, hvor en inflammatorisk/immunologisk reaktion er i gang. Da interleukin-1 (IL-1) og tumour necrosis factor α (TNFα) er kendte potente endogene stimulatorer af IL-8 og MCAF ekspression i mange celletyper er lokalisationen af disse cytokiner samt deres receptorer og det nyligt klonede interleukin 1 receptor antagonist protein (IRAP) undersøgt i biopsier fra syg og rask hud. Endelig har vi undersøgt forskellige aspekter af virkningen af en kendt 5 lipoxygenase inhibitor ETH615, samt af det aktive derivat af vitamin D_3, calcitriol, på IL-8 produktion og aktivitet.

De beskrevne undersøgelser giver anledning til følgende konklusioner:

1. Det er muligt at isolere tilstrækkelige mængder af rent og intakt total RNA fra epidermale suction blisters til at undersøge for tilstedeværelsen af en specifik messenger RNA species v.h.a. Northern blot eller dot blot teknikker. Denne metode kan anvendes når information om *in vivo* ekspression af epidermale mediatorer ønskes, uden kontaminering af prøven med dermale komponenter.

2. Ved hjælp at en nyudviklet kvantitativ dot blot teknik demonstreres det at endotel celler, stimuleret med interleukin 1α (IL-1α), *in vitro* syntetiserer store mængder interleukin 8 (IL-8) mRNA sammenlignet med stimulerede keratinocytter. Monocytter og dermale fibroblaster udtrykker intermediære mængder IL-8 mRNA på det nævnte stimuli.

3. MCAF mRNA expressionen i de ovennævnte 4 celletyper viste en fordeling som ligner IL-8 ekspressions mønsteret. Disse resultater peger på en fælles aktiverings- og regulationsmekanisme for IL-8 og MCAF, som begge tilhører en supergen familie af inflammatoriske peptider.

4. Ved immunhistokemiske undersøgelser på vævssnit demonstreres det at IL-1α, type 1 IL-1 receptoren (IL-1-R1) og IRAP er udtrykt på de samme celletyper i både normal hud og hud fra psoriasis patienter. Dette sammenfald danner mulighed for både stimulation og inhibition af IL-1 medierede virkninger og fundene understreger at en undersøgelse af begge disse forhold er vigtig når et mulig cellulært immunologisk respons ønskes klarlagt. IRAP ekspressionen i involveret psoriasis epidermis var nedsat i forhold til ekspressionen i normal hud og ikke involveret psoriasis hud.

5. TNFα og 55 kDa TNF receptoren, men ikke 75 kDa TNF receptoren, er til stede i både normal epidermis og i epidermis fra involveret psoriasis hud *in vivo*. Disse resultater viser at TNF medierede virkninger i epidermis induceres via 55 kDa TNF receptoren.

6. En kendt 5-lipoxygenase inhibitor, ETH615, er i stand til at hæmme IL-8 mRNA ekspression og biologisk aktivitet *in vitro*. Disse resultater støtter hypotesen om en cytokin-leukotrien regulatorisk cirkel og kan være medvirkende til at forklare hvorfor ETH615 har en lokal anti-inflammatorisk effekt på zymosan-induceret dermatitis hos hunde.

7. Den aktive metabolit af vitamin D_3, 1,25$(OH)_2$-cholecalciferol eller calcitriol kan hæmme IL-8 mRNA ekspression og protein produktion i humane keratinocytter, dermale fibroblaster og mononucleære blodceller *in vitro*. Disse virkninger kan sammen med den kendte virkning på celleproliferation og differentiering, medvirke til den observerede gunstige effekt af calcitriol og derivater heraf på psoriasis.

Afhandlingen viser således at en række forhold er af betydning for udviklingen af et hud-inflammatorisk respons, herunder tilstedeværelsen af specifikke cytokin receptorer, aktivatorer og inhibitor. Under normale forhold vil disse faktorer danner grundlag for en balance, der ved sygelige tilstande kan forstyrres og give anledning til inflammation. En kombination af molekylærbiologiske teknikker, biologiske assays samt immunhistokemi til måling af regulationen af cytokiners ekspression kan medvirke til en afklaring af disse forhold.

12. REFERENCES

Abe Y, Gatanaga M, Osuka Y, Kimura S, Burger RA, Granger GA, Gatanaga T. Role of 55- and 75 kDa tumor necrosis factor membrane receptors in the regulation of intercellular adhesion molecules-1 expression by HL-60 human promyelocytic leukemia cells *in vitro*. J Immunol 1993: 150: 5070-5079.

Ahuja SK, Ozcelik T, Milatovitch A, Francke U, Murphy PM. Molecular evolution of the human interleukin-8 receptor gene cluster. Nat Genet 1992: 2: 31-36.

Alam R, Lett-Brown MA, Forsythe PA, Anderson-Walters DJ, Kenamore C, Kosmos C, Grant JA. Monocyte chemotactic and activat-

ing factor is a potent histamine-releasing factor for basophils. J Clin Invest 1992: 89: 723-728.

Alexander HR, Doherty GM, Buresh CM, Venzon DJ, Norton JA. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. J Exp Med 1991: 173: 1029-1032.

Amento EP. Vitamin D and the immune system. Steroids 1987: 49: 55-72.

Anttila HSI, Reitamo S, Erkko P, Ceska M, Moser B, Baggiolini M. Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis. J Invest Dermatol 1992: 98: 96-101.

Anttila HSI, Reitamo S, Erkko P, Miettinen A, Didierjean L, Saurat J.H. Membrane and cytosolic interleukin 1 alpha and beta in normal human epidermal cells: variability of epitope exposure in immunohistochemistry. J Invest Dermatol 1990: 95: 3138.

Arend WP. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. J Clin Invest. 1990: 88: 1445-1451.

Arend WP, Joslin FG, Thompson RC, Hannum CH. An IL-1 inhibitor from human monocytes. Production and characterization of biologic properties. J Immunol 1989: 143: 1851-1858.

Arend WP, Smith MF, Janson RW, Joslin FG. IL-1 receptor antagonist and IL-1ß production in human monocytes are regulated differently. J Immunol 1991: 147: 1530-1536.

Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant human Monocyte-derived interleukin 1 receptor antagonist. J Clin Invest 1990: 85: 1694-1697.

Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A, Wolff SM, Dinarello CA. Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. Proc Natl Acad Sci USA 1984: 81: 7907-7911.

Bacon KB, Westwick J, Camp RDR. Potent and specific inhibition of IL-8-, IL-1α- and IL-1ß-induced *in vitro* human lymphocyte migration by calcium channel antagonists. Biochem Biophys Res Commun 1989: 165: 349-354.

Bajetto A, Allavena G, Cozzolino F, Rubartelli A. IL-1 receptor antagonist production by B and T lymphocytes. Cytokine 1991: 3: 498 (abstract).

Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW. Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA 1988: 85: 3294-3298.

Barker JNWN, Jones ML, Swenson CL, Sarma V, Mitra RS, Ward PA, Johnson KJ, Fantone JC, Dixit VM, Nickoloff BJ. Monocyte chemotaxis and activating factor production by keratinocytes in response to IFN-γ. J Immunol 1991 A: 146: 1192-1197.

Barker JNWN, Mitra RS, Griffiths CEM, Dixit VM, Nickoloff BJ Keratinocytes as initiators of inflammation. Lancet 1991: 337: 211-214.

Barker JNWN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ. Marked synergism between tumor necrosis factor-α and interferon-γ in regulation of keratinocyte derived adhesion molecules and chemotactic factors. J Clin Invest 1990: 85: 605-608.

Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. J Exp Med 1991: 173: 771-774.

Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: Production, distribution, and metabolic fate *in vivo*. J Immunol 1985: 135: 3972-3977.

Bigler CF, Norris DA, Weston WL, Arend WP. Interleukin-1 receptor antagonist production by human keratinocytes. J Invest Dermatol 1992: 98: 38-44.

Bikle DD, Nemanic MK, Whitney JO, Elias PW. Neonatal human foreskin keratinocytes produce 1,25 -Dihydroxyvitamin D_3. Biochemistry 1986: 25: 1545-1548.

Bischoff SC, Brunner T, De Weck AL, Dahinden CA. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. J Exp Med 1990: 172: 1577-82.

Bischoff SC, Krieger M, Brunner T, Dahinden CA. Monocyte chemotactic protein 1 is a potent activator of human basophils. J Exp Med 1992: 175: 1271-1275.

Bjerke JR, Krogh H-K, Matre R. Characterization of mononuclear cell infiltrates in psoriatic lesions. J Invest Dermatol 1978: 71: 340-343.

Brain SD, Camp RDR, Cunningham FM, Dowd PM, Greaves MW. Leukotriene B_4-like material in scale of psoriatic lesions. Br J Dermatol 1984: 83: 313-317.

Brockhaus M, Schoenfeld H-J, Schlaeger E-J, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. Proc Natl Acad Sci USA 1990: 87: 3127-3131.

Brown KA, Le Roy F, Noble G, Bacon K, Camp R, Vora A, Dumonde DC. Interleukin 8 acts on monocytes to increase their attachment to cultured endothelium and enhance their expression of surface adhesion molecules. Advances in Experimental and Cellular Biology 1991: 305: 161-162.

Camp R, Fincham N, Ross J, Bird C, Gearing A. Potent inflammatory properties in human skin of interleukin 1 alpha like material isolated from normal skin. J Invest Dermatol 1990: 94: 735741.

Carter DB, Deibel MR, Dunn CJ, Tomich C-SC, Laborde AL, Slightom JL, Berger AE, Bienkowski MJ, Sun FF, McEwan RN, Harris PKW, Yem AW, Waszak GA, Chosay JG, Sieu LC, Hardee MM, Zurcher-Neely HA, Reardon IM, Heinrikson RL, Truesdell SE, Shelly JA, Eessalu TE, Taylor BM, Tracey DE. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 1990: 344: 633-638.

Carveth HJ, Bohnsack JF, McIntyre TM, Bagglioni M, Prescott SM, Zimmerman GA: Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. Biochem Biophys Res Comm 1989: 162: 387-393.

Chomczynski P, Sacchi N. Singlestep method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987: 162: 156-159.

Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor α in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991: 39: 1125-1132.

Cooper KD, Hammerberg C, Baadsgaard O, Elder JT, Chan LS, Sauder DN, Voorhees JJ, Fisher G. IL-1 activity is reduced in psoriatic skin. Decreased IL1α an increased nonfunctional IL1ß. J Immunol 1990: 144: 4593-4603.

Dahinden CA, Kurimoto Y, De Weck AL, Lindley I, Dewald B, Baggiolini M. The neutrophil activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. J Exp Med 1989: 170: 1787-1792.

Darbonne WC, Rice GC, Mohler MA, Apple T, Hebert CA, Valente AJ, Baker JB. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest. 1991: 88: 1362-1369.

Decock B, Conongs R, Lenaerts J-P, Billiau A, Van Damme J. Identification of the monocyte chemotactic protein from human osteosarcoma cells and monocytes: Detection of a novel N-terminally processed form. Biochem Biophys Res Commun 1990: 167: 904-909.

DeForge LE, Tracey DE, Kenney JS, Remick DG. Interleukin-1 receptor antagonist protein inhibits interleukin-8 expression in lipopolysaccaride-stimulated human whole blood cells. Am J Pathol 1992: 140: 1045-1054.

Deleuran B, Kristensen M, Paludan K, Zachariae C, Zachariae E,

Thestrup-Pedersen K. The influence of second line-anti-rheumatic drugs on interleukin-8 mRNA expression and protein release in human endothelial cells and peripheral blood mononuclear cells. Cytokine 1992: 5: 405-409.

Deleuran B, Iversen L, Kristensen M, Field M, Kragballe K, Thestrup-Pedersen K, Stengaard K. Interleukin 8 secretion and 15-lipoxygenase in rheumatoid arthritis: In vitro anti-inflammatory effects by interleukin-4 and interleukin-10, but not by interleukin 1 receptor antagonist protein (IRAP). In press. Br J Rheumatol.

DeLuca HF, Schnoes HK. Vitamin D: recent advances. Annu Rev Biochem 1983: 52: 411-439.

Dembic Z, Loetscher H, Gubler U, Pan Y-CE, Lahm H-W, Gentz R, Btockhaus M, Lesslauer W. Two human TNF receptors have similar extracellular, but distinct intracellular, domain sequences. Cytokine 1990: 2: 231-237.

Detmar M, Imcke E, Ruszczak Z, Orfanos CE. Effects of tumor necrosis factor-alpha (TNFα) on cultured microvascular endothelial cells derived from human epidermis. J Invest Dermatol 1989 B: 93: 546-547 (abstract).

Detmar M, Lessing U, Stadler R, Orfanos CE. Effects of recombinant human tumor necrosis factor-alpha on normal and transformed human keratinocytes *in vitro*. J Invest Dermatol 1989 A: 92: 419 (abstract).

Detmar M, Orfanos CE. Tumor necrosis factor-alpha (TNFα) differentially modulates interferon-gamma induced antigen expression of human keratinocytes. J Invest Dermatol 1989 C: 93: 546 (abstract).

Dermers PA, Powell DE, Walz A, Clark-Lewis I, Baggiolini M, Cohn ZA. Differential effects of neutrophil-activating peptide 1/IL-8 and its homologues on leukocyte adhesion and phagocytosis. J Immunol 1991: 147: 4211-4217.

Dermers PA, Lo SK, Olsen-Egbert E; Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating peptide 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Exp Med 1990: 171: 1155-1162.

D'Eustachio P, Jadidi S, Fuhlbrigge RC, Gray PW, Chaplin DD. Interleukin-1 alpha and beta genes: linkage on chromosome 2 in the mouse. Immunogenetics 1987: 26: 339-343.

de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991: 174: 1209-1220.

Didierjean L, Salomon D, Merot Y, Siegenthaler G, Shaw A, Dayer J-M, Saurat J-H. Localization and characterization of the interleukin 1 immunoreactive pool (IL-1α and ß) in normal human epidermis. J Invest Dermatol 1989: 92: 809-816.

Didierjean L, Groves RW, Saurat JH. Interleukin 1α in normal skin. J Invest Dermatol 1991: 96: 294295.

Dixit VM, Green S, Sarma V, Holzman LB, Wolf FW, O'Rourke K, Ward PA, Prochownik EV, Marks R M: Tumor necrosis factor-α induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin. J Biol Chem 1990: 265: 2973-2978.

Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991: 77: 1627-1652.

Dinarello CA, Marnoy SO, Rosenwasser LJ. Role of arachidonate metabolism in the immunoregulatory function of human leukocyte pyrogen/lymphocyte activating factor/interleukin-1. J immunol 1983: 130: 890-896.

Djeu JY, Matsushima K, Oppenheim JJ, Shiotsuki K, Blanchard DK. Functional activation of human neutrophils by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. J Immunol 1990: 144: 2205-2210.

Dower SK, McMahan C, Flack J, Grubin C, Lupton S, Moseley B, Sims GE. Molecular characterization of two types of interleukin-1 receptor coding peptides on murine and human cells. J Leukoc Biol 1990 A: 1: 103- (Suppl).

Dower SK, Phil D, Qwarnstrom EE, Page RC, Blanton RA, Kupper TS, Raines E, Ross R, Sims JE. Biology of the interleukin-1 receptor. J Invest Dermatol 1990 B: 94: 68S-73S.

Dripps DJ, Brandhuber BJ, Thompson RC, Eisenberg SP. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J Biol Chem 1991 A: 266: 10331-10336.

Dripps DJ, Verderber E, Ng RK, Thompson RC, Eisenberg SP. Interleukin-1 receptor antagonist binds to the type II interleukin-1 receptor on B-cells and neutrophils. J Biol Chem 1991 B: 266: 20311-20315.

Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC. Primary structure and functional expression from complementary DNA of a human interleukin 1 receptor antagonist. Nature 1990: 343: 341346.

Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, Thompson RC. Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: Evolution of a cytokine control mechanism. Proc Natl Acad Sci USA 1991: 88: 5232-5236.

Elder JT, Hammerberg C, Barker J, Boman D, Ellis CN, Cooper KD, Voorhees JJ. Coordinate overexpression of IL-ß and IL-8 in psoriatic epidermis: Rapid reduction by cyclosporin prior to clinical improvement. J Invest Dermatol 1991: 96: 612 (Abstract).

Engelmann H, Aderka D, Rubinstein M, Rotman D, Wallach D. A tumor necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumor necrosis factor toxicity. J Biol Chem 1989: 264: 11974-11980.

Erikstein BK, Smeland EB, Blomhoff HK, Funderud S, Prydz K, Lesslauer W, Espevik T. Independent regulation of 55-kDa and 75-kDa tumor necrosis factor receptors during activation of human peripheral blood B lymphocytes. Eur J Immunol 1991: 21: 1033-1037.

Espevik T, Brockhaus M, loetscher H, Nonstad U, Shalaby R: characterization of binding and biological effects of monoclonal antibodies against a tumor necrosis factor receptor. J Exp Med 1990: 171: 415-426.

Farina PR, DeLeon R, Graham A, Grob P, David E, Barton R, Ksiazek J, Rothlein R, Mainolfi E, Matsushima K. Monocyte-derived neutrophil chemotactic factor (MDNCF): A stimulator of neutrophil function. FASEB J 1989: 3: A1333.

Feinberg AP, Vogelstein BA: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983: 132: 6-13.

Feldmann M, Brennan FM, Chantry D, Haworth C, Turner M, Katsikis P, Londei M, Abney E, Buchan G, Barrett K, Corcoran A, Kissonerghis M, Zheng R, Grubeck-Loebenstein B, Barkley D, Chu A, Field M, Maini RN. Cytokine assays: Role in evaluation of the pathogenesis of autoimmunity. Immunological Reviews 1991: 119: 105-123.

Fiers W. Tumor necrosis factor. Characterization at the molecular, cellular and *in vivo* level. FEBS lett 1991: 285: 199- 212.

Fogh K. Lipoxygenase products of arachidonic acid in psoriasis, atopic dermatitis and experimental arthritis. Danish Med. Bulletin 1990.

Fogh K, Larsen CG, Iversen L, Kragballe K. Interleukin-8 stimulates the formation of 15-hydroxy-eicosatetraenoic acid by human neutrophils *in vitro*. Agents Actions 1992: 35: 227-231.

Furuta R, Yamagishi J, Kotani H, Sakamoto F, Fukui T, Matsui Y, Sohmura Y, Yamada M, Yoshimura T, Larsen CG, Oppenheim JJ, Matsushima K. Production and characterization of recombinant human neutrophil chemotactic factor J Biochem 1989: 106: 436-441.

Furutani Y, Nomura H, Notake M, et al. Cloning and sequencing of

the cDNA for human monocyte chemotactic and activating factor (MCAF). Biochem Biophys Res Commun 1989: 159: 249-255.

Gahring LC, Buckley A, Daynes RA. Presence of epidermal derived thymocyte activating factor/interleukin-1 in normal human stratum corneum. J Clin Invest 1986: 76: 1585-1591.

Gayle RB, Sleath PR, Srinivason S, Birks CW, Weerawarna KS, Cerretti DP, Kozlosky CJ, Nelson N, Vanden-Bos T, Beckmann MP. Importance of the N-terminus of the interleukin-8 receptor in ligand interactions. J Biol Chem 1993: 268: 7283-7289.

Gearing AJH, Fincham NJ, Bird CR, Wadhwa M, Meager A, Cartwright JE, Camp RDR. Cytokines in skin lesions of psoriaris. Cytokine 1990: 2: 6875.

Gepner P, Amor B, Fournier C. 1,25 dihydroxyvitamin D_3 potentiates the *in vitro* inhibitory effects of cyclosporin A on T cells from rheumatoid arthritis patients. Arthritis and Rheumatism 1989: 32: 31-36.

Gillitzer R, Berger R, Mielke V, M/ller C, Wolff K, Stingl G. Upper keratinocytes of psoriatic lesions express high levels of NAP-1/IL-8 mRNA in situ. J Invest Dermatol 1991: 97: 73-79.

Gimbrone MA, Obin MS, Brock AF, Luis EA, Hass PE, Hébert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, Darbonne WC, Bechtol KB, Baker JB: Endothelial interleukin 8: A novel inhibitor of leukocyteendothelial interactions. Science 1989: 246: 1601-1603.

Granowitz EV, Clark BD, Mancilla J, Dinarello CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. J Biol Chem 1991 A: 266: 14147-14150.

Granowitz EV, Clark BD, Vannier E, Callahan MV, Dinarello CA. IL-1 receptor antagonist inhibits IL-1 induced cytokine synthesis and the binding of IL-1 to the type II IL-1 receptor in human monocytes. Cytokine 1991 B: 3: 496 (abstract).

Granowitz EV, Porat R, Mier JW, Pribble JP, Stiles DM, Bloedow DC, Catalano MA, Wolff SM, Dinarello CA. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healty humans. Cytokine 1992: 4: 353-360.

Gregory H, Young J, Schröder J-M, Mrowiez U, Christophers E. Structure determination of a human lymphocyte derived neutrophil activating peptide (LYNAP). Biochem Biophys Res Commun. 1988: 151: 883-890.

Grierson D. Gel electrophoresis of RNA. In: Rickwood D and Hames BD eds. Gel electrophoresis of nucleic acids. A practical approach. Oxford: IRL Press Limited, 1982: 1-38.

Gruaz-Chatellard D, Baumberger C, Saurat J-H, Dayer J-M. Interleukin 1 receptor antagonist in human epidermis and cultured keratinocytes. FEBS 1991: 294: 137-140.

Hammerberg C, Arend WP, Fisher GJ, Chan LS, Berger AE, Haskill JS, Voorhees JJ, Cooper KD. Interleukin-1 receptor antagonist in normal and psoriatic epidermis. J Clin Invest 1992: 90: 571-583.

Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC. Interleukin 1 receptor antagonist activity of a human interleukinl inhibitor. Nature 1990: 343: 336340.

Haskill S, Martin G, Van Le L, Morris J, Peace A, Bigler CF, Jaffe GJ, Hammerberg C, Sporn SA, Fong S, Arend WP. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc Natl Acad Sci USA 1991: 88: 36813685.

H̀bert CA, Luscinskas FW, Kiely J-M, Luis EA, Darbonne WC, Bennett GT, Liu CC, Obin MS, Gimbrone MA, Baker JB. Endothelial and leukocyte forms of interleukin 8. Conversion by thrombin and interaction with neutrophils. J Immunol 1990: 145: 3033-3040.

Heilig B, Mapara M, Brockhaus M, Krauth K, Dörken B. Two types of TNF receptors are expressed on human normal and malignant B lymphocytes. Clinical Immunology and Immunopathology. 1991: 61: 260-267.

Henderson B, Thompson RC, Hardingham T, Lewthwaite J. Inhibition of interleukin-1 induced synovitis and articular cartilage proteoglycan loss in the rabbit knee by recombinant human interleukin-1 receptor antagonist. Cytokine 1991: 3: 246-249.

Heufler C, Koch F, Schuler G. Granulocyte/macrophage colonystimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med 1988: 167: 700705.

Himmler A, Maurer-Fogy I, Kr—nke M, Scheurich P, Pfizenmaier K, Lantz M, Olsson I, Hauptmann R, Stratowa C, Adolf GR. Molecular cloning and expression of human and rat tumor necrosis factor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein. DNA and Cell Biology 1990: 9: 705-715.

Holick MF. The cutaneous photosynthesis of previtamin D_3: A unique photoendocrine system. J Invest Dermatol 1981: 76: 51-58.

Holmes WE, Lee J, Kuang W-J, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. Science 1991: 253: 1278-1280.

Huber AR, Kunkel SL, Todd RF, III, Weiss SJ. Regulation of transendothelial migration by endogenous interleukin-8. Science 1991: 254: 99-102.

Ingemansson-Nordquist B, Kiistala U, Rorsman H. Culture of adult human epidermal cells obtained from roofs of suction blisters. Acta Derm Venereol (Stockh) 1967; 47: 237-240.

Jiang J, Beller DI, Frendl G, Graves DT. Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. J Immunol 1992: 148: 2423-2428.

Jinquan T, Larsen CG, Gesser B, Matsushima K, Thestrup-Pedersen K. Human IL-10 is a chemoattractant for CD8+ T lymphocytes and an inhibitor of IIL-8 induced CD4+ T lymphocyte migration. J Immunol. 1993: 151: 4545-4551.

Kilian PS, Kaffka KL, Stern AS, Woehle D, Benjamin WR, Dechiara TM, Gubler U, Farrar JJ, Mizel SB, Lomedico PT. Interleukin 1α and interleukin 1ß bind to the same receptor on T-cells. J Immunol 1986: 136: 4509-4514.

Kiistala U, Mustakallio KK. In-vivo separation of epidermis by production of suction blisters. Lancet. 1964: 1: 1444-1445.

Kiistala U, Mustakallio KK. Dermo-epidermal separation with suction. Electron microscopic and histochemical study of initial events of blistering on human skin. J Invest Dermatol 1967: 48: 466-477.

Kimata H, Yoshida A, Ishioka C, Lindley I, Mikawa H. Interleukin-8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells. J Exp Med 1992: 176: 1227-1231.

Kirstein D, Thomsen MK, Ahnfeldt-Rønne I. Inhibition of leukotriene biosynthesis and polymorphonuclear leukocyte functions by orally active quinolylmethoxyphenylamines. Pharmacol and Toxicol 1991: 68: 125-130.

Ko Y, Mukaida N, Panyutich, Voitenok NN, Matsushima K, Kawai T, Kasahara T. Establishment of a sensitive enzyme-linked immunosorbent assay for human interleukin 8. J Immunol Method 1992: 149: 227-235.

Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Luger TA. Human keratinocytes are a source for tumor necrosis factor α: Evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 1990: 172: 1609-1614.

Kragballe K. Treatment of psoriasis by the topical application of the novel cholecalciferol analogue calcipotriol (MC 903). Arch Dermatol 1989: 125: 1647-1652.

Kragballe K, Wildfang L. Calcipotriol (MC 903), a novel vitamin D analogue stimulates terminal differentiation and inhibits proliferation of cultured human keratinocytes. Arch Dermatol Res 1990: 282: 164-167.

Krieger M, Brunner T, Bischoff SC, von-Tscharner V, Walz A, Moser B, Baggiolini M, Dahinden CA. Activation of human basophils through the IL-8 receptor. J Immunol 1992: 149: 2662-2667.

Kristensen M, Chu CQ, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM. Localization of tumour necrosis factor alpha (TNFα) and its receptors in normal and psoriatic skin: Epidermal cells express the 55 kD but not the 75 kD TNFreceptor. Clin Exp Immunol: 1993: 94: 354-362.

Kristensen M, Deleuran B, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM. Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1α in normal and psoriatic skin; Decreased expression of IRAP in psoriatic lesional epidermis. Br J Dermatol 1992: 127: 305-311.

Kristensen M, Deleuran BD, Jinquan T, Thomsen MK, Zachariae C, Paludan K, Ahnfelt-Rønne I, Matsushima K, Thestrup-Pedersen K, Larsen CG. ETH615, a synthetic inhibitor of leukotriene biosynthesis and function, also inhibits the production of and biological responses towards interleukin 8. Exp Dermatol. 1993: 2: 165-170.

Kristensen M, Deleuran BW, Larsen CG, Thestrup-Pedersen K, Paludan K. Expression of monocyte chemotactic and activating factor (MCAF) in skin related cells. A comparative study. Cytokine: 1993: 5: 520-524.

Kristensen M, Larsen CG, Jørgensen P, Paludan K. RNA purification from epidermal suction blisters. Acta Derm Venereol (Stockh) 1991: 71: 423-426.

Kristensen M, Paludan K, Larsen CG, Deleuran B, Zachariae C, Jensen PKA, Thestrup-Pedersen K. Ciclosporin can change the expression of interleukin 8 mRNA in keratinocytes, but not in fibroblasts or endothelial cells. J Invest Dermatol 1991: 96: 1023 (abstract).

Kristensen MS, Paludan K, Larsen CG, Zachariae COC, Deleuran BW, Jensen PKA, Jørgensen P, Thestrup-Pedersen K. Quantitative determination of IL-1α-induced IL-8 mRNA levels in cultured human keratinocytes, dermal fibroblasts, endothelial cells, and monocytes. J Invest Dermatol 1991: 97: 506-510.

Krzsicki RF, Hatfield CA, Bienkowski MJ, McGuire JC, Winterrowd GE, Chapman DL, Berger AE, McEwan RN, Carter DB, Chosay JG et al. Regulation of expression of IL-1 receptor antagonist protein in human synovial and dermal fibroblasts. J Immunol 1993: 150: 4008-4018.

Kuna P, Reddigari SR, Kornfeld D, Kaplan AP. IL-8 inhibits histamine release from human basophils induced by histamine-releasing factors, connective tissue activating peptide III, and IL-3. J Immunol 1991: 147: 1920-1924.

Kuna P, Reddigari SR, Rucinski D, Oppenheim JJ, Kaplan AP. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for human basophils. J Exp Med 1992: 175: 489-493.

Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, Langdon R, Lightfoot L, Gubler U. Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1 alpha and beta mRNA. Keratinocyte epidermal cell-derived thymocyte activating factor is identical to interleukin 1. J Exp Med 1986: 164: 20952100.

Kupper TS, Chua AO, Flood P, Flood P, McGuire J, Gubler U. Interleukin 1 gene expression in cultured human keratinocytes is augmented by ultraviolet irradiation. J Clin Invest 1987: 80: 430436.

Kupper TS, Lee F, Birchall N, Clark S, Dower S. Interleukin-1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. J Clin Invest 1988: 82: 1789-1792.

Lantz M, Gullberg U, Nilsson E, Olsson I. Characterization *in vitro* of a human tumor necrosis factor-binding protein. A soluble form of a tumor necrosis factor receptor. J Clin Invest 1990: 86: 1396-1402.

Larsen CG. Leukocyte activating and chemotactic cytokines in cell-mediated immune reactions of the human skin. Acta Dermato-venereol (Stockh) 1991: suppl 160.

Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophilactivating protein (NAP1) is also chemotactic for T lymphocytes. Science 1989 C: 243: 1464-1466.

Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 and tumor necrosis factor. Immunology 1989 A: 68: 31-36.

Larsen CG, Kristensen M, Paludan P, Deleuran B, Thomsen MK, Zachariae C, Kragballe K, Matsushima K, Thestrup-Pedersen K. 1,25(OH)2-D_3 is a potent regulator of interleukin-1 induced interleukin-8 expression and production. Biochem Biophys Res Commmun 1991: 176: 1020-1026.

Larsen CG, Ternowitz T, Larsen FG, and Thestrup-Pedersen K: Epidermis and lymphocyte interactions during an allergic patch test reaction. Increased activity of ETAF/IL1, epidermal derived lymphocyte chemotactic factor and mixed skin lymphocyte reactivity in persons with type IV allergy. J Invest Dermatol 1988 B: 90: 230-233.

Larsen CG, Ternowitz T, Larsen FG, Thestrup-Pedersen K. Epidermis and lymphocyte interactions during a tuberculin skin skin reaction.I increased ETAF/IL-1 like activity, expression of tissue antigens and mixed skin lymphocyte reactivity. Arch Dermatol Res 1988 A: 280: 83-89.

Larsen CG, Zachariae COC, Oppenheim JJ, Matsushima K. Production of monocyte chemotactic and activating factor (MCAF) by human dermal fibroblasts in response to interleukin 1 or tumor necrosis factor. Biochem Biophys Res Commun 1989 B: 160: 1403-1408.

Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI. Characterization of two high affinity human interleukin-8 receptors. J Biol Chem 1992: 267: 16283-16287.

Lemire JM, Adams JS, Sakai R, Jordan SC. 1,25-Dihydroxyvitamin D_3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest 1984: 74: 657-661.

Leonard EJ, Skeel A, Yoshimura T, Noer K, Kutvirt S, van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein 1. J Immunol 1990: 144: 1323-1330.

Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 1990: 11: 97-101.

Leonard EJ, Yoshimura T, Tanaka S, Raffeld M. Neutrophil recruitment by intradermally injected chemoattractant/activation protein-1. J Invest Dermatol 1991: 96: 690-694.

Lindley I, Ashauer H, Seifert J-M, Lam C, Brunowsky W, Kownatzski E, Thelen M, Peveri P, Dewald B, von Tscharner V, Walz A, Baggiolini M. Synthesis and expression in Escherichia coli of the gene encoding monocyte-derived neutrophil chemotactic factor: Biological equivalence between natural and recombinant neutrophil-activating factor. Proc Natl Acad Sci USA 1988: 85: 9199-9203.

Lomedico PT, Gubler U, Hellmann CP, Ducovich M, Giri JG, Pan YE, Collier K, Semionow R, Chua AO, Mizel SB. Cloning and expression of murine interleukin-1 in Escherichia coli. Nature 1984: 312: 458-462.

Loetscher H, Pan YE, Lahm HW, Gentz R, Brockhaus M, Tabuchi H, Lesslauer W: Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor. Cell 1990: 61: 351-359.

Longley J, Ding TG, Cuono C, Durden F, Crooks C, Hufeisen S, Eckert R, Wood GS. Isolation, detection , and amplification of intact mRNA from dermatome strips, epidermal sheets, and sorted epidermal cells. J Invest Dermatol 1991. 97; 974-979.

Luger TA, Katz SI, Oppenheim JJ: Epidermal cell (keratinocyte) derived thymocyte activating factor (ETAF). J Immunol 1981: 127: 14931498.

Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W. Tumor necrosis factor alpha (TNF alpha)-induced cell adhesion to human

endothelial cells is under dominant control of one TNF receptor type, TNF-R55. J Exp Med 1993: 177: 1277-1286.

MacLaughlin JA, Gange W, Taylor D, Smith E, Holick MF. Cultured psoriatic fibroblasts from involved and uninvolved sites have a partial but not absolute resistance to the proliferation-inhibition activity of 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA 1985: 82: 5409-5412.

Mancilla J, Ikejima T, Dinarello CA: Glycosylation of the interleukin-1 receptor type I is required for optimal binding of interleukin-1. Lymphokine Cytokine Res 1992: 11:197-205.

Marshall PJ, Griswold DE, Breton J, Webb EF, Hillegass LM, Sarau HM, Newton J, Lee JC, Bender PE, Hanna N. Pharmacology of the pyrroloimidazole SK&F 105809-I. Inhibition of inflammatory cytokine production and of 5-lipoxygenase- and cyclooxygenase-mediated metabolism of arachidonic acid. Biochem Pharmacol 1991: 42: 813-824.

Matsushima K, Akahoshi T, Yamada M, Furutani Y, Oppenheim JJ. Properties of a specific interleukin 1 (IL 1) receptor on human Epstein Barr virus-transformed B lymphocytes: Identity of the receptor for IL 1α and IL 1ß. J Immunol 1986 A: 136: 4496-4502.

Matsushima K, Larsen CG, Dubois GC, Opppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 1989: 169: 1485-1490.

Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocytederived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 1988: 167: 1883-1893.

Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. Cytokine 1989: 1: 2-13.

Matsushima K, Yodoi J, Tagaya Y, Oppenheim JJ. Down-regulation of interleukin-1 receptor expression by IL-1 and fate of internalized 125-I labeled IL-1ß in a human large granulocyte cell line. J Immunol 1986 B: 137: 3183-3188.

Medenica M, Rostenberg A. A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. J Invest Dermatol 1971: 56: 259-271.

Mehrabian M, Sparkes RS, Mohandas T, Fogelman AM, Lusis AJ. Localization of monocyte chemotactic protein-1 gene (SCYA2) to human chromosome 17q11.2-q21.1. Genomics 1991: 9: 200-203.

Mielke V, Bauman JGJ, Sticherling M, Ibs T, Zomershoe AG, Seligmann K, Henneicke H-H, Schröder J-M, Sterry W, Christophers E. Detection of neutrophil-activeting peptide NAP/IL-8 and NAP/IL-8 mRNA in human recombinant IL-1α- and human recombinant tumor necrosis factor-α-stimulated human dermal fibroblasts. J Immunol 1990: 144: 153-161.

Milde P, Hauser U, Simon T, Mall G, Ernst V, Haussler MR, Frosch P, Rauterberg EW. Expression of 1,25-Dihydroxyvitamin D_3 receptors in normal and psoriatic skin. J Invest Dermatol 1991: 97: 230-239.

Modi WS, Dean M, Seuanez HN, Mukaida N, Matsushima K, O'Brien SJ. Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily. Hum Genet 1990: 84: 185-187.

Morhenn VB, Wastek GJ, Cua AB, Mansbridge JN. Effect of recombinanant interleukin 1 and 2 on human keratinocytes. J Invest Dermatol 1989: 93: 121-126.

Morimoto S, Yoshikawa K, Kozuka T, Kitano Y, Imanaka S, Fukuo K, Kumahara Y. An open study of vitamin D_3 treatment in psoriasis vulgaris. Br J Dermatol 1986: 115: 421-429.

Moser B, Schuhmacher C, von Tscharner V, Clark-Lewis I, Baggiolini M. Neutrophil-activating peptide 2 and gro/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. J Biol Chem 1991: 266: 10666-10671.

Mukaida N, Shiroo M, Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 1989: 143: 1366-1371.

Muller K, Svenson M, Bentzen K. 1 alpha,25-Dihydroxyvitamin D_3 and a novel vitamin D analogue MC 903 are potent inhibitors of human interleukin 1 *in vitro*. Immunol Lett 1988: 17: 361-365.

Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 1991: 253: 1280-1283.

Naume B, Shalaby R, Leslauer W, Espevik T. Involvement of the 55- and the 75 kDa tumour necrosis factor receptor in the generation of lymphokine-activated killer cell activity and proliferation of natural killer cells. J Immunol 1991: 146: 3045-3048.

Nickoloff B, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS, Elder JT, Kunkel SL, Dixit VM. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor- alpha in psoriasis. Am J Pathol 1991: 138: 129-140.

Nophar Y, Kemper O, Brakebusch C, Engelmann H, Zwang R, Aderka D, Holtmann H, Wallach D. Soluble forms of tumor necrosis factor receptors (TNF-Rs). The cDNA for the type 1 TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor. EMBO J. 1990: 9: 3269-3278.

Ohlsson K, Björk P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. nature 1990: 348: 550-552.

Olsson I, Lantz M, Nilsson E, Peetre C, Thysell H, Grubb A, Adolf G. Isolation and characterization of a tumor necrosis factor binding protein fron urine. Eur J Haematol 1989: 42: 270-275.

Oxholm A, Oxholm P, Staberg B, Bentzen K: Immunohistological detection of interleukin 1-like molecules and tumour necrosis factor in human epidermis before and after UVB-irradiation *in vivo*. Br J Dermatol 1988: 118: 369-376.

Paccaud J-P, Schifferli JA, Baggiolini M. NAP-1/IL-8 induces up-regulation of CR1 receptors on human neutrophil leukocytes. Biochem Biophys Res Commun 1990: 166: 187-192.

Paludan K, Thestrup-Pedersen K. Use of the polymerase chain reaction in quantification of interleukin 8 mRNA in minute epidermal samples. J Invest Dermatol 1992: 99: 830-835.

Pennica D, Hayflick JS, Bringham TS, Palladino MA Jr, Goeddal DV. Cloning and expression in E. coli of the cDNA for murine tumor necrosis factor. Proc Natl Acad Sci USA 1985: 82: 6060-6064.

Peveri P, Walz A, Dewald B, Baggiolini M. A novel neutrophil-activating factor produced by human mononuclear phagocytes. J Exp Med 1988: 167: 1547-1559.

Piechaczyk M, Blanchard JM, Marty L, Dani C, Panabieres F, El-Sabouty S, Fort P, Jeanteur P. Postttanscriptional regulation of glyceraldehyde-3-phosphate-dehydroxygenase gene expression in rat tissues. Nucleic Acids Res 1984: 12: 6951-6963.

Pillai S, Bikle DD, Eessalu TE, Aggerwal BB, Elias PM. Binding and biological effects of tumor necrosis factor alpha on cultured neonatal foreskin keratinocytes. J Clin Invest 1989: 83: 816-821.

Piguet PF, Vesin C, Grau GE, Thompson RC. Interleukin 1 receptor antagonist (IL-1ra) prevents or cures pulmonary fibrosis elicited in mice by bleomycin or silica. Cytokine 1993: 5: 57-61.

Platt JL, Grant BW, Eddy AA, Michael AF. Immune cell populations in cutaneous delayed-type hypersensitivity. J Exp Med 1983: 158:1227-1242.

Poutsiaka DD, Clark BD, Vannier E, Dinarello CA. Production of interleukin-1 receptor antagonist and interleukin-1ß by peripheral blood mononuclear cells is differentially regulated. Blood 1991:78: 1275-1281.

Prens EP, Benne K, van Damme J, Bakkus M, Brakel K, Benner R, van Joost T. Interleukin 1 and interleukin 6 in psoriasis. J Invest Dermatol 1990: 95: 121S124S.

Re F, Mengozzi M, Muzio M, Dinarello CA, Mantovani A, Colotta FD. Expression of interleukin 1 receptor antagonist (IL-lra) by human circulating polymorphonuclear cells. Eur J Immunol 1993: 23: 570-573.

Reitamo S, Anttila HSI, Didierjean L, Saurat JH: Immunohistochemical identification of interleukin 1α and ß in human eccrine sweatgland apparatus. Br J Dermatol 1990: 122: 315323.

Reusch MK, Studtmann M, Schröder J-M, Sticherling M, Christophers E. NAP/Interleukin 8 is a potent mitogen for human keratinocytes *in vitro.* J Invest Dermatol 1990: 95: 485 (Abstract).

Ristow HJ. A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci, USA 1987: 84: 19401944.

Rigby WFC, Denome S, Fanger MW. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D_3. J Clin Invest 1987: 79: 1659-1664.

Rigby WFC, Waugh MG. Decreased accessory cell function and costimulatory activity by 1,25 dihydroxyvitamin D_3-treated monocytes. Arthritis and Rheumatism 1992: 35: 110-119.

Rigby WF, Waugh M, Graziano RF. Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25 dihydroxyvitamin D_3. Blood 1990: 76: 189-197.

Robinson EA, Yoshimura T, Leonard EJ, Tanaka S, Griffin PR, Shabanowitz, Hunt DF, Appella E. Complete amino acid sequence of a human monocyte chemoattractant, a putative mediator of cellular immune reactions. Proc Natl Acad Sci USA. 1989: 86: 1850-1854.

Rola-Pleszczynsky M, Lemaire I. Leukotrienes augment interleukin-1 production by human monocytes. J Immunol 1985: 135: 3958-3961.

Rollins BJ, Morton CC, Ledbetter DH, Eddy RL, Shows TB. Assignment of the human small inducible cytokine A2 gene SCYA2 (encoding JE or MCP-1), to 17q11.2-12: Evolutionary relatedness of cytokines clustered at the same locus. Genomics 1991 A: 10: 489-492.

Rollins BJ, Pober JS. Interleukin-4 induces the synthesis and secretion of MCP-1/JE by human endothelial cells. Am J Pathol 1991 C: 138: 1315-1319.

Rollins BJ, Walz A, Baggiolini M. Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. Blood 1991 B: 78: 1112-1116.

Rollins BJ, Yoshimura T, Leonard EJ, Pober JS. Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. Am J Pathol 1990: 136: 1229-1233.

Romero LI, Ikejima T, Pincus SH: In situ localization of interleukin 1 in normal and psoriatic skin. J Invest Dermatol 1989: 93: 518522.

Samanta AK, Oppenheim JJ, Matsushima K. Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils. J Exp Med 1989: 169: 1185-1189.

Samanta AJ, Oppenheim JJ, Matsushima K. Interleukin 8 (monocyte-derived neutrophil chemotactic factor) dynamically regulates its own receptor expression on human neutrophils. J Biol Chem 1990: 265: 183-189.

Sauder DN, Carter CS, Katz SI, Oppenheim JJ. Epidermal cell production of thymocyte activating factor (ETAF). J Invest Dermatol 1982: 79: 3439.

Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC, Wong GHW, Gatanaga T, Granger GA, Lentz R, Raab H, Kohr WJ, Goeddel DV. Molecular cloning and expression of a receptor for tumor necrosis factor. Cell 1990: 61: 361-370.

Schall TJ, O'Hehir RE, Goeddel DV, Lamb JR. Uncoupling of cytokine mRNA expression and protein secretion during the induction phase of T cell anergy. J Immunol 1992: 148: 381-387.

Scheurich P, Köbrich G, Pfitzenmaier. Antagonistic control of tumor necrosis factor receptors by protein kinases A and C. J Exp Med 1989: 170: 947-958.

Scheynius A, Fischer T, Forsum U, Klareskog L. Phenotypic characterization in situ of inflammatory cells in allergic and irritant contact dermatitis in man. Clin Exp Immunol 1984: 55: 81-90.

Schröder J-M. The monocyte-derived neutrophil activating peptide (NAP/Interleukin 8) stimulates human neutrophil arachidonate5-lipoxygenase, but not the release of cellular arachidonate. J Exp Med. 1989 A: 170: 847863.

Schröder JM, Christophers E: Identification of $C5_{ades\ arg}$ and anionic neutrophil activating peptide (ANAP) in psoriatic scales. J Invest Dermatol 1986; 87: 53-58.

Schröder J-M, Christophers E. Secretion of novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells. J Immunol 1989 C: 142: 244-251.

Schröder JM, Gregory H, Young J, Christophers E. Neutrophil-activating proteins in psoriasis. J Invest Dermatol 1992: 98: 241-247.

Schröder JM, Mrowietz U, Christophers E: Purification and partial biological characterization of a human lymphocytederived peptide with potent neutrophilstimulating activity. J Immunol 1988: 140: 3524 -3540.

Schröder JM, Mrowietz U, Morita E, Christophers E: Purification and partial biochemical characterization of a human monocyte-derived neutrophil-chemotactic peptide that lacks interleukin 1 activity. J Immunol 1987: 139: 3474-3483.

Schröder JM, Sticherling M, Henneicke HH, Preissner WC, Christophers E. IL-α or tumor necrosis factor-α stimulate release of three NAP-1/IL-8 related neutrophil chemotactic proteins in human dermal fibroblasts. J Immunol 1990: 144: 2223-2232.

Schröder J-M, Young J, Gregory H, Christophers E. Aminoacid sequence characterization of two structurally related neutrophil activating peptides obtained from lesional psoriatic scales. 1989 B: 92: 515 (Abstract).

Seitz M, Dewald B, Gerber N, Baggiolini M. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatiod arthritis. J Clin Invest 1991: 87: 463-469.

Shalaby MR, Sundan A, Loetscher H, Brockhaus M, Lesslauer W, Espevik T. Binding and regulation of cellular functions by monoclonal antibodies against human tumor necrosis factor receptors. J Exp Med 1990: 172: 1517-1520.

Shieh J-H, Gordon MS, Peterson RHF, Jakubowski AA, Gabrilove JL, Moore MAS. Modulation of cytokine receptors and superoxide production in neutrophils treated with IL-1 *in vitro* and *in vivo.* Blood 1990: 76: 165a (Abstr, suppl).

Shields J, Bernasconi LM, Benotto W, Shaw AR, Mazzei GJ. Production of a 26,000-dalton interleukin-1 inhibitor by human monocytes is regulated by granulocyte-macrophage colony-stimulating factor. Cytokine 1990: 2: 122-128.

Shyy Y-J, Li Y-S, Kolattukudy PE. Structure of human monocyte chemotactic protein gene and its regulation by TPA. Biochem Biophys Res Commun 1990: 169: 346-351.

Sica A, Matsushima K, Wang JM, Dejana E, Colotta F, Mantovani A. IL1 transcriptionally activates the monocytederived neutrophil chemotactic factor/IL8 gene in endothelial cells. Immunology 1990 A: 69: 548-553.

Sica A, Wang JM, Colotta F, Dejana E, Mantovani A, Oppenheim JJ, Larsen CG, Zachariae CO, Matsushima K. Monocyte chemotactic and activating factor gene expression in endothelial cells by IL-1 and tumor necrosis factor. J Immunol 1990 B: 144: 3034-3038.

Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM; March CJ, Dower SK. Cloning the interleukin 1 receptor from human T cells. Proc Natl Acad Sci USA 1989: 86: 8946-8950.

Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, Grabstein KH, Dower

SK. Interleukin 1 signalling occurs exclusively via the type I receptor. Immunology 1993: 90: 6155-6159.

Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM, Friend D, Alpert AR, Gillis S, Urdal DL, Dower SK. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 1988: 241: 585-589.

Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jrezy R, Dower SK, Cosman D, Goodwin RG. A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 1990: 248: 1019-1023.

Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. J Biol Chem 1987: 262: 6951-6954.

Smith WB, Gamble JR, Clark-Lewis I, Vadas MA. Interleukin-8 induces neutrophil transendothelial migration. Immunology 1991: 72: 65-72.

Smith WB, Gamble JR, Clark-Lewis I, Vadas MA. Chemotactic desensitization of neutrophils demonstrates interleukin 8 (IL-8)-dependent and IL-8-independent mechanisms of transmigration through cytokine-activated endothelium. Immunology 1993: 78: 491-497.

Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. Oral or topical use of 1,25-dihydroxyvitamin D_3 can be a safe and effective therapy for psoriasis. J Am Acad Dermatol 1988: 19: 516-528.

Smith EL, Walworth NC, Holick MF. Effect of 1,25-dihydroxyvitamin D_3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. J Invest Dermatol 1986: 86: 709-714.

Snyderman R, Altman LC, Hausman MS, Mergenhagen SE. Human mononuclear leukocyte chemotaxis: A quantitative assay for humoral and cellular chemotactic factors. J Immunol 1972: 108: 857-860.

Sozzani I, Luini W, Molino M, Jilek P, Bottazzi B, Cerletti C, Matsushima K, Mantovani A. The signal transduction pathway involved in the migration induced by a monocyte chemotactic cytokine. J Immunol 1991: 147: 2215-2221.

Standiford TJ, Strieter RM, Kasahara K, Kunkel SL. Disparate regulation of interleukin 8 gene expression from blood monocytes, endothelial cells, and fibroblasts by interleukin 4. Biochem Biophys Res Commun 1990 A: 171: 531-536.

Standiford TJ, Strieter RM, Chensue SW, Westwick J, Kasahara K, Kunkel SL. IL-4 inhibits the expression of IL-8 from stimulated human monocytes. J Immunol 1990 B: 145: 1435-1439.

Sticherling M, Bornscheuer E, Schröder J-M, Christophers E. Localization of neutrophil-activating peptide-1/interleukin-8-immunoreactivity in normal and psoriatic skin. J Invest Dermatol 1991: 96: 26-30.

Sticherling M, Bornscheuer E, Schröder J-M, Christophers E. Immunohistochemical studies on NAP-1/IL-8 in contact eczema and atopic dermatitis. Arch Dermatol Res 1992: 284: 82-85.

Strieter RM, Kasahara K, Allen R, Showell HJ, Standiford TJ, Kunkel SL. Human neutrophils exhibit disparate chemotactic factor gene expression. Biochem Biophys Res Commun 1990: 173: 725-730.

Strieter RM, Kasahara K, Allen RM, Standiford TJ, Rolfe MW, Becker FS, Chensue SW, Kunkel SL. Cytokine-induced neutrophil-derived interleukin-8. Am J Pathol 1992: 141: 397-407.

Strieter RM, Kunkel SL, Showell HJ, Remick DG, Phan SH, Ward PA, and Marks RM: Endothelial cell gene expression of a neutrophil chemotactic factor by TNFα, LPS, and IL1ß. Science 1989 B: 243: 1467-1469.

Strieter RM, Phan SH, Showell HJ, Remick DG, Lynch JP, Genord M, Raiford C, Eskandari M, Marks RM, and Kunkel SL. Monokine induced neutrophil chemotactic factor gene expression in human fibroblasts. J Biol Chem 1989 A: 264: 10621-10626.

Strieter RM, Wiggins R, Phan SH, Wharram BL, Showell HJ, Remick DG, Chensue SW, Kunkel SL. Monocyte chemotactic protein gene expression by cytokine-treated human fibroblasts and endothelial cells. Biochem Biophys Res Commun 1989 C: 162: 694-700.

Swensson O, Schubert C, Christophers E, Schröder J-M. Inflammatory properties of neutrophil-activating protein- 1/interleukin 8 (NAP-1/IL-8) in human skin: A light- and electronmicroscopic study. J Invest Dermatol 1991: 96: 682-689.

Symons JA, Wood NC, Di Giovine FS, Duff GW. Soluble IL-2 receptor in rheumatoid arthritis. Correlation with disease activity, IL-1 and IL-2 inhibition. J Immunol 1988: 141: 2612-2618.

Ternowitz T, Thestrup-Pedersen K. Epidermis and lymphocyte interactions during a tuberculin skin reaction. I. Homogenized epidermal tissue supernatant has increased chemotactic activity for lymphocytes. J Invest Dermatol 1986: 87: 613-617.

Thomsen MK. The role of neutrophil-activating mediators in canine health and disease, with special reference to the role of leukotrienes in inflammatory dermatoses. J Vet Pharmacol Therap 1991: 14: 113-133.

Thelen M, Peveri P, Kernen P, von Tscharner V, Walz A, Baggiolini M. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. FASEB J 1988: 2: 2702-2706.

Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF. Glycocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6, but not of M-CSF in human fibroblasts. Blood 1992: 79: 45-51.

Tracey KJ, Vlassara h, Cerami A. Cachetin/tumour necrosis factor. Lancet 1989: 1122-1126.

Trefzer U, Brockhaus M, Loetscher H, Parlow F, Kapp A, Schöpf E, Krutmann J. 55 kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. J Invest Dermatol 1991: 97: 911-916.

Turner M, Chantry D, Katsikis P, Berger A, Brennan FM, Feldmann M. Induction of the interleukin 1 receptor antagonist protein, by transforming growth factor ß. Eur J Immunol 1991: 21: 16351639.

Tuschil A, Lam C, Haslberger A, Lindley I. Interleukin-8 stimulates calcium transients and promotes epidermal cell proliferation. J Invest Dermatol 1992: 99: 294-298.

Valente AJ, Rozek MM, Schwartz CJ, Graves DT. Characterization of monocyte chemotactic protein-1 binding to human monocytes. Biochem Biophys Res commun 1991: 76: 309-314.

Van Damme J, Van Beeumen J, Opdenakker G, Billiau A. A novel, NH_2-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive, and granulocytosis-promoting activity. J Exp Med 1988: 167: 1364-1376.

Vannier E, Miller LC, Dinarello CA. Coordinated antiinflammatory effects of interleukin 4: Interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. Proc Natl acad Sci USA 1992: 89: 4076-4080.

Vilcek J, Palombella VJ, Henriksen-DeStefano D, Swenson C, Feinman R, Makoto H, Tsujimoto M: Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. J Exp Med 1986: 163: 632-643.

von Asmuth EJU, Leeuwenberg JFM, van den Linden CJ, Buurman WA. Tumour necrosis factor-α induces neutrophil mediated injury of cultured human endothelial cells. Scand J Immunol 1991: 34: 197-206.

Wahl SM, Costa GL, Corcoran M, Wahl LM, Berger AE. Transforming growth factor-beta mediates IL-1 dependent induction of IL-1 receptor antagonist. J Immunol 1993: 150: 3553-3560.

Wakefield PE, James WD, Samlaska CP, Meltzer MS: Tumor necrosis factor. J Am Acad Dermatol 1991: 24: 675-685.

Walz A, Peveri P, Aschauer H, Baggiolini M: Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun 1987: 149: 755 -761.

Wang JM, Oppenheim JJ, Kelvin D, Matsushima K. Properties of MCAF-receptor on human monocytic cells. Cytokine 1991: 3: 483 (Abstract).

Wang JM, Sherry B, Fivash MJ, Kelvin DJ, Oppenheim JJ. Human recombinant macrophage inflammatory protein-1 alpha and -beta and monocyte chemotactic and activating factor utilize common and unique receptors on human monocytes. J Immunol 1993: 150: 3022-3029.

White MV, Yoshimura T, Hook W, Kaliner MA, Leonard EJ. Neutrophil attractant/activating protein-1 (NAP-1) causes human basophil histamine release. Immunol Ltrs 1989: 22: 151-154.

Wong GH, Tartaglia LA, Lee MS, Goeddel DV. Antiviral activity of tumor necrosis factor (TNF) is signaled through the 55-kDa receptor, type I TNF. J Immunol 1992: 149: 3350-3353.

Wong HL, Costa GL, Lotze MT, Wahl SM. Interleukin (IL) 4 differentially regulates monocyte IL-1 family gene expression and synthesis *in vitro* and *in vivo*. J Exp Med 1993: 177: 775-781.

Ye K, Koch K-C, Clark BD, Dinarello CA. Interleukin-1 down-regulates gene and surface expression of interleukin-1 receptor type 1 by destabilizing its mRNA whereas interleukin-2 increases its expression. Cytokine 1991: 3: abstract 51.

Yoshimura T, Leonard EJ. Identification of high affinity receptors for human monocyte chemoattractant protein-1 on human monocytes.. J Immunol 1990 B : 145: 292-297.

Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ. Neutrophil chemotactic factor produced by lipopolysaccaride (LPS)-stimulated human blood mononuclear leucocytes: Partial characterization and separation from interleukin 1 (IL-1). J Immunol 1987 A: 139: 788-793.

Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ: Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defence cytokines. Proc Natl Acad Sci USA 1987 B: 84: 9233-9237.

Yoshimura T, Robinson EA, Appella E, Matsushima K, Showalter SD, Skeel A, Leonard EJ. Three forms of monocyte-derived neutrophil chemotactic factor (MDNCF) distinguihed by different length of the amino-terminal sequence. Molecular Immunology 1989 A: 26: 87-93.

Yoshimura T, Robinson EA, Tanaka S, Appella E, Leonard EJ. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. J Immunol 1989 B: 142: 1956-1962.

Yoshimura T, Yuhki N, Moore SK, Appella A, Lerman MI, Leonard EJ. Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEB 1989 C: 244: 487-493.

Zachariae COC, Anderson AO, Thompson HL, Appella E, Mantovani A, Oppenheim JJ, Matsushima K. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. J Exp Med 1990: 171: 2177-2182.

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

Zachariae COC, Thestrup-Pedersen K, Matsushima K. Expression and secretion of leukocyte chemotactic cytokines by normal human melanocytes and melanoma cells. J Invest Dermatol 1991: 97: 593-599.

Zipfel PF, Bialonski A, Skerka C. Induction of members of the IL-8/NAP-1 gene family in human T lymphocytes is suppressed by cyclosporin A. Biochem Biophys Res Commun 1991: 181: 179-183.