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STUDIES ON THE MOLECULAR PATHOGENESIS OF PSORIASIS

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Academic dissertation

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in the auditorium of the Department of Dermatology and Venereology, Meilahdentie 2, on May 14th at 12 noon.

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To my family

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1 List of original publications

This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I-V). Some unpublished data are also included.

I Asumalahti K, Veal C, Laitinen T, **Suomela S**, Allen M, Elomaa O, Moser M, de Cid R, Ripatti S, Vorechovsky I, Marcusson JA, Nakagawa H, Lazaro C, Estivill X, Capon F, Novelli G, The Psoriasis Consortium, Saarialho-Kere U, Barker J, Trembath R, Kere J: "Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus." *Hum Mol Genet*, 2002: 11: 589-97.

II **Suomela S**, Elomaa O, Asumalahti K, Kariniemi A-L, Karvonen S-L, Peltonen J, Kere J, Saarialho-Kere U: "HCR, a candidate gene for psoriasis, is expressed differently in psoriasis and other hyperproliferative skin disorders and is downregulated by IFN-γ in keratinocytes. " *J Invest Dermatol*, 2003: 121:1360-1364.

III **Suomela S**, Kariniemi A-L, Snellman E, Saarialho-Kere U: "Metalloelastase (MMP-12) and 92-kDa gelatinase (MMP-9) as well as their inhibitors, TIMP-1 and -3, are expressed in psoriatic lesions." *Exp Dermatol*, 2001: 10: 175-183.

IV **Suomela S**, Kariniemi A-L, Impola U, Karvonen S-L, Snellman E, Uurasmaa T, Peltonen J, Saarialho-Kere U: "MMP-19 is expressed by keratinocytes in psoriasis." *Acta Derm Venereol*, 2003: 83: 108-114.

V **Suomela S**, Cao L, Bowcock A, Saarialho-Kere U: "Interferon alpha – inducible protein 27 (IFI27) is up-regulated in psoriatic skin and certain epithelial cancers." *J Invest Dermatol, J Invest Dermatol*, 2004: 122: 717-721.

2   Abbreviations

APC   antigen presenting cell
BCC   basal cell carcinoma
BM   basement membrane
bFGF   basic fibroblast growth factor
cDNA   complementary deoxyribonucleic acid
CDSN   corneodesmosin
CLA   cutaneous lymphocyte antigen
DNA   deoxyribonucleic acid
ECM   extracellular matrix
EGF   epidermal growth factor
ERK   extracellular signal-regulated kinase
GM-CSF   granulocyte macrophage colony stimulating factor
HCR   α-helical coiled-coil rod homologue (also known as Pg8 and SBP)
HLA   human leukocyte antigen
ICAM-1   intercellular adhesion molecule-1
IFN-γ   gamma-interferon
IFI27   interferon α-inducible protein 27 (also known as ISG12 and p27)
IGF   insulin-like growth factor
IL   interleukin
IP-10   IFN-γ-inducible protein 10
ISG   interferon stimulated gene
JNK   c-Jun N-terminal kinase
KGF   keratinocyte growth factor
LC   Langerhans cell
LD   linkage disequilibrium
LFA   leukocyte function associated antigen
MCP-1   monocyte chemotactic protein-1
MDC   macrophage-derived chemokine
MGS/GROα   melanoma growth stimulatory activity
MHC   major histocompatibility complex
MIG   monokine induced by IFN-γ
MIP-1   macrophage inflammatory protein-1
MMP   matrix metalloproteinase
mRNA   messenger ribonucleic acid
MT-MMP   membrane type matrix metalloproteinase
NK   natural killer
PCR   polymerase chain reaction
PDGF   platelet-derived growth factor
PSORS   psoriasis susceptibility locus
RANTES   regulated upon expression, normal T cell expressed and secreted
RNA   ribonucleic acid
RT-PCR   reverse transcriptase polymerase chain reaction
SCC   squamous cell carcinoma
SNP   single nucleotide polymorphism
TARC   thymus- and activation-regulated chemokine
TCR   T cell receptor
TDT   transmission disequilibrium test
TGF-α/β   transforming growth factor-alpha/beta
Th   T helper
TIMP   tissue inhibitor of metalloproteinases
TNF-α   tumor necrosis factor-alpha
VEGF   vascular endothelial growth factor
VCAM   vascular cell adhesion molecule

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3   Abstract

Psoriasis is a chronic skin disease characterized by keratinocyte hyperproliferation and dedifferentiation, neoangiogenesis, and inflammation. T cell-mediated immunity, in which cytokines play an essential role, is considered to be the key element in the disease process, but the exact pathogenesis still remains unsolved. Aggregation in families is typical but Mendelian laws cannot explain the inheritance in most cases. Rather, psoriasis is a multifactorial disease requiring environmental trigger factors and genetic susceptibility to become manifested. This study aimed at evaluating a positional candidate gene and certain psoriasis-associated proteins in the pathobiology of psoriasis.

The human genome contains several psoriasis susceptibility loci, of these PSORS1 is responsible for up to 50% of the familial psoriasis. Of the eight genes in PSORS1, the alpha-helix coiled coil rod homologue (HCR) has proved to be a good candidate gene for psoriasis based on its location, polymorphism, and a disease-associated allele. In this study, we demonstrated a specific allelic variant of HCR, HCR*WWCC, to be strongly associated with psoriasis, and in linkage disequilibrium (LD) with two other susceptibility alleles in the region, HLA-Cw*6 and CDSN*5, in different populations. The secondary structure of the HCR protein differs between the nonrisk allele and the HCR*WWCC allele, possibly affecting the antigenic properties of the protein. The HCR protein localized basally and suprabasally above the dermal tips *in vivo* in psoriatic lesions, a pattern not found in normal skin or other hyperproliferative skin disorders. Its expression was opposite to the hyperproliferation marker Ki67 and the cell-adhesion protein β-catenin, suggesting that HCR may have antiproliferative functions and that the adhesive properties of cells expressing HCR were abnormal. In regulation studies, interferon gamma (IFN-γ) downregulated HCR mRNA expression in primary keratinocytes two-fold.

Many of the cytokines involved in the pathobiology of psoriasis are important inducers of matrix metalloproteinases (MMPs) as well. Moreover, the role of MMPs in wound healing is well established, but little is known about MMPs in psoriasis although these conditions share many common histopathological features. In this study, MMP-12 was upregulated in psoriatic lesions in macrophages, possibly aiding macrophage migration through the basement membrane (BM). The basal and suprabasal keratinocytes of hyperproliferative areas of psoriatic lesions expressed MMP-19, suggesting that this upregulation could represent a response to changes in the extracellular matrix (ECM). The upregulation was verified with quantitativeTaqMan PCR, in which a 15-fold upregulation of MMP-19 was demonstrated in psoriatic keratinocytes. Macrophages and neutrophils expressed MMP-9. MMP-3 was expressed in basal psoriatic keratinocytes in a few samples, associating with areas of BM disruption. Keratinocyte hyperproliferation did not induce MMP-21 or –26 expression or that of MMP-8, which was expressed in neutrophils. Expression of TIMP-1 (tissue inhibitor of matrix metalloproteinase-1) mRNA and TIMP-3 mRNA was upregulated in psoriatic lesions, possibly reflecting the anti-angiogenic properties of TIMPs.

IFN-γ and IFI27, an interferon inducible gene, are upregulated in psoriasis. In this study, IFI27 mRNA was expressed in the psoriatic epidermis, other hyperproliferative skin disorders, healing wounds, and squamous cell carcinomas (SCCs) in a spatially and temporally regulated manner. IFN-γ, TNF-α, and TGF-β1 upregulated IFI27 mRNA expression in primary cultured keratinocytes, possibly affecting their proliferation. Thus, IFI27 may be a novel marker of epithelial proliferation and of cancer.

4   Introduction

Psoriasis is a chronic skin disorder with unknown etiology affecting 1 to 3% of Caucasians (Christophers 2001). The most common form of psoriasis is psoriasis vulgaris, which is characterized by sharply demarcated, red, and scaly symmetrical plaques on the elbows, knees or scalp (Lomholt 1963). Histologically, keratinocytes show abnormal differentiation and hyperproliferation, inflammatory infiltrates invade the dermis and the epidermis, and a marked neoangiogenesis is present (Ortonne 1999). The basement membrane (BM) structures are altered, and a complex network of cytokines, mainly Th1 type, are involved in various disease related processes (Hertle et al. 1992, Pellegrini et al. 1992, Mondello et al. 1994, Bonifati and Ameglio 1999). Psoriasis worsens the quality of life and may even lead to hospitalization in severe cases, although the disease is rarely fatal. No curative therapy exists and the localized treatments are laborious and ineffective in a significant proportion of patients. Systemic or UV phototherapies may have unacceptable side effects; hepatotoxicity and nephrotoxicity may follow metotrexate or cyclosporine treatments, teratogenicity is a risk of oral retinoids, and skin cancer may be caused by frequent PUVA (psoralen and long wave ultraviolet radiation) treatments (Barker 2001, Krueger and Callis 2003). Due to hospitalization, time spent on medical leave, and expensive therapies the economic burden of psoriasis on society is considerable.

A strong genetic component associates with psoriasis, although only the susceptibility is inherited and additional trigger factors are needed for the disease to occur (Henseler 1998, Bowcock and Barker 2003). Several candidate genes and proteins for psoriasis exist on the basis of either their location in the genome, coinciding with the location of susceptibility genes in genome scans, or their putative role in the pathophysiology of psoriatic skin. In this study we have focused our analyses on a novel positional candidate gene, HCR, which is one of the eight genes in the most important psoriasis susceptibility locus, PSORS1 in chromosome 6p21.3. By different molecular methods we have investigated the role of HCR together with various functional candidates, the matrix remodeling MMPs and an interferon-stimulated gene IFI27, in the pathobiology of psoriasis.

5   Review of the literature

5.1   *Clinical characteristics of psoriasis*

Psoriasis is a relatively common skin disorder affecting 1 to 3% of individuals worldwide, males and females equally (Lomholt 1963, Brandrup and Green 1981, Christophers 2001). Prevalence is among the highest in Caucasoid populations, up to 4.7% of Canadians (Christophers 2001). The disease is rarer among those of Asian (0.4% of Chinese) or African origin (0.7% of Afro-Americans), and is absent from the native populations of Samoa (Christophers 2001). Because its prevalence has not varied during the last century, in contrast to atopic dermatitis, environmental changes seem to have had a minor effect (Christophers 2001).

The overall clinical course of psoriasis is highly variable inter-individually: the symptoms may worsen, wane, and occasionally go into spontaneous remission. A variable age of onset, precipitation of active psoriatic disease by different trigger factors, variable clinical response to therapy, patchy appearance, i.e, local variation in the skin, are common features as well (Farber and Nall 1974, Christophers 2001). Generally only the skin is affected, but with extensive skin involvement systemic symptoms including thermoregulation, fluid balance, and protein metabolism disturbances may occur.

5.1.1   Different subtypes

Sharply demarcated erythematous and scaly lesions with a symmetrical distribution on the extensor surfaces or scalp are characteristic of **chronic plaque** psoriasis (psoriasis vulgaris), a form of the disease affecting 90% of patients (Lomholt 1963). The size of the plaques may vary to a great extent, and sometimes the entire skin is inflamed, leading to **erythrodermic** psoriasis**.**

The sudden, generalized appearance of small red lesions on the trunk and proximal limbs is typical for **guttate** psoriasis (eruptive psoriasis), which is the most frequent disease form in children and young adults. Many of these patients (appr. 70%) may develop the chronic plaque form later in life, and chronic plaque patients may also present with guttate flares (Martin et al. 1996, Naldi et al. 2001).

Erythematous lesions in the intertriginous areas with no scaling are typical of **inverse** psoriasis (seborrhoeic psoriasis). The sides of the nose, mouth, and eyes or the center of the chest may be affected. This form often coexists with chronic plaque psoriasis.

Pustular psoriasis is characterized by localized or generalized sterile pustules in erythematous and scaly skin (Mengesha and Bennett 2002).

5.1.2   Associated symptoms

Almost half of psoriasis patients develop **nail changes**, which include pitting, discoloration, subungual hyperkeratosis, and onycholysis (Larko 1995, Lavaroni et al. 1994). These, in turn, are associated with **psoriatic arthritis**, a seronegative spondylarthropathy present in

5 to 20% of psoriatic patients with affected skin (Lavaroni et al. 1994). This arthritis is either an oligoarthritis of distal interphalangeal joints or a polyarthritis of small joints in the hands, feet, and spine, which can lead to severe deformation (Ruderman 2003). With cutaneous improvement, arthritis often improves (Perlman et al. 1979).

5.1.3 Trigger factors

Psoriasis has been associated with several triggering factors. **Cutaneous trauma** (sunburn, surgery, trauma from scratching) can cause the development of a psoriatic plaque in about 40% of psoriatics (Koebner's phenomenon) (Raychaudhuri and Gross 2000a). According to many patients and clinicians, psoriasis is exacerbated by **psychological stress**, probably via neuroimmunological mechanisms (Farber and Nall 1974, Raychaudhuri and Gross 2000b, Kirby and Griffiths 2001)**. Several drugs** (beta-blockers, angiotensin-converting enzyme inhibitors, antimalarials, lithium) as well as **bacterial, viral, and yeast infections** are triggers (Ortonne 1999). Acute guttate psoriasis and exacerbation of chronic plaque psoriasis associate especially with group A beta hemolytic streptococcal infections (Valdimarsson et al. 1997, Skov and Baadsgaard 2000). In several studies, **alcohol consumption, smoking,** and **obesity** have also been associated with increased frequency of psoriasis (Raychaudhuri and Gross 2000a).

5.1.4 Type I psoriasis

Type I psoriasis, also called early-onset type psoriasis, begins before the age of 40 (usually at 16-22 years of age). The majority of patients with a positive family history demonstrate positivity to human lymphocyte antigen-Cw6 (HLA-Cw6) (Henseler and Christophers 1985). Cw6-positive patients typically present with a guttate-type onset and persistent disseminated guttate-like papules. These patients tend to develop more extensive plaques and a more severe disease which worsens during or after throat infections. Koebner's phenomenon is more often present in Cw6-positive patients, and they benefit from UV-phototherapy (Gudjonsson et al. 2002). Type I psoriasis has an irregular course and tends to generalize.

5.1.5 Type II psoriasis

The onset of type II psoriasis occurs after the age of 40 (usually at 57-60 years). This late-onset type presents with minor hereditary association and no family history. The frequency of the Cw6 allele is the same as in the control population. Compared to the early-onset type, type II psoriasis is considered to be mild (Henseler and Christophers 1985).

5.2 *Histopathological features of psoriasis*

5.2.1 Epidermis

Keratinocytes are the main cell type in the epidermis undergoing terminal differentiation during the outward migration from the basal cell layer to the spinous, granular, and cornified layers. Especially in chronic plaque lesions, a marked **epidermal thickening** is observed (Christophers and Mrowietz 1995, Ortonne 1999). This thickening is a result of **hyperplasia** of basal and suprabasal keratinocytes. At the same time, **abnormal**

keratinocyte **differentiation** with marked **parakeratosis** (nuclei in the stratum corneum) and an **absent granular layer** is demonstrated. The psoriatic keratinocytes in the mid and upper levels are senescent, contributing to the **resistance of plaques to apoptosis** as well as the resistance to transformation (Nickoloff 2001). **Hyperkeratosis** and **increased shedding** of epidermal scale are also characteristic epidermal changes in psoriasis, suggesting abnormal adhesion properties of psoriatic keratinocytes (Allen et al. 2001).

Neutrophils invade the epidermis and accumulate as small spongiform **Kogoj pustules,** or migrate up to the stratum corneum forming **Munro's microabscesses**. Munro's microabscesses are found only in parakeratotic areas of the epidermis (van de Kerkhof and Lammers 1987). With exacerbation of the plaque the number of infiltrating neutrophils increases. Infiltration of neutrophils is striking in pustular and in guttate psoriasis (Christophers and Mrowietz 1995).

The number of epidermal *lymphocytes* of the psoriatic lesion is significantly increased and comprises both CD8+ (killer) T lymphocytes and CD4+ (helper) T lymphocytes, proportionally more of CD8+ type (de Boer et al. 1994, Austin et al. 1998). *Langerhans cells* and a macrophage subpopulation called *non-Langerhans dendritic cells* represent the epidermal dendritic cells. In psoriasis, fully mature Langerhans cells are increased in number and have enhanced antigen-presenting capacity due to overexpression of HLA class II molecules (MgGregor et al. 1992, Paukkonen et al. 1992, Dieu-Nosjean et al. 2000). *CD68 positive monocytes/macrophages,* being in close contact with keratinocytes, are increased in number (de Boer et al. 1994), and some scattered *NK-cells* can also be encountered in the epidermis of a psoriatic lesion.

Most authors agree that premature expression of involucrin, decreased expression of filaggrin, and abnormalities in transglutaminase, psoriasin, calgranulins, cystatins, psoriasis-alpha associated fatty acid binding protein, cornifin, lipocortin-1, tissue plasminogen activator, elastase, and cyclophilin are indicators of hyperproliferation of cells (Kadunce and Krueger 1995). Hyperproliferation is also associated with an abnormal keratin pattern. The proliferation-specific keratins K5 and K14 are expressed basally, and with increased differentiation, replaced by keratins K1 and K10 in normal skin (Rao et al. 1996). In psoriasis, however, the suprabasal K1 and K10 are decreased and keratins associated with hyperproliferation, K6 and K16, are upregulated. IFN-γ induced suprabasal expression of K17, which is normally confined to hair follicles, is characteristic as well (Komine et al. 1996).

5.2.2 Dermal compartment

Neoangiogenesis is characteristic of psoriatic plaque formation, and in lesional skin increased numbers of dilated, tortuous vessels are thus encountered. Indeed, a four-fold increase in endothelium of the superficial, but not of the deeper, microvasculature in lesional skin is reported (Creamer et al. 1997). **The dermal papillae** are extremely **high and oedematous,** and **in the tips of dermal papillae a prominent perivascular infiltrate of mainly mononuclear cells** with a few neutrophils can be seen. Scattered *B cells* are present but the main lymphocyte type are *T cells* (de Boer et al. 1994). Increased numbers of T cells in the psoriatic plaque are mainly CD4+ lymphocytes belonging to the Th1 subset, while CD8+ T lymphocytes constitute about 25% of all T cells (Christophers and Mrowietz 1995).

Mast cells emerge and degranulate as the first cells around the postcapillary venules (Schubert and Christophers 1985) and they are found in increased numbers beneath the epidermis. ***Monocyte/macrophages*** migrate into the tissue as the first leukocytes in a developing plaque lesion (Schubert and Christophers 1985). In fully developed lesions, these ***dermal dendrocytes***, representing monocytes from the peripheral blood and serving as antigen-presenting cells, and ***dermal dendritic cells*** (dermal Langerhans cells) are found in increased numbers in papillary dermis (Christophers and Mrowietz 1995). ***NK-cells*** are scattered in the dermis and in perivascular infiltrate in the lesional skin (de Boer et al. 1994).

5.2.3 Basement membrane (dermal-epidermal junction)

Basal keratinocytes are in contact with dermal HLA-DR expressing cells called ***lining macrophages***. These cells, most often observed in the tips of epidermal rete ridges, are bandlike monocytes forming cellular protrusions through the BM (Christophers and Mrowietz 1995). Other cells attached to the BM and in close contact to basal keratinocytes are ***T cells*** and scattered ***NK cells*** (de Boer et al. 1994).

Epithelial cells attach to the ***lamina lucida*** of the BM via actin, $\alpha3\beta1$ integrin, and laminins 10/11, 5 and 6/7. These connect to network-forming type IV collagen via nidogen/entactin in ***lamina densa*** (Burgeson and Christiano 1997). Specialized junctional complexes, ***hemidesmosomes,*** promote the adhesion of epithelial cells to the underlying BM. They consist of **1**. cytoplasmic plaque proteins (plectin, BPAG1, intermediate filament associated protein, HD1), **2**. transmembrane proteins ($\alpha6\beta4$ integrin, BPAG2 = collagen XVII) connecting the cell interior to the ECM, and **3**. BM associated proteins of the ECM (laminin-5 =kalinin) linked to type VII collagen (Rousselle et al. 1997) (**Figure 1**). In psoriatic lesional skin, ***type IV collagen*** staining is discontinuous, while in nonlesional skin the staining is the same as in normal skin (Mondello et al. 1994, Mondello et al. 1996).

Laminin 5 is a major ligand for $\alpha6\beta4$ integrin but it can also bind to the $\alpha6\beta1$ and $\alpha3\beta1$ forms (Borradori and Sonnenberg 1999). $\alpha6\beta4$ integrin transmits signals from the ECM to the cell interior, modulating proliferation, apoptosis, differentiation, and organization of the cytoskeleton via gene expression. The association of laminin-5 with $\alpha6\beta4$ integrin is crucial for the maintenance of stable adhesion (Baker et al. 1996). In psoriasis, ***$\alpha6\beta4$ integrin*** is located pericellularly instead of basally, suggesting that no proper interactions with the BM exist (Giannelli et al. 1994). Interestingly, integrin expression is already altered in nonlesional skin (Hertle et al. 1992, Pellegrini et al. 1992). In addition to this, ***laminin-1*** is missing (Mondello et al. 1994) and, in fact, in nonlesional skin the staining for laminin 1 is already discontinuous and fragmented (Mondello et al. 1996).

Fibronectin is overexpressed in the papillary dermis where the laminin interruptions of the BM occur (Vaccaro et al. 2002). In the stratum corneum above the rete ridges, intraepidermal fibronectin synthesis by keratinocytes and Langerhans cells is also increased (Pellegrini et al. 1992). Concordantly, in psoriatic keratinocytes, ***alpha 5 integrin fibronectin receptor*** is overexpressed (Bata-Csorgo et al. 1998, Kellner et al. 1991), as in neoplastic keratinocytes (Savoia et al. 1993), in keratinocytes involved in wound healing (Hertle et al. 1992), and in rapidly dividing fetal keratinocytes (Hertle et al. 1991). In normal human epidermis, fibronectin does not exist and the $\alpha5\beta1$ fibronectin receptor is poorly expressed (de Luca et al. 1994), but during development adhesion of embryonic cell integrins to fibronectin is essential for their migration through matrixes

(George et al. 1993). Aberrant localization of the embryonic form of cellular fibronectin is seen in proliferating, developing tissue and in wound healing and in nonlesional psoriatic skin *in vivo* (Ting et al. 2000).

Tenascin C, a large glycoprotein of the ECM binding to fibronectin and proteoglycans, is markedly upregulated in psoriatic skin (Latijnhouwers et al. 1998) where a continuous band of tenascin is seen in the upper dermis along the BM in the dermal papillae. In normal skin, only patchy, if any, tenascin C is seen. The distribution of heparan sulfate proteoglycans such as perlecan are also altered in psoriasis (Seyger et al. 1997).
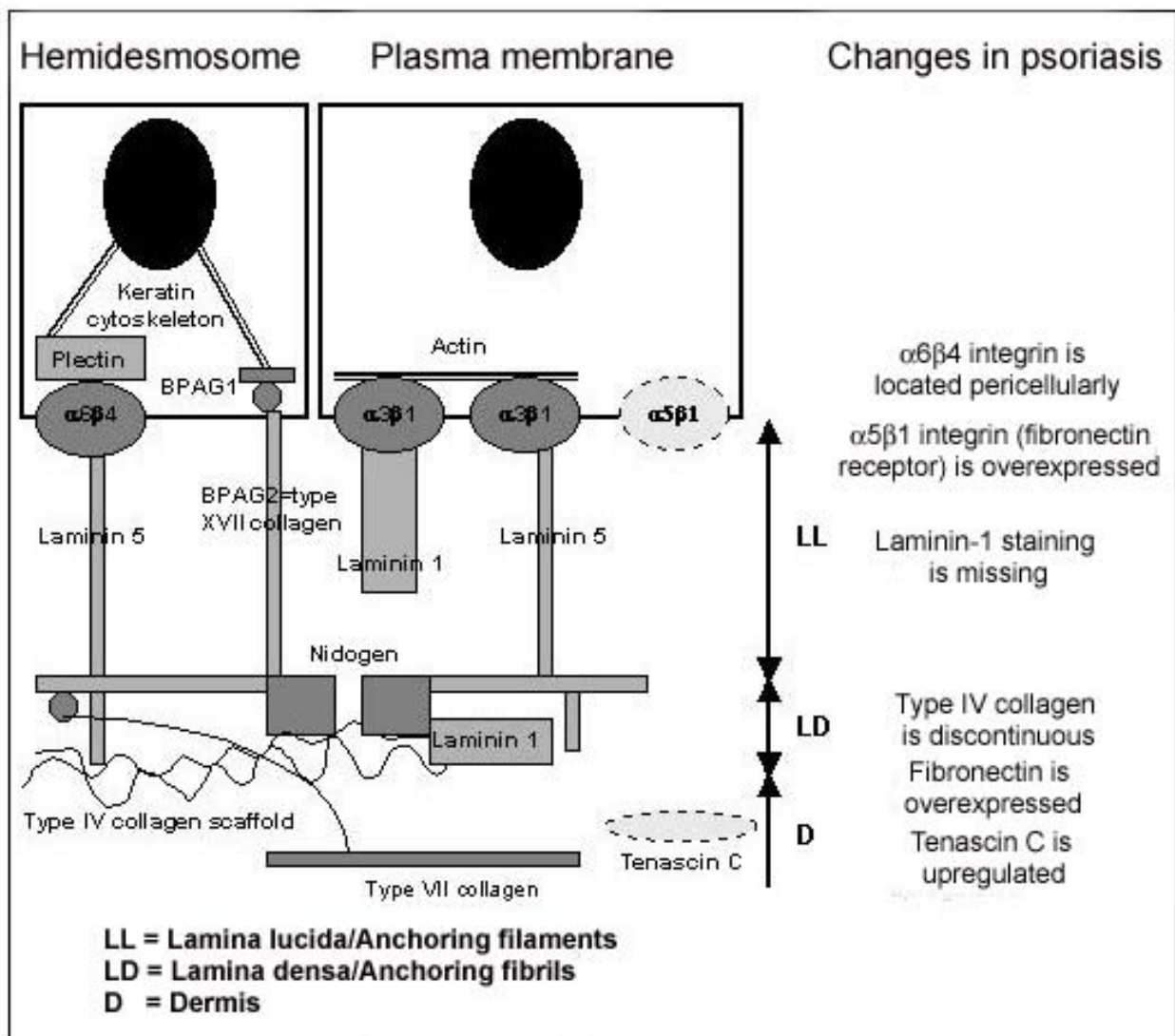

Figure 1. The basement membrane. The changes in BM structure in psoriasis are indicated (partly modified from Burgeson and Christiano 1997).

Epidermal BM defects may have resulted from leukocyte migration into the epidermis. Thus, regenerative maturation of the epidermis, as in an injury response (i.e., wound healing), could be brought about (Krueger 2002). This would not, however, properly explain the reported alterations in integrin expression already noted in nonlesional skin.

Migrating lymphocytes disrupt desmosome connections between adjacent keratinocytes causing a membrane defect that is considered as an injury (Krueger 2002). In accordance, integrin-dependent intercellular contacts are altered in psoriasis when the integrins $\alpha2\beta1$ and $\alpha3\beta1$ are no longer restricted to lateral surfaces of basal keratinocytes (Giannelli et al. 1994). $\beta2$-integrins have been suggested to play a role as well (Bullard 2002).

5.2.4 Histopathological features of differential diagnostic skin disorders

In *lichenoid chronic dermatitis* the epidermis is thickened with thorn-like rete ridges, parakeratosis, and hyperkeratosis. Neutrophils can be encountered in the epidermal compartment, but these do not form Munro's microabscesses. In the upper dermis, marked bandlike leukocyte infiltration is seen and capillaries are dilated. Fibrosis is characteristic.

In *lichen planus* the stratum granulosum is thick and the epidermis is acanthotic with hyperkeratosis and saw-like rete ridges. Round inflammatory cells accumulate in the upper dermis and cause characteristic basal vacuoles protruding through the BM. A continuous band of lymphocytes can be detected in the dermis.

In *chronic eczema* the epidermis is thick and acanthotic with hyperkeratosis. Occasional parakeratosis can be found. In contrast to psoriasis, stratum granulosum exists. In the dermal compartment lymphocytes infiltrate around capillaries.

Pityriasis rubra pilaris is characterized by so-called shoulder parakeratosis lining at the opening of a hair follicle. The epidermis is acanthotic and stratum granulosum is present. In the upper dermis, a mild inflammatory reaction comprising round cells is encountered.

In *mycosis fungoides*, acanthosis is milder and hyperkeratosis slighter than in psoriasis. No neutrophils, eosinophils or plasma cells are encountered, but a marked infiltration of lymphocytes is characteristic. Lutzner cells and Pautrier's microabscesses as well as pigmentary incontinens are histopathological features of mycosis fungoides.

In *ichtyosis vulgaris* the stratum corneum is thickened, and mild to moderate hyperkeratosis is present. In contrast to psoriasis, epidermis is of normal thickness. In *lamellar ichtyosis*, focal parakeratosis is seen along with thickened stratum granulosum. Acanthosis is moderate.

5.3   Pathogenesis of psoriasis

The primary pathogenetic mechanism for psoriasis is still unknown. Keratinocytes, fibroblasts, antigen-presenting cells, T cells, and endothelial cells have all been proposed as candidates for the primary defect. It is likely, however, that abnormal regulation of T cell-keratinocyte interaction with a complex cytokine network is involved (Ortonne 1999, Bos and De Rie 1999).

Hypothesizing that the primary defect resides in **keratinocytes**, the defective epidermal keratinocytes could be activated by physical or chemical injury increasing the synthesis and release of cytokines. This results in antigen-independent activation of T lymphocytes, which, in turn, releases additional cytokines stimulating inflammation and proliferation of keratinocytes and T lymphocytes. As evidence for a keratinocyte defect, Chang et al. (1992) have demonstrated that cytokines secreted by psoriatic epidermal cells potentiate T lymphocyte activation to a greater extent than cytokines secreted from normal epidermal cells. It is also postulated that only psoriatic keratinocytes respond to activated T cells messages with hyperproliferation, because of their specific receptors or signal-transducing mechanisms (Bos and DeRie 1999). This is in accordance with studies by Bata-Csorgo et al. (1995), who demonstrated that normal keratinocytes did not respond to psoriatic T cell supernatants. On the other hand, skin lesion-derived and stimulated T cell clone supernatants have been shown to stimulate keratinocyte growth *in vitro* (Strange et al. 1993).

Fibroblasts derived from either psoriatic lesional or nonlesional skin (but not from normal skin) have been demonstrated to induce hyperproliferation of normal keratinocytes in a skin-equivalent system *in vitro* (Saiag et al. 1985). This has been confirmed by other *in vitro* studies (Krueger and Jorgensen 1990).

On the other hand, whether or not the primary defect resides in the immune system, persistent **T-lymphocyte** stimulation could cause epidermal hyperproliferation by direct interaction with keratinocytes or indirectly via autoantigens, superantigens, or **antigen presenting cells** (APCs), or all of these. It may be equally true, however, that psoriasis results from the direct uncontrolled interaction between T cells and stem-cell keratinocytes, and neither autoantigens, superantigens, nor APCs are involved (Bos and De Rie 1999).

5.3.1 Hyperproliferation of keratinocytes

The precise pathogenetic mechanism of epidermal hyperproliferation in psoriasis remains unsolved (Bos and De Rie 1999). **The cell cycle time** of hyperproliferating psoriatic keratinocytes is short, and while maturation and shedding of keratinocytes takes 26 days in normal epidermis it occurs in 4 days in psoriatic epidermis (Ortonne 1999). In the basal cell layer of normal epidermis, only a small fraction of stem cells are active in cell cycling, but the fraction of cycling stem cells can increase upon stimulation such as wound healing. In psoriatic hyperplastic epidermis, an increase in **the fraction of cells dividing**, or in **the number of cell cycles** before the dividing cells enter terminal apoptosis, or both, is plausible. There is some controversy whether the cell type undergoing this increased number of cell cycles are **stem cells** (Bata-Csorgo 1993) or **transiently amplifying cells** (McKay and Leigh 1995). Growth factors, coming from various cell types, are believed to control the increased proliferation.

Currently available anti-psoriatic drugs act on keratinocyte proliferation. Calcipotriol, a vitamin D3 analogue, and retinoids, natural and synthetic vitamin A derivatives, modulate keratinocyte hyperproliferation and differentiation (Creamer et al. 2002). Cyclosporine has strong antiproliferative effects on human epidermal keratinocytes in addition to immunomodulatory effects (Khandke et al. 1991).

5.3.2 Inflammation

Currently psoriasis is considered to be an autoimmune disease, although the trigger within psoriatic plaques remains unknown. Candidate skin autoantigens that have cross-reactivity with bacterial antigens include keratins. The importance of T cell activation has been demonstrated in the pathogenesis of psoriasis (Krueger 2002, Mehlis and Gordon 2003). Cyclosporine, which inhibits T cell proliferation and cytokine production (Ellis et al. 1986), and DAB389IL-2, a toxin which kills activated T cells (Gottlieb et al. 1995), reduce psoriasis symptoms. Transmission or clearance of psoriasis with bone marrow transplantation supports the pathogenetic role of the immune system (Kanamori et al. 2002).

How could T cells then cause psoriatic plaques to develop? ***T cells are activated*** in three phases (**Figure 2**). First T cells bind to an antigen-presenting cell (APC) in a reversible process mediated by LFA-1 and CD2 of T cells and ICAM-1 and LFA-3 of APC cells. Next, T cell receptor (TCR) recognizes an antigen presented by the major histocompatibility complex (MHC) of an APC in an antigen specific interaction. Finally, a non-antigen specific cell-cell interaction takes place involving CD28, CD86, CD2, and IL-2R of the T cell and CD80, CD28, and LFA-3 of the APC and IL-2. ***T cells migrate to the skin*** and slow down, in a process called rolling. This involves CLA, cutaneous lymphocyte antigen, on T cells and E-selectin (ELAM, endothelial leukocyte adhesion molecule-1) on the endothelial cell. In order for T cells to bind to the endothelium, the T cell surface proteins must be activated by chemokines, and LFA-1 /ICAM and VLA/VCAM interact (LFA-1 = leukocyte function associated antigen, ICAM = intercellular adhesion molecule, VLA = very late antigen, VCAM = vascular cell adhesion molecule). Diapedesis, migration through the vessel wall, occurs after binding. As a final step, activated T cells and secretions of other inflammatory cells, such as local macrophages, dendritic cells, and vascular endothelial cells, and keratinocytes, ***induce the keratinocyte changes*** (hyperproliferation) and the expression of adhesion molecules by endothelial cells. Activated keratinocytes produce growth factors stimulating neutrophil influx, vascular alterations, and keratinocyte hyperplasia (Bos and De Rie 1999, Krueger 2002, Mehlis and Gordon 2003).

Langerhans cells are the dendritic APC cells of the epidermis. After antigen exposure, their ability to stimulate T cell activation increases with increased synthesis of cell surface counterreceptors (i.e., CD80 (B7-1), CD86 (B7-2), CD40, ICAM-1) in a maturation process. Activated Langerhans cells migrate from skin to lymph nodes presenting the antigen to nodal naïve T cells (cells that have not been activated by antigen previously). These T cells recognize antigens bound to class I or II MHC molecules, which leads to T cell activation, CLA expression (making the access to skin possible), and differentiation into type 1 or 2 effector lymphocytes (Krueger 2002).
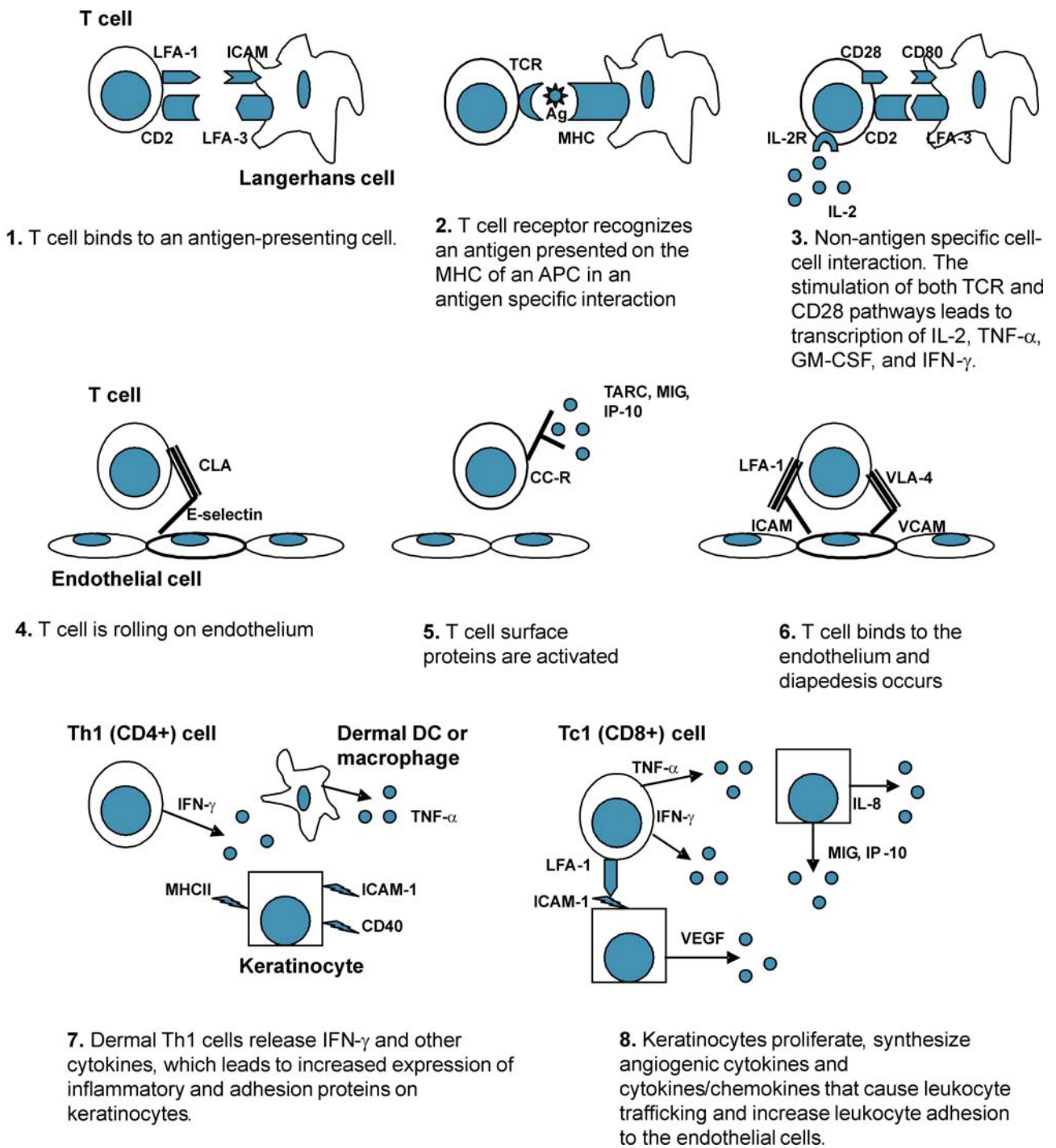
Figure 2. Current hypothesis of the pathogenesis of psoriasis (modified from Mehlis and Gordon 2003 and Krueger 2002).

Superantigens derived from bacteria can form a bridge between the APC and the T cell by binding to MHC class II and the TCR (an antigen-independent mechanism of T cell activation in psoriasis). Defined by the TCR rearrangements, the T cell population involved in psoriasis seems to be, however, clonally expanded to such an extent that a common psoriatic antigen may be causing effects in different patients and in different tissues affected by psoriasis (Prinz et al. 1999, Tassiulas et al. 1999, Prinz 2001). This supports an antigen-specific T cell response and not an inflammation mediated by superantigens. In fact, lesional epidermal and dermal CD8+ T cells react with group A streptococcal antigens (Ovigne et al. 2002). The T cells activated by non-antigen dependent mechanism may, however, become antigen-specific memory cells that react with a crossreactive autoantigen such as keratin (molecular mimicry) (Valdimarsson et al. 1995, Sigmundsdottir et al. 1997, Prinz 2001).

Several new treatments reducing or eliminating the pathogenic effects of T cells are being investigated as possible anti-psoriatic drugs. **Alefacept** binds to CD2 on T cells, blocking the LFA-3/CD2 interaction (Krueger and Callis 2003). Alefacept also binds to FcgRIII IgG receptors on NK cells and macrophages, resulting in apoptosis of those T cells expressing high levels of CD2. **Efalizumab** is an antibody directed against the alpha subunit of LFA-1 (Werther et al. 1996, Leonardi 2003). **Etanercept** and **infliximab** act as competitive inhibitors of tumor necrosis factor-alpha (TNF-α), which is an important pro-inflammatory cytokine in the pathobiology of psoriasis (Goffe and Cather 2003, Gottlieb 2003). Unfortunately, only one third of patients benefit dramatically from the new, expensive immunomodulatory drugs. At least in the long term, increased risk of infection and possible reactivation of tuberculosis and lymphomas must be taken into account, as these new drugs are potentially immunosuppressive (Boehncke 2003).

5.3.3 Angiogenesis

Keratinocytes are thought to be a major source of pro-angiogenic cytokines (VEGF, IL-8) but the precise mechanism for angiogenesis in psoriasis is still unknown. In a developing psoriatic plaque, endothelial cells swell and become activated showing prominent Golgi apparatus and Weibel-Palade bodies (Christophers and Mrowietz 1995). Activated endothelial cells migrate, sprout, and lay down a BM with pericytes for structural support to form novel vessel networks (Longo et al. 2002). Activation and swelling of endothelial cells results in widening of the intercellular spaces, and dermal blood vessels dilate. The lesional capillary loops adopt a venous phenotype, including bridged fenestrations, and express E-selectin, making it easier for leukocytes to migrate into the skin (Creamer et al. 2002).

Although angiogenesis may not be the primary event in the pathogenesis of psoriasis, understanding the pathways leading to angioproliferation may help in finding novel antipsoriatic drugs (Nickoloff 2000, Creamer et al. 2002). In fact, vitamin D, retinoids, and cyclosporin all possess anti-angiogenic activity as well as antiproliferative and anti-inflammatory effects (Creamer et al. 2002).

5.3.4 Cytokine mediators

Several cytokines form a complex and multi-dimensional network in psoriasis pathobiology, none of which alone can be considered to be the causative mechanism (Bonifati and Ameglio 1999). Both activated CD4+ and CD8+ T cells produce IL-2 and IFN-

γ, producing cytokine profile type 1 in psoriasis (Bonifati and Ameglio 1999). Infiltrating T cells are thus type 1 helper T cells (Th1)(CD4+) and type 1 cytotoxic T cells (Tc1)(CD8+) (Austin et al. 1999). Highly effective immune responses are usually associated with a strong type 1 immune response that generates Th1 and Tc1 T cells. It is speculated that the APC or local concentrations of cytokines may have an effect on the T cell pathway (Krueger 2002). Thus, mature Langerhans cells producing abundant IL-12 would provide the appropriate cellular and cytokine background for direct stimulation of Th1 and Tc1 T cells in lesional psoriatic skin (Krueger 2002).

Cytokines are essential in the various steps of psoriasis pathobiology. They influence keratinocyte proliferation, induce neutrophil and T cell chemotaxis, keep T cells in type 1 differentiation, enhance angiogenesis and upregulate adhesion molecules on endothelial cells, and stimulate the release of other chemokines. In psoriatic lesional skin, a large number of cytokines are reported to be up- or downregulated (**Table 1**).

Table 1. Cytokines upregulated in psoriatic skin, their putative effects in psoriasis, and relevance in the context of cutaneous MMPs.

Cytokine	Production	Hyperproliferation	Inflammation	Angiogenesis	MMP*
TNF-α	Tcell, LC, kc, mf, mc	+	+	+	1,2,3,7,9, 10,12,13,19,28
γ-IFN	Tcell, kc, NK cell	?	+		1,3, 9,13
GM-CSF	Tcell, kc	+	+	+	1,9,12
IL-1	kc	+	+	+	1,2,3,9,12,13
IL-2	Tcell	-	+		9
IL-6	Tcell, kc, fb, ec	+	+		1, 3
IL-8	kc, nf	+	+	+	9
IL-12	LC		+		2,14
TGF-α	kc	+	-	+	3,9,10
Amphiregulin	kc	+	+	?	2,9,10**
VEGF	ec, kc		+	+	12
bFGF	Tcell, kc, mc, mf	+		+	1,3
NGF	kc	+	+	+	
Endothelin 1	ec	+	+/-	vasoconstr	2

kc = keratinocyte, mf = macrophage, mc = mast cell, fb = fibroblast, ec = endothelial cell, nf = neutrophil, ? = controversial data

* For references, see section 5.5.8 and Study III

** EGF induces these MMPs

TNF-α production is an early event in cutaneous inflammation and is increased in psoriasis as well (Nickoloff 1991, Bonifati and Ameglio 1999). TNF-α stimulates the keratinocytes to produce IL-8, ICAM-1, TGF-α, β-defensins (antimicrobial peptides), and PAI2 (plasminogen activator inhibitor-2), which is thought to protect cells from apoptosis (Nickoloff et al. 1991, Bonifati and Ameglio 1999, Gottlieb 2003). In addition, TNF-α upregulates CD40 and MHC-II proteins on keratinocytes (Krueger 2002). Macrophages also enhance their proinflammatory cytokine and chemokine production when stimulated by TNF-α. Endothelial cells express adhesion molecules and increase production of VEGF leading to increased angiogenesis and erythema. VEGF may also further promote leucocyte migration into skin. On T cells, TNF-α stimulation causes proinflammatory cytokine production, increased nuclear transcription factor activation, and T cell activation leading to increased inflammation. Langerhans cells respond to TNF-α stimulation by maturing, more efficiently presenting antigen, and upregulating their T cell co-stimulatory

surface molecules. TNF-α is also capable of stimulating Langerhans cells to migrate from skin to the lymph nodes. Langerhans cells also increase proinflammatory cytokine production, leading to increased inflammation (Goffe and Cather 2003). TNF-α is produced by stimulated Langerhans cells, macrophages, monocytes, T cells, and keratinocytes (Nickoloff et al. 1991, Krueger 2002). Some investigators have shown TNF-α to be inhibitory for normal keratinocyte proliferation, speculating that psoriatic keratinocytes may have an altered response to TNF-α (Symington 1989, Pillai et al. 1989, Detmar and Orfanos 1990). Others have reported that TNF-α increases keratinocyte proliferation (Gottlieb 2003).

IFN-γ induces the expression of the adhesion molecule ICAM-1 on keratinocytes and endothelial cells, influencing the trafficking of T lymphocytes into lesional epidermis (Barker et al. 1990, Bonifati and Ameglio 1999). IFN-γ and IL-2 activated keratinocytes secrete IL-1, IL-6, IL-8, IFN-γ, TNF-α, and TGF-α and chemokines MIG and IP-10, which influence both themselves and other cell types including T lymphocytes (Ortonne 1999, Krueger 2002). IFN-γ also stimulates TNF-α release from dermal macrophages or monocytes (Krueger 2002). Interestingly, injection of IFN-γ into nonlesional skin of a patient with psoriasis induced new lesions (Fierlbeck et al. 1990), although IFN-γ is reported to have antiproliferative effects on normal keratinocytes *in vitro* (Nickoloff 1991, Krueger 2002). Psoriatic keratinocytes, however, do not seem to be as susceptible to the growth-inhibitory effects of IFN-γ as normal keratinocytes (Baker et al. 1988). These findings suggest an altered response by psoriatic keratinocytes to γ-IFN, which could contribute to both the increased proliferation and impaired differentiation of epidermal cells in psoriasis. IFN-γ stimulates APC activity and upregulates a number of TNF-α receptors (Bonifati and Ameglio 1999).

GM-CSF, produced by various cell types including keratinocytes, in response to IL-1 or TNF-α among others, increases keratinocyte proliferation and activates neutrophils (Kadunce and Krueger 1995, Bonifati and Ameglio 1999). It also stimulates migration and proliferation of endothelial cells (Werner and Grose 2002). Therefore, psoriasis is exacerbated with GM-CSF therapy (Kelly et al. 1993).

IL-1 family consists of IL-1α, IL-1β, and IL-1-receptor-antagonist, which are produced mainly by keratinocytes in skin. IL-1 induces vascular endothelial cell adhesion molecules for leukocytes (E-selectin, VCAM-1, ICAM-1) on keratinocytes and stimulates expression of KGF and GM-CSF in fibroblasts. These fibroblast-derived factors in turn stimulate keratinocyte proliferation and differentiation (Werner and Smola 2001). In addition to this, IL-1 is a direct keratinocyte mitogen itself (Kadunce and Krueger 1995, Krueger 2002) and mediates angiogenesis, activates T lymphocytes, and induces other cytokines (i.e., TNF-α, IL-2, IFN-γ, TGF-α, IL-6, IL-8, GM-CSF) (Bonifati and Ameglio 1999). Some controversy exists concerning biological levels of IL-1 in psoriasis: some report decreased IL-1α activity and increased non-functional IL-1β (Cooper et al. 1990, Baker and Fry 1992), others have shown that freshly isolated epidermal cells from lesional skin release increased amounts of active IL-1α and IL-1β compared to normal skin (Debets et al. 1995) and that IL-1α transgenic mice develop psoriasis-like histologic features (Groves et al. 1995).

IL-2 is a growth factor and chemoattractant for T cells and induces T cell cytotoxicity as well as stimulating NK cell activity. (Bonifati and Ameglio 1999). High doses of IL-2 may induce psoriasis in predisposed patients (Lee et al. 1988).

IL-6, as a major mediator of the host response to injury and infection, enhances the activation, proliferation, and chemotaxis of T lymphocytes in dermal infiltrate. It also enhances proliferation and activation of B cells and macrophages. IL-6 is produced by keratinocytes, fibroblasts, endothelial cells, and by T cells (Nickoloff et al. 1991, Bonifati and Ameglio1999). It stimulates keratinocyte proliferation *in vitro* as well (Grossman et al. 1989, Krueger 2002). Indeed, psoriatic keratinocytes seem to be more sensitive to the growth-promoting effect of IL-6 than normal ones (Bonifati and Ameglio 1999).

IL-8 is the main chemotactic signal for neutrophils to migrate into the epidermis but acts as a potent T cell chemoattractant as well (Gillitzer et al. 1996, Bonifati and Ameglio 1999). In addition to chemotaxis, it enhances the activation and proliferation of T lymphocytes and is reported to stimulate angiogenesis (Nickoloff et al. 1994). IL-8 is produced by keratinocytes and is mitogenic for them *in vitro* (Kadunce and Krueger 1995, Bonifati and Ameglio 1999). Neutrophils in psoriatic lesions also produce IL-8. Anti-IL-8 therapy has been suggested to be an effective antipsoriatic drug because of the diverse and powerful effects of IL-8, but the results of Phase I/II studies have not been satisfactory (Krueger 2002).

IL-12 is a product of mature Langerhans cells, which enhances T cell activation and differentiation stimulating the type 1 T cell maturation pathway (Krueger 2002).

The **EGF** family comprises EGF, TGF-α, amphiregulin, and heparin-binding EGF-like growth factor. Of these, TGF-α and amphiregulin are increased in the lesional epidermis (Bonifati and Ameglio 1999). Moreover, the hyperplastic epidermis in psoriasis has increased EGF/TGFα receptors (Nanney et al. 1986, Krane et al. 1992). High expression of **amphiregulin**, which functions via the EGF receptor, correlates with a psoriasis-like phenotype, and transgenic amphiregulin overexpressing mice present a psoriasis-like phenotype (Cook et al. 1997). **TGF-α** is produced by keratinocytes, induces IL-1, and has mitogenic and angiogenic properties (Kadunce and Krueger 1995, Bonifati and Ameglio 1999). TGF-α requires a costimulatory signal driven by IGF-1r (insulin growth factor-1 receptor) and importantly, in psoriasis increased expression of IGF-1r has been reported (Krane et al.1992).

VEGF, along with other angiogenic cytokines, regulates vascular growth and remodeling in psoriasis lesions (Kirby and Grifftihs 2001, Krueger 2002). Leukocytes show increased adhesion to selectins and VCAM expressed on new vessels in skin, and therefore VEGF may be the link between angiogenesis and cell-mediated inflammation in psoriasis (Detmar et al. 1998). VEGF together with bFGF enhances Ang2 (angiopoietin) and Tie 2 (endothelial-specific receptor) expression in dermal microvascular endothelial cell cultures. Ang2, in turn, destabilizes vessels by blocking Tie2 signaling and acts with VEGF to initiate angiogenesis (Creamer et al. 2002).

bFGF has mitogenic and angiogenic properties and is found not only basally but also suprabasally in psoriasis (Yaguchi et al. 1993, Creamer et al. 2002). It can be produced by T cells (Bos and De Rie 1999).

NGF (nerve growth factor) induces chemokine expression in keratinocytes (Raychaudhuri and Raychaudhuri 2004), and keratinocytes in lesional and nonlesional psoriatic tissue, in turn, express high levels of NGF. NGF stimulates keratinocyte and endothelial cell proliferation and adherence molecule expression (Pincelli 2000, Werner and Grose 2002). A marked upregulation of NGF receptors, p75 neurotrophin receptor (p75NTR) and tyrosine kinase A (TrkA), are also encountered in the terminal cutaneous nerves of psoriatic lesions (Raychaudhuri and Raychaudhuri 2004). In transplanted psoriatic plaques, a marked proliferation of terminal cutaneous nerves is seen, and NGF and substance P may contribute to the activation of T cells (Raychaudhuri and Raychaudhuri 2004).

Endothelin 1, produced in part by endothelial cells, is mitogenic to keratinocytes and a chemoattractant to neutrophils (Davenport 2002, Bonifati and Ameglio 1999). Serum levels of endothelin-1 correlate with PASI (Psoriasis Area and Severity Index) scores (Bonifati and Ameglio 1999).

Chemokines attract leukocytes to psoriatic lesions, and are divided into two classes, C-X-C and C-C, depending on the presence or absence of a single amino acid between two cysteine residues. Several chemokines are known to be upregulated in psoriatic skin: activated dermal endothelial cells can synthesize TARC, MIG, and IP-10, which are chemoattractants to T cells. MIG and IP-10 are also synthesized by epidermal keratinocytes in response to IFN-γ (Krueger 2002). The synthesis of MGS/GROα is stimulated by TNF-α and IL-1 *in vitro*, and it attracts neutrophils to the psoriatic epidermis (Bonifati and Ameglio 1999). MCP-1 is a monocyte chemoattractant stimulated by IL-1, TNF-α, IFN-γ, and TGF-α.

Table 2. Chemokines upregulated in psoriasis and their receptors (adapted from Krueger 2002* and Bonifati and Ameglio 1999**).

Chemokine receptor of T cells	Chemokines
CLA+T cells: CCR6	MIP3α**
CCR5	RANTES*
CCR4	MDC, TARC, MIG, IP-10*
CCR2	MCP-1**
CXCR3	MDC, TARC, MIG, IP-10*
CXCR2	MGS/GROα**

5.3.5 Mouse models

Several transgenic mouse models for psoriasis have been developed, while no naturally occurring animal model exists. **Flaky skin, abesia,** and **chronic proliferative dermatitis** mice have some psoriasis-like characteristics but lack T cell-based immunopathogenesis and dermal vascular changes (Schön 1999, Mizutani et al. 2003).

Xenotransplantation of psoriatic lesions to **severe combined immunodeficiency mice (SCID)** is the only model reproducing human psoriatic lesions, though pustule formation is rare in this model (Mizutani et al. 2003). In the SCID mouse model, normal skin is grafted from a patient susceptible to psoriasis onto an immunodeficient mouse. When autologous

immunocytes are then activated *in vitro* (by IL-2 and superantigens) and injected into the transplanted skin, the skin phenotype is nearly identical to clinical and histological psoriasis (Nickoloff 2000, Nickoloff et al. 1995, Gilhar et al. 1997, Wrone-Smith and Nickoloff 1996). Activated only by IL-2, **natural killer cells** from a psoriatic patient autologously injected into a skin graft of a SCID mouse also resulted in classic psoriasis histology (Gilhar et al. 2002). In addition, injecting with naïve CD4+ T lymphocytes mismatched for the minor histocompatibility antigens causes an erythrosquamous skin disorder that clinically, histopathologically, and in cytokine level resembles psoriasis without a primary epithelial abnormality (Schön 1997).

Most transgenic mouse models overexpress growth factors important in the pathogenesis of psoriasis under the keratin-14 (K14) or involucrin promoter. Mice overexpressing **amphiregulin**, **IL-1α**, **TGF-α**, **caspase-1** (converting pro-IL1β to mature IL-1β), **β1-integrin** alone or in combination with α2 and α5 integrins, **IL-20,** or bone morphogenetic protein-6 (**BMP-6**, under K10 promoter) share some features characteristic of psoriasis (Cook et al. 1997, Groves et al. 1995, Vassar and Fuchs 1991, Yamanaka et al. 2000, Carroll et al. 1995, Blumberg et al. 2001, Blessing et al. 1996). Overexpression of **TGF-β1**, under the K5 promoter, also results in a psoriasis-like phenotype (Li et al. 2003). TGF-β1 promotes differentiation and appears overexpressed in psoriatic skin by immunohistochemistry but not by mRNA studies. TGF-β serum levels, however, have been demonstrated to correlate with the PASI scores (Bonifati and Ameglio 1999).
In a recent report by Xia et al. (2003), transgenic delivery of **VEGF** to mouse skin resulted in a psoriasiform condition with characteristic vascular and epidermal alterations and inflammatory infiltrates. The VEGF transgenic mice as reported previously do not, however, produce psoriasiform lesions (Larcher et al. 1998). In addition, **IL-6** and **IFN-γ** transgenic mice fail to demonstrate a psoriatic phenotype (Turksen et al. 1992, Carroll et al. 1997). Ablation of **IkB kinase 2** from epidermis and overexpression of constitutively active **Rac1** in basal keratinocytes of transgenic mice results in a psoriatic phenotype instead (Pasparakis et al. 2002, Dey et al. 2003).

5.4   Genetics of psoriasis

An epidemiological study comprising more than 10 000 inhabitants of the Faroe Islands demonstrated that the incidence of psoriasis was greater amongst first-and second-degree relatives of psoriatics than unaffected control subjects (Lomholt 1963). Thereafter, studies in other populations have confirmed this finding, estimating the lifetime risk for psoriasis to be approximately 24 to 28% among first-degree relatives (Farber and Nall 1974, Melski and Stern 1981, Brandrup 1984, Swanbeck et al. 1994, 1997).

Monozygotic **twins** have a concordance rate of 63 to 73% compared with the 17 to 20% concordance rate of dizygotic twins, further supporting the genetic background of this disease (Farber et al. 1974, Brandrup et al. 1978, Brandrup et al. 1982). In addition, the clinical pattern is amazingly similar in monozygotic twins compared with the clinical picture of dizygotic twins. The concordance rate is, however, never 100%, implicating that environmental factors must have an impact on the induction of psoriasis symptoms.
Despite the clear familial aggregation of psoriasis, the precise **inheritance model** has been under debate. Currently most investigators agree that psoriasis belongs to the group of complex diseases, the inheritance being multifactorial: genetic variants in multiple genes

interact both with each other and the environment. (Henseler 1998, Barker 2001, Elder et al. 2001, Bowcock and Barker 2003). Concordant with this view, genome scans in psoriasis have revealed several psoriasis susceptibility loci in the human genome (see sections 5.4.2 and 5.4.3) in addition to the previously established HLA-association with psoriasis (Tiilikainen et al. 1980, Enerbäck et al 1997, Mallon et al. 1997).

5.4.1 SNPs in mapping of complex diseases

Detecting genetic factors underlying complex diseases is difficult due to clinical and genetic locus heterogeneity, the impact of environmental factors, and the existence of phenocopies. Linkage mapping with microsatellite markers and genome-wide scans are often used as one tool to identify susceptibility loci in complex diseases, and have revealed several susceptibility loci for psoriasis as well (see sections 5.4.2 and 5.4.3). Once a locus has been identified in such a scan, single nucleotide polymorphisms (SNPs) are used in refined mapping to identify the candidate genes.

SNPs are single base pair variations in genomic DNA for which different alleles exist in healthy individuals, and the least frequent allele has a frequency of 0.01 or greater in the general population (Collins et al. 1998). SNP alleles can be genotyped, and because of their frequency (appr. one SNP exists per every 1 kb) are good markers for genome-wide mapping of complex diseases (Sachidanandam et al. 2001). Most **association analyses** of candidate genes to date have been utilizing non-synonymous (i.e., cause amino acid change) coding SNPs, but SNPs in promoters or regulatory regions may be disease-causing variants as well (Enattah et al. 2002). In association analysis, carefully selected and matched case-controls are studied. To diminish the bias, isolated populations or prospective study cohorts with a sufficiently large number of participants can be recruited (Peltonen et al. 2000, Cardon and Bell 2001). Family-based controls reduce the effect of population stratification but might require even larger sample sizes due to weaker power. The TDT-test (transmission disequilibrium test) is the most common family-based association test (Cardon and Bell 2001).

Two or more linked loci can occur together in the same chromosome more often than expected by chance, a phenomenon called **linkage disequilibrium** (LD). LD is disrupted by recombination, mutation, and gene conversion. Genomic regions with rare recombination and high LD are called haplotype blocks. Within these blocks, a few common haplotypes account for over 80% of all haplotypes of the block, which are defined by less than 10% of the total SNPs in the block. Thus, only two or three SNPs (tag SNPs) are needed to identify a block. These advances in defining haplotype blocks provide new tools for the mapping of complex diseases (Daly et al. 2001, Patil et al. 2001, Reich et al. 2001).

5.4.2 The PSORS1 locus in HLA-C

The PSORS1 (psoriasis susceptibility 1) locus at 6p21.3 is the major susceptibility locus for psoriasis. It is estimated that this locus accounts for 30% to 50% of the genetic predisposition to psoriasis (Trembath et al. 1997). In linkage studies of different populations, significant linkage to the PSORS1 locus has been reported (Nair et al. 1997, Trembath et al. 1997, Burden et al. 1998, Samuelson et al. 1999, Lee et al. 2000, Veal et al. 2001). In association studies, psoriasis patients with early onset disease have a higher frequency of the HLA-Cw*0602 allele than those with late onset disease (Tiilikainen et al.

1980, Henseler and Christophers 1985, Enerbäck et al. 1997, Mallon et al. 1997), and the HLA-Cw*0602 positive patients are reported to have even more severe symptoms (Gudjonsson et al. 2002). The symptoms were not more severe, however, in Cw*6 homozygous patients but the HLA-Cw*6 homozygotes had a 2.5 fold increased risk of developing psoriasis over HLA-Cw*6 heterozygotes (Gudjonsson et al. 2003). Association analyses with densely spaced markers across the MHC region have further refined the PSORS1 locus (Balendran et al. 1999, Oka et al. 1999, Nair et al. 2000). Based on these studies, the psoriasis susceptibility gene in PSORS1 locates most likely in a region of approximately 200 kb telomeric to HLA-C. In this region, eight genes have been identified: HLA-C, TCF19 (SCI), OTF3 (POU5FI), HCR (Pg8), CDSN, SEEK1, SPR1, and STG (Zhou and Chaplin 1993, Krishnan et al. 1995, Guillaudeux et al. 1998, Consortium 1999, Oka et al. 1999, Holm et al. 2003, Chang et al. 2003a) (**Figure 3**).
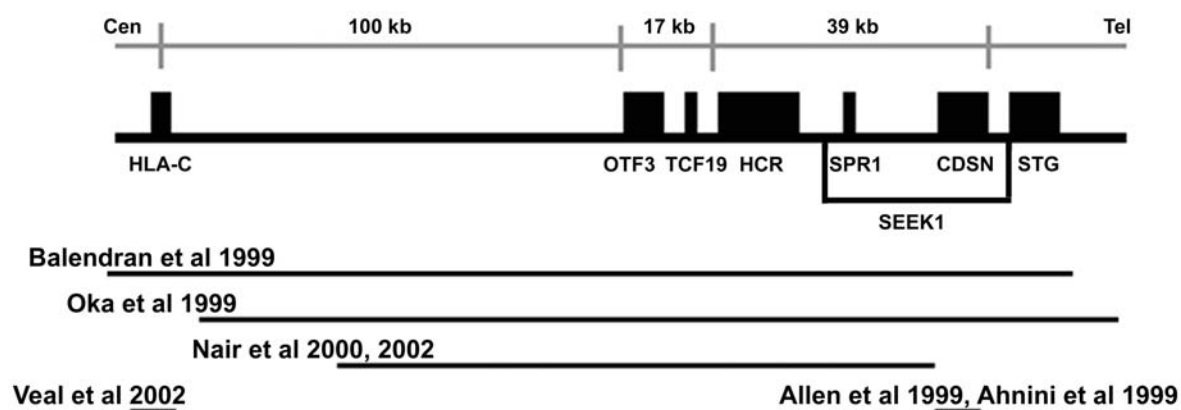

Figure 3. Map of the PSORS1 locus, with different PSORS1 regions refined in different studies (modified from Bowcock and Barker 2003).

It has been suggested that the **HLA-Cw*6** allele itself is not the direct determinant of susceptibility, but rather that it is in tight linkage disequilibrium with it (Jenisch et al. 1998). Despite the universal and strong HLA-Cw*6 association, little is known about its expression in psoriatic and normal skin. If HLA-C is proven to be "the psoriasis gene", the defined antigen would no doubt have an important role in the pathogenesis of psoriasis and this knowledge could be utilized in drug development.

HCR (alpha-helix coiled-coil rod homologue, Pg8) was first predicted from genomic sequence by Guillaudeux et al. and verified and renamed by Oka et al. (Guillaudeux et al. 1998, Oka et al. 1999). It is a novel gene in PSORS1 with non-significant structural homologies to trichohyalin, myosin, laminin, and plectin but with unknown function. In a case-control study of the Kainuu subpopulation in Finland, we demonstrated that the HCR gene was highly polymorphic with 12 SNPs; two of these showed a significant association with psoriasis (Asumalahti et al. 2000). The HLA-Cw*6 allele, however, showed even

stronger association being more rare in the control population, but the difference was not significant. In addition, HCR mRNA was expressed in psoriatic skin making HCR a tempting candidate gene (Asumalahti et al. 2000).

Corneodesmosin (CDSN, S gene) is another putative candidate gene for psoriasis. It is expressed at the late stages of keratinocyte differentiation in cornified squamous epithelia, influencing the cohesion of the corneal layer and desquamation (Zhou and Chaplin 1993, Simon et al. 2001). In addition, CDSN is expressed differently in lesional psoriatic skin than in normal or non-lesional skin or other inflammatory skin diseases (Allen et al. 2001). Allen et al. (1999) showed a significant association with three non-synonymous CDSN SNPs (CDSN-619, -1240, -1243, collectively termed CDSN*5) and psoriasis in a TDT analysis. The CDSN association with psoriasis has been confirmed, but with tight LD with HLA-Cw*6 (Jenisch et al. 1999, Tazi Ahnini et al. 1999). In a Japanese population and in the Kainuu population, however, none of the CDSN polymorphisms were associated with psoriasis (Ishihara et al. 1996, Asumalahti et al. 2000). The associations of CDSN with psoriasis in North-eastern Thai patients could be explained by LD to a nearby gene (Romphruk et al. 2003).

The other genes in the region have proved to be less tempting candidate genes for psoriasis. **OTF3** (octamer-binding transcription factor 3) is a POU transcription factor important in embryogenesis and thus major changes in this gene would probably be lethal (Niwa et al. 2000). The beta-allele of OTF3 has been shown to associate with psoriasis, but in strong LD with the HLA-Cw*0602 allele (Gonzalez et al. 2000). In Chinese patients, no differences in allele frequencies between patients and controls could be found (Chang et al. 2003b). **TCF19** (transcription factor 19, SC1) is also believed to have an important function in cell regulation (Ku et al. 1991). It is expressed in the G1S phase of the cell cycle and is involved in the transcription of genes required for the later stages of cell cycle progression (Ku et al. 1991). No disease-specific variants of these genes have been found (Nair et al. 2000, Teraoka et al. 2000). **SPR1** has four amino acid changing SNPs, but in a case-control study of Chinese patients allelic distributions did not differ between patients and controls and HLA-Cw6 remained the major risk allele (Chang et al. 2003a). In Swedish patients, one of the four SNPs showed association but this was Cw*0602 dependent (Holm et al. 2003). Five SNPs of **SEEK1** associated with psoriasis in Swedish patients, two of which even showed Cw*0602 –independent association. These SNPs were located, however, in the second exon of SEEK1, which seems to be untranslated (Holm et al. 2003). Both SPR1 and SEEK1 are expressed in skin. The **STG** gene still remains poorly characterized.

5.4.3 Other PSORS loci

A single gene alone is not responsible for the disease process, more likely several genes interplay in the pathogenesis of psoriasis. Some of these genes could be protective, others disease-causing. Several genome-wide scans have been performed in order to find new loci for psoriasis susceptibility. These linkage studies have found multiple loci in different populations (**Table 3**).

Table 3. Psoriasis susceptibility loci by genome-wide linkage scans.

Locus	Location	Reference
PSORS1	6p21.3	Trembath et al. 1997, Nair et al. 1997, Jenisch et al. 1998, Enlund et al. 1999, Balendran et al. 1999, Samuelsson et al. 1999, Oka et al. 1999, Nair et al. 2000
PSORS2	17q24-q25	Tomfohrde et al. 1994, Nair et al. 1997, Enlund et al. 1999, Samuelsson et al. 1999
PSORS3	4q34	Matthews et al. 1996
PSORS4	1q21	Bhalerao and Bowcock 1998, Capon et al. 1999
PSORS5	3q21	Samuelsson et al. 1999, Veal et al. 2001
PSORS6	19p13	Lee et al. 2000
PSORS7	1p35-p34	Veal et al. 2001
PSORS8	16q12-q13	Nair et al. 1997, Karason et al. 2003

PSORS2 in 17q24-q25 has been confirmed by linkage analysis in different Caucasian patients (Tomfohrde et al. 1994, Enlund et al. 1999, Nair et al. 1997, Samuelsson et al. 1999). Helms et al. (2003) showed associating SNPs in two peaks: the proximal peak located in or near SLC9A3R1 - involved in epithelial membrane biology and immune synapse formation in T cells - and NAT9 (a new member of the N-acetyltransferase family), and the distal peak in RAPTOR (p150 target of rapamycin (TOR)-scaffold protein containing WD-repeats). A disease-associated SNP in the proximal peak causes loss of the RUNX1 (runt-related transcription factor) binding, suggesting defective regulation of SLC9A3R1 or NAT9 by RUNX1 as a susceptibility factor for psoriasis (Helms et al. 2003).

Matthews et al. performed a genome-wide scan in six of the 17q unlinked multiplex Irish families and found positive linkage at **PSORS3** in 4q34 (Matthews et al. 1996).

In Italian families, the genome-wide scan and fine mapping with LD showed evidence for linkage at another locus, **PSORS4** in 1q21 (Capon et al. 1999, Capon et al. 2001). The same region had been identified with families from the USA (Bhalerao and Bowcock 1998). PSORS4 resides within the region of the Epidermal Differentiation Complex (Mischke et al. 1996), in which genes encoding structural proteins of epidermal cornification (such as involucrin and trichohyalin) and S100 proteins are located.

Two genome-wide scans in Swedish families suggested linkage at 3q21 **(PSORS5)** (Enlund et al. 1999, Samuelsson et al. 1999). Hewett et al. narrowed the locus to a 250-kb interval by TDT analysis (Hewett et al. 2002). The SLC12A8 gene occurs in this region. The predicted protein shares 30% to 40% homology with the family of cation/chloride cotransporters, making the suggested susceptibility interesting in the sense of understanding the possible biochemical pathways in psoriasis.

In German families **PSORS6** at 19p13 was reported in addition to two other possible loci on chromosomes 8q and 21q (Lee et al. 2000).

In families from the UK, a novel susceptibility locus on 1p35-p34 (**PSORS7**) was identified along with two other novel loci at 2p and 14q (Veal et al. 2001).

In a large Icelandic family, another possible susceptibility locus was found at 16q12-q13 (**PSORS8**) (Karason et al. 2003). Crohn's disease variant gene (NOD2) is also on 16q, but no evidence for association between NOD2 and psoriasis has been found (Nair et al. 2001).

We searched for other psoriasis susceptibility loci in our families unlinked to PSORS1 by a genome scan, and identified linkage to **18p11**, suggested also in British patients (Asumalahti et al. 2003b, Veal et al. 2001). Moreover, a Swedish study showed a susceptibility locus for atopic dermatitis to be on 18p (Bradley et al. 2002), and psoriasis is already known to share other susceptibility loci with atopic dermatitis: PSORS4, PSORS5 and PSORS2 (Cookson and Moffat 2002). Interestingly, another autoimmune disease, rheumatoid arthritis, shares susceptibility loci PSORS5 and PSORS2 (Bowcock and Barker 2003). These connections remain to be solved.

5.5   *Matrix metalloproteinases in the proteolytic remodeling of the ECM*

Spatially and temporally regulated remodeling of the ECM macromolecules requires cooperation of many different exo- and endopeptidases (proteinases). **Endopeptidases** can be divided into four subgroups (cysteine proteases, aspartic proteases, serine proteases, metalloproteinases) according to the amino acid residue or cofactor required for catalytic activity. **Metalloproteinases** contain a metal ion in the catalytic site. They can be further divided into 40 families, one of which is the superfamily of **metzincins**, members of which bind zinc at the catalytic site, have conserved "Met-turn" motifs and conserved structural topology. Metzincins consist of serralysins, matrixins (**matrix metalloproteinases**), astacins, and adamalysins (Sternlicht and Werb 2001). Matrix metalloproteinases currently comprise 23 human members, which can be divided into six subgroups according to their structure and substrate specificity (McCawley and Matrisian 2001) (**Figure 4**).

Upon translation, the matrix metalloproteinase (MMP) is in a prepro-form with the N-terminal hydrophobic signal peptide, which leads the protein for secretion out of the cell. The secreted pro-MMP is inactive, possessing a highly conserved sequence which has a cysteine residue binding the catalytic zinc ion (the so-called "cysteine switch"). Activation occurs when the Cys-zinc bond disrupts and the pro-domain cleaves off leading to a conformational change (Sternlicht and Werb 2001, Egeblad and Werb 2002). The catalytic core-domain contains a metal-binding site for Zn^{2+}, which is crucial for the proteolytic activity, and a conserved methionine forming the "Met-turn" structure (Sternlicht and Werb 2001).

Figure 4. Matrix metalloproteinases: structures, families, and common substrates (modified from Raza and Cornelius 2000, Sternlicht and Werb 2001, McCawley and Matrisian 2001).

MMPs can collectively cleave most ECM and BM macromolecules and therefore participate in such physiological processes as fetal development and morphogenesis, endometrial cycling, ovulation, and mammary development, tooth eruption and bone remodeling, inflammatory cell function, apoptosis, nerve growth, and angiogenesis. Destructive processes can also be mediated by MMPs, some examples are tumor cell invasion and metastasis, atherosclerosis, rheumatoid arthritis, gastric ulceration, chronic wounds, bullous skin diseases, and dermal photoageing (Kähäri and Saarialho-Kere 1997, 1999).

MMPs are important regulators of wound healing (Ågren et al. 2001, Lund et al. 1999) and MMP expression is elevated especially in chronic wounds (Saarialho-Kere 1998, Trengove et al. 1999). The hyperproliferative keratinocytes of normal re-epithelialization resemble proliferating keratinocytes seen in psoriasis. In addition to this, MMPs permit tunneling of new vessels through dermal matrix, a process in neoangiogenesis encountered in wound healing as well as in psoriasis (Burbridge et al. 2002). An inflammatory reaction is characteristic for both wound healing and psoriasis, and various inflammatory cells are capable of expressing an impressive number of MMPs: monocytes express MMP-1, -7, -8, and -9, macrophages MMP-1, -2, -3, -8, -9, -10, -12, and -13, and neutrophils store MMP-8, -25 and -9 (Barrick et al. 1999, Prikk et al. 2001). In addition, retinoids, which are used as antipsoriatic drugs, can suppress MMP synthesis (Li et al. 1999). Currently the safety and efficacy of systemic and topical metalloproteinase inhibitors are being investigated as possible new drugs for psoriasis (Kirby and Griffiths 2001, Sawa et al. 2002, Sauder et al. 2002).

5.5.1 Collagenases

Collagenases -1 (MMP-1), -2 (MMP-8), and -3 (MMP-13) degrade native fibrillar collagens (type I, II, III, V, and IX) at a specific site to produce N-terminal and C-terminal triple helical fragments, which spontaneously denaturate to gelatin.

MMP-1 is expressed in many normal cells, such as migrating epidermal keratinocytes, fibroblasts, endothelial cells, monocytes, and macrophages, and is detected in various physiological situations such as wound healing (Pilcher et al. 1998, Saarialho-Kere 1998). MMP-1 preferentially cleaves type III collagen. Fibrillar collagen induces MMP-1 expression of migrating keratinocytes via $\alpha2\beta1$ integrin (Pilcher et al. 1998). MMP-1 is associated with pathological conditions as well - these include various inflammatory diseases and cancers (Johansson et al. 2000a).

MMP-8 (neutrophil collagenase) is synthetizised by polymorphonuclear leukocytes in bone marrow, and - distinctly from the two other collagenases - stored intracellularly in granules and released upon extracellular stimulation (Hasty et al. 1990). MMP-8 preferentially cleaves type I and type II collagens, possessing a high affinity for type I collagen (Armstrong and Jude 2002). The level of MMP-8 is a 100-fold higher than the MMP-1 level in wound exudates and tissue extracts (Nwomeh et al. 1999).

MMP-13 preferentially cleaves type II collagen and gelatin but has a potent degrading activity against a wide spectrum of substrates (i.e., type IV, X, XIV collagens, tenascin, fibronectin, and aggrecan core protein) (Kähäri and Saarialho-Kere 1997, Sternlicht and Werb 2001). MMP-13 is expressed in culture by skin fibroblasts and keratinocytes (Johansson et al. 2000b). Activated fibroblasts as well as macrophage-like cells in chronic wounds express MMP-13 abundantly, but in acute human wounds MMP-13 mRNA expression is low (Saarialho-Kere 1998, Vaalamo et al. 1997). MMP-13 production and

activation is induced in fibroblasts cultured within collagen gel, mediated by $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins requiring p38 activity (Ravanti et al. 1999).

5.5.2 Gelatinases

Gelatinases A (MMP-2, 72 kDa gelatinase) and B (MMP-9, 92 kDa gelatinase) contain three fibronectin-type II domains in the hemopexin domain, which are important for elastolytic activity. They preferentially cleave gelatin and type IV collagen, but cleave other BM components as well (Shipley et al. 1996a, Birkedal-Hansen 1995). The expression of gelatinases correlates with the invasiveness of several tumors (Stetler-Stevenson and Yu 2001) and they are suggested to contribute to the deformation of skin structure in several skin diseases. Indeed, psoriatic papillomatosis may be caused by keratinocyte overexpression of MMP-2 and MMP-9 gelatinases together with the absence of TIMP-1 and TIMP-2 (Feliciani et al. 1997). T cells can also secrete both gelatinases (Leppert et al. 1995).

MMP-2 is produced by many cell types including keratinocytes, fibroblasts, and endothelial cells (Kähäri and Saarialho-Kere 1997), and is believed to have a unique role in cancer invasion cleaving native type I collagen (Aimes and Quigley 1995). In fact, MMP-2 knock-out mice have reduced angiogenesis, impaired tumor growth, and decreased metastasis (Sternlicht and Werb 2001). In both acute and chronic wounds, MMP-2 is expressed by fibroblasts of the granulation tissue (Oikarinen et al. 1993, Salo et al. 1994) and is seen in the leading epidermal wound edge as well (Ashcroft et al. 1997, Oikarinen et al. 1993, Salo et al. 1994). When the production of MMP-2 is inhibited by tetracycline analogues, the migration of mucosal and skin keratinocytes is inhibited. MMP-2 is also reported to be present in the papillary dermis, but not in epidermis, in psoriasis (Feliciani et al. 1997).

MMP-9 is expressed by keratinocytes but also by different inflammatory cells including lymphocytes, macrophages, polymorphonuclear leukocytes, eosinophils, mast cells, and malignant cells (van den Steen 2002). Like MMP-8, MMP-9 is synthesized during neutrophil development, stored within granules and released upon stimulus. In other cell types, MMP-9 expression requires transcriptional activity. In acute wounds, oral mucosal wounds and in the epidermis of various blistering diseases (but not in chronic wounds), MMP-9 is seen in leading edge keratinocytes and in neutrophils and macrophage-like cells in the wound granulation tissue (Ashcroft et al. 1997, Oikarinen et al. 1993, Salo et al. 1994). In MMP-9 knock-out mice apoptosis, vascularization, and ossification are delayed (Vu et al. 1998), and they are resistant to blister formation in a bullous pemphigoid model (Liu et al. 1998). MMP-9 substrates include interleukin-1 (Schonbeck et al. 1998) and TGF-β (Yu and Stamenkovich 2000), and it possesses angiostatin converting enzyme activity (Patterson and Sang 1997). MMP-9 has also been reported in keratinocytes and in the upper dermis around blood vessels in psoriasis *in vivo* (Feliciani et al. 1997) and in the culture fluid of psoriatic lesional skin *in vitro* (Varani et al. 1998).

5.5.3 Stromelysins

Stromelysins -1 (MMP-3), -2 (MMP-10), and -3 (MMP-11) share domain structure with collagenases but are incapable of degrading native fibrillar collagens.

MMP-3 activates proMMPs -1, -3, -7, -8, -9, and -13 (McCawley and Matrisian 2001) and is expressed by many cells including keratinocytes and fibroblasts (Kähäri and Saarialho-Kere 1999). MMP-3 knock-out mice show impaired wound contraction when challenged and are susceptible to arthritis (Sternlicht and Werb 2001). MMP-3 is involved in the proliferative phase of wound healing (Vaalamo et al. 1996).

MMP-10 degrades the protein core of proteoglycans, type IV and IX collagens, laminin-1, and fibronectin (McCawley and Matrisian 2001). In response to cytokines, T cells are capable of producing MMP-10. Migrating epithelial cells express MMP-10 during wound healing and it most likely participates in the remodeling of newly formed BM (Rechardt et al. 2000, Vaalamo et al. 1998).

MMP-11 does not degrade ECM components but cleaves serine proteinase inhibitors and insulin-like growth factor binding protein-1 and releases matrix-bound growth factors (Manes et al. 1997, Boulay et al. 2001). MMP-11 has a furin cleavage site and thus is processed intracellularly before release as a mature enzyme. Knock-out mice are more resistant to chemically induced tumors and in fact, high MMP-11 expression is associated with promoted homing of malignant epithelial cells and decreased cancer cell death (Masson et al. 1998, Boulay et al. 2001). MMP-11 is expressed during wound healing as well (Luo et al. 2002).

MMP-12 (human metalloelastase, HME) is able to degrade elastin, type IV collagen, gelatin, fibronectin, laminin-1, entactin, vitronectin, and proteoglycans (Sternlicht and Werb 2001). MMP-12 is expressed mainly by macrophages in intestinal ulceration and cutaneous diseases associated with granulomas or macrophage migration (Vaalamo et al. 1998, Vaalamo et al. 1999). GM-CSF stimulates macrophages to produce metalloelastase, and thus MMP-12 may inhibit angiogenesis of tumours by converting plasminogen to angiostatin and by reducing the number of urokinase-type plasminogen activator receptors on endothelial and tumor cells (Cornelius et al. 1998, Koolwijk et al. 2001). In MMP-12 knock-out mice macrophages show reduced ability to invade through BMs *in vivo* and *in vitro* (Shipley et al. 1996b), and the mice are not susceptible to aortic aneurysm formation (Sternlicht and Werb 2001).

5.5.4 Matrilysins

Matrilysin -1 (MMP-7) and -2 (endometase, MMP-26), the smallest MMPs, lack the hinge region and hemopexin domain (Park et al. 2000). They also have a unique threonine residue adjacent to the Zn-binding site.

MMP-7 cleaves fibronectin and laminin-1, among others (Sternlicht and Werb 2001). It is expressed constitutively by normal exocrine and mucosal epithelium-like skin and salivary glands and can be stored in the secretory epithelial cells. Interestingly, MMP-7 activates alpha-defensins, having a role in innate host defence (Lopez-Boado et al. 2000) in the small intestine. MMP-7 is upregulated in many tumors of epithelial origin such as breast (Basset et al. 1990), lung and upper respiratory tract (Muller et al. 1991), skin (Karelina et al. 1994), stomach and colon (Newell et al. 1994, McDonnell et al. 1991) cancers, but may inhibit tumor angiogenesis as well by generating angiostatin (Patterson and Sang 1997, Pozzi et al. 2000). MMP-7 knock-out mice show reduced intestinal tumorigenesis (Wilson et al. 1997), and are impaired in activating prodefensins and kill exogenous bacteria in the small intestine (Wilson et al. 1999).

MMP-26 cleaves fibrinogen and fibronectin, type IV collagen, gelatin, and vitronectin (Park et al. 2000, Marchenko et al. 2001). MMP-26 is expressed in malignant tumors and tumor cell lines (Marchenko et al. 2001) but also in many normal tissues, including peripheral blood leukocytes (Marchenko et al. 2001).

5.5.5 MT-MMPs

MMP-14, MMP-15, MMP-16, and **MMP-24** together form a subgroup of type I transmembrane- MMPs, and differ structurally from other MMPs by having a furin-sensitive motif cleaving of which leads to activation, a transmembrane domain, and a cytoplasmic tail at the C-terminus (Sternlicht and Werb 2001). **MMP-17** and **MMP-25** are glycosylphospatidylinositol-anchored (GPI) MMPs bound to the cell surface. Collectively MT-MMPs cleave a wide variety of ECM molecules *in vitro*, but MMP-17 and MMP-25 seem to have fewer substrates than the others. MMP-14 and MMP-15 can degrade gelatin, fibronectin, tenascin, nidogen, aggrecan, perlecan, laminin, and are able to cleave proTNF-α into active form (d'Ortho et al. 1997). All but MMP-17 and MMP-25 activate proMMP-2, and MMP-14 together with MMP-15 activates MMP-13. MMP-14 deficient mice have impaired angiogenesis and endochondrial ossification (Zhou et al. 2000).
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MMP-23 (CA-MMP) lacks the transmembrane domain and GPI-anchor, but has instead two novel motifs, a cysteine array (CA) and an Ig-fold (Velasco et al. 1999). The mouse orthologue for MMP-23, CA-MMP, is a type II transmembrane MMP (Pei et al. 2000). MMP-23 is expressed mainly in ovary, testis, and prostate (Velasco et al. 1999).

5.5.6 Other MMPs

The novel members of the MMP family differ from the other MMP subcategories structurally or functionally or both, and are proposed to form a new subclass of MMPs.

MMP-19 (RASI) was first cloned from mammary gland and liver (Cossins et al. 1996, Pendas et al. 1997) and is detected in the placenta, lung, pancreas, ovary, spleen, and intestine by northern hybridization (Pendas et al. 1997). It has previously been detected in endothelial and vascular smooth muscle cells *in vivo* suggesting a role in angiogenesis (Kolb et al. 1999). MMP-19 has a wide substrate specificity, cleaving many components of the BM and ECM, e.g., type IV collagen, laminin, nidogen, tenascin-C, fibronectin, type I gelatin, aggrecan, and cartilage oligomeric protein (COMP), but does not seem to activate any proMMPs (Stracke et al. 2000a, Stracke et al. 2000b). TIMP-2, -3, -4, and to a lesser extent TIMP-1, are able to inhibit MMP-19 (Stracke 2000a).

MMP-20 (enamelysin) is an MMP involved in tooth enamel formation. It is expressed by ameloblasts and odontoblasts of developing teeth and is capable of degrading amelogenin (enamel matrix protein), aggrecan, and COMP (Stracke et al. 2000b). MMP-20 is found in human tongue SCC cells, tooth pulp, and placenta (Väänänen et al. 2001).

MMP-21 expression is detected in leukocytes, brain, lung, testis, ovary, colon, and kidney, but is seen in various cancers (such as prostatic, ovarian, breast, colon, pancreatic and skin carcinomas) and several cancer cell lines as well (Ahokas et al. 2002). MMP-21 is homologous to the Xenopus MMP (MMP-18) and may play a role in fetal development (Ahokas et al. 2002). In addition, it seems to be expressed mainly in epithelial structures and it is regulated at least by TGF-β1 in keratinocytes (Ahokas et al. 2003).

MMP-28 (epilysin) was cloned from testis, keratinocyte, and lung cDNA libraries (Lohi et al. 2001, Marchenko and Strongin 2001). Structurally MMP-28 is related to MMP-19 (Illman et al. 2001), and seems to be alternatively spliced and cleaved by a furin-like proprotein convertase (Illman et al. 2003). MMP-28 is associated with proliferative cells in epithelial wound repair (Lohi et al. 2001, Saarialho-Kere et al. 2002) and various carcinomas express MMP-28 (Marchenko and Strongin 2001). Because of its wide expression in normal tissues, it has been claimed that MMP-28 functions in physiological tissue homeostasis and turnover.

5.5.7 Regulation of matrix metalloproteinases

The MMPs are tightly regulated because of their potential to degrade tissues, and the regulation occurs temporally and spatially at several levels. Transcription, mRNA stabilization, translation, trafficking of membrane-bound forms (secretion and endocytosis), shedding, localization, zymogen activation, and inhibition of proteolysis can all be regulated. Normally, MMPs are expressed only at low levels but upon stimulation i.e. when tissue remodeling is needed, their production and activation is enhanced (Nagase and Woessner 1999, Sternlicht and Werb 2001). Most MMPs are transcriptionally regulated, but MMP-8 and MMP-9 are stored in granules of neutrophils and macrophages and MMP-7 is expressed constitutively in the ductal epithelium of exocrine glands.

Transcription The expression of MMPs is mainly transcriptionally regulated and MMP protein levels generally correlate with mRNA levels *in vivo* (Saarialho-Kere et al. 1993, Sternlicht and Werb 2001). Many cytokines and growth factors (e.g. TNF-α, EGF, bFGF, IL-1, PDGF, IL-6 and TGF-β), hormones, oncogenes, cell-cell and cell-matrix interactions, cellular transformation, bacterial endotoxin and other virulence factors, and extrinsic chemical and physical factors (e.g. UV radiation) induce production of MMPs (Kähäri and Saarialho-Kere 1999). This regulation can even be cell type specific (Sternlicht and Werb 2001).

When the *ligand* binds to the appropriate *receptor*, it (or other external stimuli) launches a signal cascade, which involves *phosphorylation* of cell surface serine/threonine kinases (mitogen-activated protein kinases, *MAPK*). Either ERK1,2 (activated by mitogens and phorbol esters), JNK/SAPK, or p38 (activated by inflammatory cytokines or cellular stress) MAP-kinase pathway is activated. Phosphorylation of MAP-kinase induces *transcriptional factors AP-1* (activator protein-1) *and ETS* (E26 transformation specific), which bind to the conserved promoter regions of inducible MMP genes (*AP-1 binding element* and *PEA3 element* - polyoma enhancer A-binding protein-3 - respectively), inducing *MMP expression*. ETS proteins form a complex with other transcription factors before they bind to DNA (Westermarck and Kähäri 1999).

In addition to AP-1 and ETS, nuclear factor of kappabeta (NFκB) signal transducers and activators of transcription, p53, and TGF-β inhibitory element are important transcription factors controlling MMP expression (Johansson et al. 2000b, Kähäri and Saarialho-Kere 1999, Van den Seen et al. 2002). Alternative splicing also contributes to MMP expression (Sternlicht and Werb 2001, Luo et al. 2002). SNPs in several MMP gene promoters influencing transcription have been associated with increased susceptibility to different cancers (Ye 2000, Sternlicht and Werb 2001).

MMP-2, MMP-11, MMP-28, and MMP-14 lack the AP-1 site. MMP-11 is transcriptionally activated only by retinoic acid or by stromal cell interactions with tumor cells (Anglard et al. 1995, Ahmad et al. 1997)

Modulation of mRNA stability EGF and phorbol esters can stabilize MMP-1 and MMP-3 mRNA transcripts and PDGF MMP-13 mRNA transcripts (Sternlicht and Werb 2001). The AU-rich sequences in the 3'-untranslated region regulate at least the stability of MMP-1 mRNA (Sternlicht and Werb 2001).

MMP secretion Only activated neutrophils secrete pre-formed MMP-8 and MMP-9 while protein kinase C, plasmin, and thrombin release MMP-12 from macrophages (Sternlicht and Werb 2001).

Proenzyme activation Most MMPs are secreted in latent form and the cysteine residue in the prodomain needs to be removed from the Zn^{2+}-ion before extracellular activation (Nagase and Woessner 1999, Sternlicht and Werb 2001). In contrast, MMP-11, MMP-28, and MT-MMPs are activated intracellularly by furin-like proteases (Velasco et al. 1999, Sternlicht and Werb 2001, Illman et al. 2003). MMP-2 is activated by the MT-MMPs and TIMP2 (Sternlicht and Werb 2001). *In vivo*, tissue and plasma proteinases, such as the uPA/plasmin system, or bacterial proteinases activate MMPs and many MMPs activate pro-forms of other MMPs (Carmeliet et al. 1997, Nagase and Woessner 1999, Sternlicht and Werb 2001). Mere substrate binding to proMMP-9 can activate it without removal of the prodomain (Bannikov et al. 2002).

Inhibitors TIMPs (tissue inhibitor of matrix metalloproteinase) are the physiological, local and reversible inhibitors of MMPs, and the inhibition occurs by binding noncovalently to the active site in 1:1 stochiometry. The hemopexin domain is responsible for MMP substrate and inhibitor specificity, but the inhibition of MMP activity takes place due to binding to the catalytic domain. Four TIMPs are known to date; TIMP-3 binds to ECM, the others are soluble (Nagase and Brew 2002). TIMPs can inhibit the activity of all MMPs *in vitro*, with only a few exceptions: TIMP1 inhibits only poorly MMPs -14, -15, -16, -17, and -24 as well as MMP-19 (Lee et al. 2004). An imbalance between TIMP and MMP activities is considered to result in excessive degradation of ECM components in tumor invasion and metastasis (Egeblad and Werb 2002). Other functions of TIMPS include matrix binding, inhibition of angiogenesis and the induction of apoptosis (Nagase and Woessner 1999). TIMPs are, however, capable of inducing VEGF secretion as well (Egeblad and Werb 2002).

TIMP-1 promotes keratinocyte growth, but overexpression of TIMP-1 reduces tumor cell growth (Gomez et al. 1997). The expression is upregulated by TGF-β, EGF, TNF-α, ILs, retinoids, and glucocorticoids. TIMP-1 is not expressed in psoriasis (Feliciani et al. 1997).

TIMP-2 also has cell growth-promoting activity and is mainly constitutively expressed (Birkedal-Hansen 1995, Wang et al. 2002). TIMP-2 is not expressed in psoriasis (Feliciani et al. 1997).

TIMP-3 is the most potent in inhibiting MMP-9 but inhibits shedding of the TNF-α receptor, L-selectin, the IL-6 receptor, and syndecans-1 and -4, and ADAM-17 (TACE = TNF-α converting enzyme) (Nagase and Woessner 1999, Borland et al. 1999, Fitzgerald et al. 2000, Sternlicht and Werb 2001). Overexpression of TIMP-3 induces apoptosis in several cancer cell lines (Nagase and Woessner 1999). Expression is upregulated by TGF-β, EGF, TNF-α, ILs, retinoids, and glucocorticoids.

TIMP-4 is mainly expressed in heart and inhibits particularly MMP-2, -7, and -26 activity but MMP-1, -3 and -9 as well (Greene et al. 1996, Rahkonen et al. 2002).

Serine protease inhibitors (serpins) block plasminogen activation regulating MMP activity. These include alpha1-antitrypsin and plasminogen activator inhibitors (PAI)-1 and -2. *Alpha2-macroglobulin* is a non-specific proteinase inhibitor, which is the major inhibitor of MMPs in tissue fluids (Sternlicht and Werb 2001).

5.5.8 Cytokines relevant both in MMP biology and psoriasis

MMPs may play a role in the release of various growth factors from the ECM and mediate receptor turn over (Vu and Werb 2000, Egeblad and Werb 2002). Moreover, MMPs can be induced by different cytokines, several of which are important in the pathobiology of psoriasis.

At least MMP-1, -2, -3, -7, -9, -12, and -14 cleave pro**TNF-α** into the biologically active form (Sternlicht and Werb 2001). TIMP3, instead, prevents TNF-α shedding in cell-culture models (Nagase and Woessner 1999, Sternlicht and Werb 2001). On the other hand, TNF-α is capable of activating proMMP-9 to active enzyme in human skin (Han et al. 2001a) and promoting activation of proMMP-2 by inducing MT1-MMP via the NFκB pathway when embedded in type I collagen in dermal fibroblasts (Han et al. 2001b). In addition, TNF-α and **TGF-β** synergistically induce proMMP-9 in human skin as well as in isolated dermal fibroblasts and epidermal keratinocytes (Han et al. 2002). In activated, *in vitro* generated Langerhans cells (LC), TNF-α also initiates proMMP-2 and proMMP-9 production before migration. Moreover, activation of LC induces synthesis of TNF-α and expression of TNFRII on the cell membrane (Noirey et al. 2002). In human gingival mucosal keratinocytes, TNF-α and **IL-1β** stimulate MMP-9, but not MMP-2 production, while **IFN-γ** inhibits MMP-9 production (Mäkelä et al. 1998, Salo et al. 1994). MMP-9 expression is induced and MMP-2 expression augmented in fibroblasts by TNF-α, **TGF-β,** and **EGF** (Kobayashi et al. 2003).

TIMP-1 is down-regulated by TNF-α in skin (Han et al. 2002) but on the other hand, TNF-α is also reported to enhance TIMP-1 expression together with MMP-1 and MMP-3 expression in fibroblast cultures (Dasu et al. 2003, Reunanen et al. 2002). In transformed keratinocytes, MMP-1 is enhanced by TNF-α (Ala-aho et al. 2000). TNF-α stimulates MMP-13 expression in transformed keratinocytes and endothelial cells (Ala-aho et al. 2000, Hattori et al. 2003) and microvascular endothelial cells can be stimulated to migrate by exposure to TNF-α. These migrating cells express collagenase mRNA but not TIMP-1. **IFN-γ** instead upregulates TIMP1 but blocks upregulation of collagenase caused by TNF-α (Cornelius et al. 1995). TNF-α, **EGF**, and **TGF-β** induce MMP-10 expression in keratinocyte cultures (Rechardt et al. 2000), and MMP-12 expression is induced by TNF-α and **TGF-β** in epithelial MCF10 cells (Kerkelä et al. 2002). In human epidermal keratinocytes **EGF** stimulates MMP-9 (Zeigler 1999).

IFN-γ induces MMP- 1 and MMP-3 in cultured keratinocytes both at mRNA and protein level (Tamai et al. 1995). In addition, MMP-13 is downregulated in transformed human keratinocytes and human cutaneous SCC cells by IFN-γ (Ala-aho et al. 2000).

GM-CSF enhances MMP-9 in monocytes (Zhou et al. 2003) and activated neutrophils (Takafuji et al. 2003). It also induces MMP-1 in monocytes when in combination with TNF-α or IFN-γ (Zhou et al. 2003) and MMP-12 in macrophages (Wu et al. 2000, Jost et al. 2003).

IL-1 induces MMP-12 in macrophages (Wu et al. 2000) and IL-1α stimulates MMP-13 in endothelial cells (Hattori et al. 2003). IL-1β induces MMP-1 expression via transactivation of the EGF receptor and ERK pathway in human keratinocytes (Wan et al. 2001). IL-1β may be activated by MMP-2, MMP-3, and MMP-9 (Vu and Werb 2000). **IL-2** can cleave IL-2 receptor-α on T cells, depressing the IL-2 dependent proliferation (McCawley and Matrisian 2001). **IL-6** enhances MMP-1 and TIMP1 in fibroblast cultures (Dasu et al. 2003). **IL-8** stimulates neutrophils to secrete MMPs, and MMP-9 potentiates the activity of IL-8 (Van den Steen et al. 2002). **IL-12** in monocytes enhances MMP-2 and MMP-14 (Abraham 2002).

MCP-1 enhances gene expression of MMP1 as well as TIMP1 in primary human dermal fibroblasts (Yamamoto et al. 2000). **IL-8, RANTES, MIP1α, MIP1β,** and **MCP1** induce

expression of metalloproteinases in various leukocyte subtypes (Xia et al. 1996, Johnatty et al. 1997, Wu et al. 2000). CC chemokine ligand **(CCL)5/RANTES** induces MMP-19 in human monocytes (Locati et al. 2002). MIG, IP-10, IL-8, MCP-3 are cleaved by MMP-9 and MIG and IP-10 by MMP-8 (Van den Steen et al. 2003).

MMP-1 and MMP-3 cleave perlecan releasing **bFGF** (Whitelock et al. 1996), and **endothelin-1** can be cleaved by MMP-2 to produce a variant with greater vasoconstrictor effects (Vu and Werb 2000). MMP-2 is capable of shedding FGF receptor type I from the cell surface as well (McCawley and Matrisian 2001).

TGF-β1 reduces the expression of collagenase genes and activity in cultured cells while simultaneously elevating the expression of TIMPs (Verrechia and Mauviel 2002, Salmela et al. 2003). MMP-2, -3, and -7 cleave decorin releasing TGF-β (Vu and Werb 2000), and MMP-2 and MMP-9 process TGF-β into an active ligand (Van den Steen et al. 2002, Yu and Stamenkovic 2000). TGF-β increases keratinocyte migration, as well as MMP-2 secretion and cell-associated production in keratinocytes (Mäkelä et al. 1999). It also upregulates both MMP-2 and MMP-9 activities in cultured fibroblasts and also, transiently, MMP-2 mRNA expression (Salo et al. 1991, Van den Steen et al. 2002, Verrecchia and Mauviel 2002). TGF-β induces MMP-2 and -9 production in human gingival mucosal keratinocytes (Salo et al. 1991) and increases MMP-2 and -9 expression in peripheral blood monocytes and in various tumorigenic and non-tumorigenic cell lines (Van den Steen et al. 2002). TGF-β activates MMP-13 and -1 (Ala-aho 2000) and upregulates MMP-12 in epithelial cells (Kerkelä et al. 2002).

5.6   IFI27 (ISG12, p27) as a member of the ISG family

The IFI27 gene is located at 14q32, where genome-wide linkage scans have reported one of the psoriasis susceptibility loci to reside (Veal et al. 2001). Moreover, in a large gene expression study, IFI27 was one of the most abundantly expressed genes in psoriatic skin along with keratin 6, 16, 17, β2-defensin, and psoriasin; IFI27 was also upregulated in nonlesional skin (Bowcock et al. 2001).

The interferons mainly act by stimulating over 1000 interferon-stimulated genes (ISGs), which can be responsive to type I IFNs (α/β, via IFN-stimulated regulatory elements mediated pathway, ISRE) or type II IFNs (γ, via gamma activated sequence mediated pathway, GAS) or both (Stark et al. 1998, Smidt 2003, Parker and Porter 2004). Members of the interferon cytokine family have antitumoral, antiviral, antiparasite, immunomodulatory, and antiproliferative functions (Stark 1998). They represent part of the natural defence to tumors, and α/β-IFNs are used as antiproliferative agents in cancer treatment, hepatitis C, and multiple sclerosis (Gjermandsen et al. 2000, Parker and Porter 2004).

IFI27, originally cloned as an estrogen-induced gene from the human cell line MCF7, is a new member of the small ISGs - other small ISGs include 6-16, 9-27, 1-8, 15, and 6-26 (Rasmussen et al. 1993). High IFI27 expression levels were demonstrated in many breast carcinomas, although the expression was independent of the presence of estradiol receptors. IFI27 was found to be induced by α-IFN in several cell lines, and expressed in

colon, stomach, and lung. Gjermandsen et al. (2000) have reported IFI27 and 6-16 mRNA to be highly inducible by IFN-α, slightly inducible by IFN-γ and not induced by IL-6, IL-2, TNF-α, GM-CSF, or EGF in several cell lines including the SVK-14 keratinocyte cell line. IFI27 and 6-16 EST (expressed sequence tag) sequences were found in the UniGene database from uterus, testis, placenta, breast, colon, brain, and kidney and in many libraries of carcinogenic origin (Gjermandsen et al. 2000).

Another small ISG, 6-16, shares 33% structural homology with IFI27 and is highly inducible by type I IFNs (α/β-IFN). The IFI27 gene encodes a putative hydrophobic protein of 122 amino acids. The 5'end of the IFI27 gene is alternatively spliced, leading to a shorter transcript in HeLa and AMA cells or a longer one in MCF7 cells, but the coding region could not be extended to include a signal peptide as with the homologous 6-16 cDNA. This signal peptide localizes 6-16 to the plasma membrane of the cell. The IFI27 protein, however, was found on the nuclear membrane when studied with a polyclonal antibody against a synthetic peptide identical to the N-terminal sequence of IFI27 (Martensen et al. 2001).

The exact function of 16-6 and IFI27 proteins remains unknown. IFI27 homologies are found in rat (IRG1) and hamster, and it has been reported very recently that humans possess altogether four ISG12 genes: 6-16, ISG12a, ISG12b, and ISG12c, of which 6-16 and ISG12a are upregulated by type I IFNs (Parker and Porter 2004). Type I IFN elevated ISG12 expression two-fold in a STAT1 negative manner in human 2fTGH cells (Kim et al. 2003).

6   Aims of the study

The principal purpose was to study the function of HCR, a novel psoriasis candidate gene in the PSORS1 locus, in the skin. The contribution of various matrix metalloproteinases and IFI27 in the pathobiology of psoriasis was also studied. This was achieved by investigating:

1. the structure of HCR and psoriasis-associated HCR polymorphisms and other susceptibility alleles in the PSORS1 locus in different populations

2. HCR protein expression in lesional and nonlesional psoriatic skin compared to other psoriasis-like hyperproliferative skin disorders and normal skin

3. HCR expression and regulation *in vitro* in normal and psoriatic keratinocytes

4. expression of various matrix metalloproteinases and their natural inhibitors in psoriatic skin

5. expression and regulation of an interferon-stimulated gene, IFI27, in psoriasis compared to other inflammatory skin disorders and wound repair

7   Materials and Methods

7.1   Patients

During 1999 - 2000 the Finnish Psoriasis Consortium (Helsinki, Turku, Tampere, Oulu University Hospitals and the Central Hospital of Päijät-Häme) examined 480 isolated psoriasis patients, 200 families with one affected member, and 30 multiple pedigree psoriatics. In Study I, we analyzed 91 nuclear families with a proband and parents (trios). One of the parents was also affected in 21 families.

Inclusion criteria for the study were: 1.Onset of psoriasis before the age of 40 yr. 2. At least two typical psoriasis plaques present when the patient was examined and interviewed. 3. The patient was over 15 years of age.

Exclusion criteria were: 1. The patient had pustulosis palmoplantaris. 2. Presence of scalp psoriasis only. 3. The diagnosis was uncertain.

To study associations of the PSORS1 candidate alleles in patients of different ethnic origin (Study I), DNA samples from British (n = 175), Swedish (n = 64), Italian (n = 48) and Gujarati Indian (n = 27) trios and 52 multiplex Spanish families were obtained. We also analyzed case-control samples of 61 Gujarati Indian patients with 73 population-based controls and 83 Japanese patients with 70 population-based controls. The non-Finnish patients predominantly had early-onset, plaque psoriasis with a familial background (Table I of Study I).

Studies were approved by the Ethical Committees of Helsinki, Turku, Tampere, and Oulu University Central Hospitals and Central Hospital of Päijät-Häme and the Department of Medical Genetics, University of Helsinki. The collection of foreign patient samples was approved by the local Ethics Review Boards in each country. The studies followed the Declaration of Helsinki Guidelines.

7.2   Tissue samples

Psoriatic lesional, nonlesional and normal skin samples (I-V)
Finnish skin samples (obtained from the Departments of Dermatology, Helsinki, Turku and Oulu University Central Hospitals, and Central Hospital of Päijät-Häme) were fixed in formalin and embedded in paraffin. Twenty-nine patients with known systemic and topical treatments had agreed to give a biopsy from the center of a psoriatic lesion, and those specimens were examined in Studies I, III-V. At the time of biopsy, the disease was either newly diagnosed or had lasted longer, up to 41 years. Skin samples were also taken from normal looking skin approximately 10 cm away from the psoriasis lesion or from the border between the nonlesional and lesional skin (non-lesional psoriatic skin samples, n = 16). The normal skin samples were obtained from the Department of Dermatology, University of Helsinki.

The punch biopsies for TaqMan analysis were taken from untreated psoriatic lesions and non-lesional skin approximately 10 cm away from the lesion and frozen in liquid nitrogen.

Table 4. Psoriatic, non-lesional and normal skin samples used in Studies I, III-V.

Study	Normal skin	Lesional psoriatic skin	Non-lesional psoriatic skin	Without treatment	Local treatment*	Systemic treatment
I	7	16	16	16	-	-
III	-	29	9	17	7 + 0 + 1	1 + 1 + 2**
IV	6	29	14	17	8 + 3 + 1	-
V	6	23	16	12	5 + 2 + 2	1 SUP + 1 PUVA

* x + y + z : x = corticosteroid, y = calcipotriol, z = corticosteroid + calcipotriol
** x + y + z : x =methotrexate, Y = acithretin and UVB, z = acithretin and bath PUVA

Samples of other hyperproliferative skin disorders and skin cancers (II, IV- V)
Formalin-fixed, paraffin-embedded specimens of lichen planus (II n = 6; IV n = 5; V n = 3), lichenoid chronic dermatitis (neurodermatitis) (II, IV, V n = 3), chronic eczema (II n = 8; V n = 3), mycosis fungoides (II n = 3), pityriasis rubra pilaris (II n = 6; V n = 3), chronic ulcers (II n = 7; V n = 5), ichthyosis vulgaris (II n = 4), palmoplantar pustulosis (V n = 3), normally healing timed wounds (V n = 6) (Vaalamo et al. 1996), Morbus Bowen (II n = 4), basal cell carcinoma (BCC) (II n = 6; V n = 4), squamous cell carcinoma (SCC) (II grade I n = 2, grade II n = 5, grade III n = 6; V n = 13) and extramammary Paget's disease (II n = 3) were obtained from the Department of Dermatopathology, Helsinki University Central Hospital. The diagnoses were confirmed by an experienced dermatopathologist.

Samples of other cancers studied (II)
Specimens of lung adenocarcinoma (n = 4), lung SCC (n = 2), ductal adenocarcinoma of the breast (n = 2), mucinous (n = 1) and serous (n = 2) ovarial cancers, colon carcinoma (n = 6), esophageal adenocarcinoma (n = 3), and SCC (n = 6) were obtained from the Department of Pathology, Helsinki University Central Hospital. The diagnoses were confirmed by an experienced cancer pathologist.

7.3   Genotyping and SNPs (I)

Reverse Transcriptase (RT)-PCR, PCR amplification and direct sequencing
Total RNA was extracted from a primary keratinocyte cell line with the Rneasy Mini Kit (Qiagen, Valencia, CA) to amplify the HCR cDNA. cDNA was synthesized with random hexamer primers and M-MLV reverse transcriptase enzyme (Promega, Madison, WI) at 70ºC for 5 min, followed by a 60 min incubation at 37ºC. PCR amplifications were performed in 20 µl volumes containing 50 ng of genomic DNA or 2 µl of cDNA, 1 x PCR buffer (10mM Tris-HCl, 50 mM KCl, 0.1% triton X-100), 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.6 µM primer mix and 0.6 U of DNA polymerase (DyNAzyme, Finnzymes, Espoo, Finland). The samples were denatured for 5 min at 94ºC, followed by 35-38 cycles of 30 s at 94ºC and 30 s at 55-68ºC followed by 30-180 s of annealing and elongation at 72ºC. The PCR products were either gel extracted for purification or purified with a PCR purification kit (Qiagen, Valencia, CA). Sequencing was done in both directions with dye-terminator chemistry and an ABI 373A or an ABI 377 sequencer.
PCR amplification for SNP genotyping was carried out in 10 µl volumes containing 25 ng of genomic DNA, 1x PCR buffer (10mM Tris-HCl, 50 mM KCl, 0,1% triton X-100), 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM primer mix (for primer sequences see Study I), 1%

DMSO, and 0.3 U of DNA polymerase (DyNAzyme II, Finnzymes). One primer from each pair had a fluorescent label.

Digestion reactions with altered restriction site recognizing enzymes were carried out overnight in 10 µl volumes containing 5 µl of PCR product, manufacturer's buffer (New England Biolabs, Beverly, MA) and 0.125-1 U of either BstUI (HCR*307), AvaII (HCR*325), Tsp509I (HCR*477), BsmFI (HCR*771) MsII (HCR*1723), HhaI (HCR*1911), MwoI (HCR*2327), or HphI (CDSN*1243). The pooled digestion products were separated by **electrophoresis** in an ABI 377 sequencer and allele calling was performed using the **Genotyper program** (Applied Biosystems, Foster City, CA).
Restriction enzymes MnlI (CDSN*619), MspA1I, and DdeI were used in genotyping the CDSN*619 and HLA-Cw*6 alleles, respectively. The digestion products were analyzed in agarose gels and visualized under UV light.
The British, Japanese, and Gujarati Indian samples were genotyped in Leicester, Great Britain, in the laboratory of Professor Richard Trembath by an allele-specific hybridization assay (Jeffreys et al. 2000) with the exception of HLA-Cw6, genotyped by SSP-PCR (Tonks et al. 1999). A control sample set was blindly genotyped with both assays in both laboratories for validation.

7.4   Statistical analyses (I, II)

Haplotype association analyses were performed with the Haplotype Pattern Mining (HPM) algorithm (Toivonen et al. 2000), which determined for combinations of marker alleles more frequent in psoriasis-associated than in control chromosomes using a χ^2 test. The haplotypes best associated with the phenotype (i.e. exceeding a given threshold level for χ^2) predicted the disease susceptibility gene location with a non-parametric model. Four independent chromosomes were obtained from each trio, and a chromosome was considered trait-associated if it occurred only in affected family members, and a control if it occurred only in unaffected individuals. Trios were haplotyped with an in-house computer program written by Petteri Sevon (unpublished), the larger pedigrees (Spanish families in Study I) were first divided into trios by randomly selecting one trio per pedigree.

Transmission Disequilibrium Tests (TDT), evaluating the transmission of an associated marker allele from a heterozygous parent to an affected offspring compared with the transmission of the alternative marker allele, were computed with GENEHUNTER 2.1 (Kruglyak et al. 1996). If the haplotype contained more than four markers, the TDT analyses were done manually.

X^2 tests or **Fisher's exact tests** (when the number of expected observations was less than five) were used to calculate the statistical significance of the allele associations for single SNPs or alleles between the case and control groups. **Relative risk (RR)** and **odds ratios (OR)** were also determined for each association with 95% confidence intervals (95% CI). RR = [a/(a+b)]/[c/(c+d)]; a = number of patients with the risk allele; c = the number of patients without the risk allele; b and d = the equivalent values in the controls, respectively; OR = $f_{(aff)}/(1-f_{(aff)})$:$f_{(contr)}/(1-f_{(contr)})$, f = frequency.

Unpaired t-tests were used to measure the statistical significance of the differences in HCR mRNA expression levels of differently grown cells in TaqMan analyses. The test

compares the means of two different sample sets (i.e. control and treated) when they are not linked to each other.

Linkage disequilibrium (LD), the non-random association of alleles at linked loci, was calculated by different statistical measures (D, D´ and r^2). D (coefficient of LD) is the difference between the observed haplotype frequency and the expected haplotype frequency under statistical independence, dependent on allele frequencies. D´ (normalized measure of LD, D/D $_{max}$) depends on sample size and r^2 (square of the correlation coefficient) is affected by allele frequencies, as for D (Ardlie et al. 2002, Weiss and Clark 2002).

7.5 RNA probes (III,V)

The generation and specificity of the antisense human MMP-1 (Saarialho-Kere et al. 1992), MMP-3 (Saarialho-Kere et al. 1994), MMP-7 (McDonnel et al. 1991), MMP-10 (Saarialho-Kere et al. 1994), MMP-12 (Vaalamo et al. 1998), MMP-13 (Johansson et al. 1997)**,** TIMP-1 (Saarialho-Kere et al. 1992), and TIMP-3 (Airola et al. 1998) probes have been described previously. For *in situ* hybridizations to detect IFI27, we amplified a 482 bp cDNA fragment of IFI27 with PCR and subcloned it into pBluescript SK+ plasmid digested with EcoRI/BamHI. PvuII restriction enzyme released the insert, and its integrity was confirmed by DNA sequencing. T3 was used for the antisense probe and the sense probe was transcribed as a T7 run-off. A commercial kit (Promega Corp. Madison, WI) was used for *in vitro* transcription of the cDNAs.

Table 5. Probes for *in situ* hybridization

Probe (Study)	Length bp/ part of the sequence	Genbank accession no.
MMP-1 (III)	550 (1-150)	NM_002421
MMP-3 (III)	217 (1584-1801)	XM_006271
MMP-7 (III)	800 (14-813)	NM_002423
MMP-10 (III)	175 (1568-1743)	NM_002425
MMP-12 (III)	651 (600-1250)	NM_002426
MMP-13 (III)	511 (1532-2042)	NM_002427
TIMP-1 (III)	313 (1-313)	NM_003254
TIMP-3 (III)	636 (282-917)	NM_000362
IFI27 (V)	482 (72-552)	NM_005532

7.6 In situ *hybridization (III,V)*

In situ hybridizations were performed on 4 µm sections as described previously (Prosser et al. 1989, Saarialho-Kere et al. 1993). All samples were treated with proteinase K (1 µg/ml) and washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. ^{35}S-labeled probes (4-5 x 10^4 cpm/µl of hybridization buffer)were hybridized to the sections at 50°C or 55°C for at least 18 h in a humidified chamber. Slides were then washed under stringent conditions, including treatment with RNAse A to remove unhybridized probe.

Photographic emulsion was developed after 11-47 d of autoradiograpy and the slides were stained with hematoxylin and eosin. Samples previously positive for MMP-12 (sarcoidosis), MMP-7 (sweat gland tumors), MMP-1, MMP-3, MMP-10, MMP-13, TIMP-1, and TIMP-3 (chronic ulcers), were used as positive controls in each experiment. The slides were analyzed independently by two investigators.

7.7   Immunohistochemistry (I-V)

Immunostaining was performed using the avidin-biotin-peroxidase complex technique (Vectastain ABC Kit; Vector laboratories, Inc., Burlingame, CA), Histostain-Plus Bulk Kit (Zymed laboratories, Inc., San Francisco, CA), or StreptABComplex/HRP Duet (Mouse/Rabbit) Kit, DAKO, A/S Glostrup, Denmark, no. K0492). Type IV collagen, Ki67, p63, and β-catenin immunoanalyses were performed on sections serial to those used for MMP-19 or HCR. CD68 immunohistochemistry was performed on sections serial to those used for MMP-12, and laminin-5 on sections serial to MMP-3 or IFI27. Samples were deparaffinized, dehydrated and pretreated, if necessary, with trypsin (10 mg/ml) or antigen retrieval. Endogenous peroxidase was blocked with 0.5% hydrogen peroxide, and treatment with normal serum blocked non-specific staining. The sections were incubated with the primary antibodies (**Table 6**) for 1 h at 37ºC or overnight at 4ºC in a humidified chamber. Secondary biotinylated antibody was applied, followed by avidin-biotin-peroxidase complex. Diaminobenzidine (DAB) or aminoethylcarbazole (AEC) were used as chromogenic substrates and the tissues were counterstained with Harris hematoxylin as described (Saarialho-Kere et al. 1993). Controls were performed with normal mouse or rabbit immunoglobulins or with pre-immune serum.

Table 6. Antibodies used in immunohistochemistry

Antibody (Study)	Source	Dilution / pretreatment
HCR (I, II)	See the text "7.8 Production of antibodies" and "7.9 Western blotting"	4 µg/ml / trypsin
Ki-67 (I, II)	A047, Dako, A/S Glostrup, Denmark	1:200 / antigen retrieval
p63 (IV)	MS-1081-P1, Neomarkers, Fremont, CA, USA	1:250
MMP-9 (III)	DB-2211, Diabor, Oulu, Finland	1:400 / trypsin
MMP-8 (unpublished results)	IM38L, Oncogene, San Diego, CA, USA	1:20 / trypsin
MMP-19 (IV)	RDI-MMP19abR, Research Diagnostics Inc., Flanders, NJ	1:60
MMP-28 (IV)	Gift from Doc. Jouko Lohi	0.25 µg/ml / antigen retrieval
CD-68 (III)	KP-1, M0814, Dako, A/S Glostrup, DK	1:300 / trypsin
γ- chain of laminin-5 (III, V)	Dr. Karl Tryggvason, Karolinska institutet, Sweden	1:600 / trypsin
type IV collagen (IV)	M785, Dako, A/S Glostrup, DK	1:75 / trypsin
β-catenin (II)	C19220, Transduction laboratories, Lexington, KY	1:1000 / antigen retrieval

7.8 Production of antibodies (I)

Rabbits were immunized against HCR protein with a synthetic 18-mer peptide ERDVSSDRQEPGRRGRSW (amino acids 62-79, Sigma Genosys Cambridge, UK). Affinity-purification of the antibodies was performed with the peptide bound to an epoxy-activated sepharose column according to the manufacturer's instructions (Pharmacia Biotech, Uppsala, Sweden).

7.9 Western blotting (I)

The specificity of the HCR antibodies was confirmed by SDS-PAGE and Western blotting in standard procedures. HCR cDNA was cloned into the pCMV5 vector, and by lipofection technique (Fugene, Roche, Indianapolis, IN) expressed transiently in COS-1 cells. Affinity-purified HCR antibodies (1 µg/ml) were primary antibodies and peroxidase-conjugated anti-rabbit IgG secondary antibodies detected with enhanced chemiluminiscence (Boehringer Mannheim, Mannheim, Germany).

7.10 Cell cultures (II, IV-V)

Primary keratinocytes were established from adult skin obtained during mammoplasties for non-malignant disease (Rechardt et al. 2000). Subcutaneous fat and deep dermis were removed, and the remaining tissue was incubated overnight in 0.25% trypsin in solution A (Gibco BRL, Life Technologies, Paisley, Scotland). The following day, keratinocytes were scraped off the epidermis and suspended in Keratinocyte Growth Medium (KGM, Gibco BRL) containing 2% decalcified fetal calf serum (FCS) and supplemented with 5 ng/ml epidermal growth factor (EGF) and 50 mg/ml bovine pituitary extract (BPE) (supplied by the vendor). Keratinocytes were maintained in KGM (with EGF and BPE) until confluency. Normal keratinocytes were also fixed with formalin for HCR immunostaining after two days in KGM (Gibco BRL) or Dulbecco's modified Eagle's medium (Ca^{2+} 1.8 mM) (Gibco BRL).

Psoriatic lesional and non-lesional keratinocyte cell lines were established from psoriatic plaque lesions and non-lesional skin from four patients at Oulu University Hospital. Cell lines were cultured in calcium-free Keratinocyte Basal Medium-2 (KBM-2, Clonetics, BioWhittaker, Inc., Walkersville, Maryland, USA), as previously described (Karvonen et al. 2000), supplemented with bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, transferrin, epinephrine, gentamicin sulfate, and amphotericin B (all from Clonetics), and containing 1% penicillin-streptomycin.

Normal, nonlesional, and lesional keratinocytes were grown on Lab-Tec chamber slides in calcium-free KBM-2 medium (Clonetics) in passages 2-3 for comparison of HCR protein expression between these cell lines. In Study IV, these cell lines were grown until confluency in 24-well plates and the MMP-19 mRNA levels were determined by TaqMan real time PCR.

7.11 Cytokines and growth factors (II,V)

The regulation of HCR and IFI27 expression was studied by plating equal amounts of normal keratinocytes in passages 2-4 on 24-well plates and allowing them to attach for 24 h. The confluent cell cultures were washed with PBS and incubated in medium without supplements overnight. Therafter, the cells were treated with different cytokines, growth factors or anti-psoriatic agents (**Table 7**). Cells without any treatment were used as control. We also grew normal keratinocytes in both low Ca^{2+} KGM (0.09mM) and in KGM with $CaCl_2$ (Ca^{2+} 1.8 mM) to evaluate the effect of keratinocyte differentiation on HCR expression. The effect of different confluencies of seeded normal keratinocytes on HCR mRNA expression was also studied. In every experiment each treatment was performed in at least three separate wells. After 24 h and 48 h total RNA was extracted from the cells.

Table 7. Cytokines and growth factors used in cell culture

Cytokine / growth factor / drug (study)	Concentration	Source
1,25-$(OH)_2$ D3 =calcitriol (II, V)	10^{-7}M	Sigma, St Louis, MO
bFGF (II, V)	10 ng/ml	Sigma
dexamethasone (II, V)	10^{-5}M	Sigma
EGF (II, V)	10 ng/ml	Sigma
IFN-γ (II)	1 ng/ml	Promega, WI, USA
IFN-γ (II, V)	10-100 U/ml (II) 100 U/ml (V)	Roche Molecular Biochemicals, Mannheim, Germany
IGF-1 (II, V)	100 ng/ml	R&D Systems, Minneapolis,
IL-1β (II, V)	5 U/ml	Roche Molecular Biochemicals
IL-10 (II, V)	10 ng/ml	R&D Systems
KGF (II, V)	10 ng/ml	Sigma
LPS=lipopolysaccharide (II, V)	2,5 µg/ml	Sigma
PMA=phorbol 12 myristate 13-acetate (II, V)	10 ng/ml	Sigma
retinoic acid=RA (II, V)	10^{-6} M	Sigma
TGF-α (II, V)	30 ng/ml	Sigma
TGF-β1 (II, V)	10 ng/ml	Sigma
TNF-α (II, V)	10 ng/ml	Sigma
VEGF (II, V)	10 ng/ml	R&D Systems

7.12 Quantitative RT-PCR (II, IV,V)

PCR primers and **probes** (PE Biosystems, Warrington, UK) for HCR, MMP-19, and IFI27 were designed using the computer program Primer Express (Applied Biosystems, Foster City, CA). The HPLC-purified fluorogenic probes contained a reporter dye (FAM) covalently attached at the 5'end and a quencher dye (TAMRA) covalently attached at the 3' end. The starting material was quantitated by amplifying endogenous controls in the PCR reactions. Pre-developed TaqMan assay reagents for endogenous control human GAPDH (MMP-19, HCR), β-actin (HCR), or 18S rRNA (IFI27) were labeled with VIC reporter dye (Applied Biosystems).

In Study IV, lesional and non-lesional punch biopsies, maintained in liquid nitrogen, were crushed with a mortar and pestle. Total cellular RNA was extracted from punch biopsies as well as from keratinocytes using the RNAeasy Miniprep-Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions (Studies II, IV, V). RNA was then reverse-transcribed to cDNA with TaqMan Reverse Transcriptase enzyme and oligonucleotides (Applied Biosystems) and used as a template for PCR. Real time quantitative PCRs were performed with the ABI PRISM® 7700 Sequence Detector System (Applied Biosystems) as previously described (Rechardt et al. 2000). PCR reactions were performed in a total volume of 20 μl, containing 5 μl 1:5 cDNA sample, 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems), 100 nM (HCR, IFI27) or 200 nM (MMP-19) of each primer and 100 nM (HCR, IFI27) or 200 nM (MMP-19) of fluorogenic probe. The endogenous control reagents were used as control genes from the same samples in separate reactions. MicroAmp® Optical 96-Well Reaction Plates and Optical Caps (Applied Biosystems) were used for reactions. The PCR started with 2 min at 50°C and the initial 10 min denaturating temperature at 94°C, followed by a total of 40 cycles of 15 s at 94°C and 1 min at 60°C.

In Study IV, the normal keratinocyte and psoriatic keratinocyte reactions were also done by conventional PCR with the same primers but without fluorogenic probes. The PCR was performed in 39 cycles with an initial 10 min denaturing temperature of 95°C followed by 15 seconds of denaturing, 1 min of annealing and elongation (60°C) and 2 min of final elongation to produce a product of 99 bp. In the presence of 5 ng ethidium bromide per ml, 20 μl aliquots of the products were run in a 2% low melting point agarose gel and visualized under ultraviolet light.

Table 8. Primers and probes for Taqman quantitative RT-PCR

	Forward primer (nts)	Probe	Reverse primer (nts)
HCR	5'-GCCCACCAGGGA GTCCATA-3' (2202-2220)	AAGGGTCCCTCTCTGT CCTGCTCG (2222-2245)	5'-GGCTTCACTC AGGTCCTGCA-3' (2252-2271)
MMP-19	5'-GCTTCCTACTCC CCATGACAGT-3' (127-148)	CCCGTGGACTACCTG (183-197)	5'-GGCTTCTGTAGGT ACCCATATTGT-3' (223-200)
IFI27	5'-TGCCTCGGGCAG CCT-3' (300-314)	TGGGTACTCTGCAGTC ACTGGGAGCA (317-342)	5'-TTGGTCAATCCGG AGAGTCC-3' (365-346)

7.13 Northern blotting (V)

Keratinocytes were cultured in KGM supplemented with EGF and BPE until confluency. The cells were then washed twice with PBS and transferred to Keratinocyte Basic Medium without supplementation. Keratinocyte cultures were split and 10 ng per ml TNF-α was added to one half of the culture. Total cellular RNA was extracted 24 hours later with TRIzol Reagent according to manufacturer's instructions (Invitrogen Corporation, California). RNA was denatured and fractioned in a 1% agarose gel containing 2.2% formaldehyde, and transferred to a nylon supported-nitrocellulose membrane (Osmonics, Massachusetts). The membrane was hybridized to IFI27 cDNA probe labeled with [α-^{32}P]dCTP using PerfectHyb Plus buffer, according to the protocol provided by the manufacturer (Sigma, MO). Hybridization signal was visualized by autoradiography.

8 Results and Discussion

8.1 The gene structure of HCR (I)

We verified the existence of two novel exons, predicted by two different prediction programs (GENSCAN and FGENES), in the HCR gene. The coding sequence comprised 2349 bp in 18 exons encoding a protein of 782 amino acids (GenBank AY029160) with the translation initiation codon in exon 2. No novel polymorphic sites in the gene were identified.

8.2 Statistical analysis of PSORS1 susceptibility alleles (I)

We genotyped 419 trios from Great Britain (29 of these were of Gujarati Indian origin), Finland, Sweden, Italy, and Spain to verify the HCR associations in different populations.
In **allele association analyses**, the HLA-Cw*6, HCR-307*T, HCR-325*T, and HCR-1723*T alleles showed strongest association with psoriasis ($P<10^{-10}$, OR>2) (I, Table 2).
In **haplotype analysis**, HCR-307*T, HCR-325*T, HCR-*1723*T, and HCR-2327*G were inherited almost exclusively in the same chromosomes (I, Table 3). The susceptibility allele HCR*WWCC was found in 35% of patient and 18% of control chromosomes ($P = 10^{-10}$, OR 2.5, 95% CI 1.9-3.3) and showed a significant association in TDT analysis as well (178 transmitted vs. 82 untransmitted chromosomes, $P = 10^{-11}$). The HLA-Cw*6 allele association, however, was slightly stronger, with $P = 10^{-11}$ and OR 2.9 (95% CI 2.1-3.9). In TDT analyses the P-value for HLA-Cw*6 was $P = 10^{-13}$. The CDSN*5 showed slightly weaker association both in haplotype analysis ($P = 10^{-9}$, OR 2.0, 95%CI 1.6-2.5) and in TDT analysis (275 transmitted vs. 180 untransmitted, $P = 10^{-7}$) (**Figure 5**).
The association was the strongest when these three alleles were analyzed as a one shared haplotype, (OR 3.9, 95% CI 2.1-7.3), though probably due to the lower overall number of chromosomes the P-value remained 10^{-6} (I, Table 3).

In **LD analyses**, HLA-Cw*6 and HCR*WWCC were in stronger disequilibrium (D´ = 0.73, r^2 = 0.46 in patients) than HCR*WWCC and CDSN*5 (D´=0.41, r^2=0.08 in patients). Little recombination was evident between the three haplotypes based on inspection of the haplotypes or LD measurement of the three loci, indicating that the genes are probably included in the same haplotype block (Daly et al. 2001, Patil et al. 2001, Reich et al. 2001). Because of the strong LD between these genes they could not be separated with statistical confidence from each other - even with our material of approximately 1700 chromosomes.
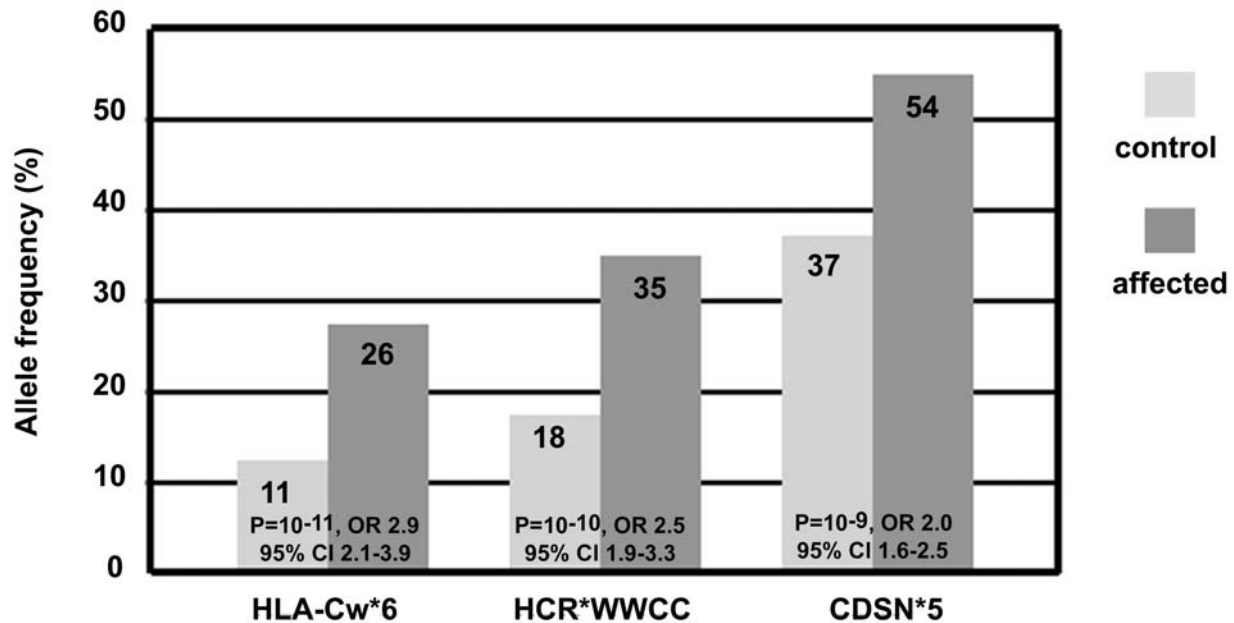
Figure 5. Haplotype association results (908 control, 772 patient chromosomes)

We continued our studies investigating the possible association of HCR with palmoplantar pustulosis and guttate psoriasis. The association of guttate psoriasis with PSORS1 was even stronger than that of plaque psoriasis, suggesting that differences in these two subtypes of psoriasis are due to factors other than PSORS1 (Asumalahti et al. 2003a). HLA-Cw*6 showed the strongest association, but the three susceptibility alleles (HLA-Cw*6, HCR*WWCC, CDSN*5) most likely occurred together. In contrast, palmoplantar pustulosis showed no association with HLA-C alleles, suggesting a different genetic background although similarities in pathogenesis may occur (Asumalahti et al. 2003a).

Two studies have questionned the role of HCR as a susceptibility gene for psoriasis. O'Brien et al. (2001) concluded that HCR is unlikely to have an independent effect on psoriasis based on stratification of the HCR alleles over HLA-Cw*6 and vice versa in their study of 42 patients and 38 controls. The study lacked, however, the power to differentiate between HCR and HLA-C because of the small sample size and absence of chromosomal data. Chia et al. (2001) concluded that variations in HCR are not causal for psoriasis because the two risk haplotypes in PSORS1 identified in their previous study (Nair et al. 2000) did not include HCR susceptibility SNPs. The association with cluster 17 to psoriasis had, however, a not highly significant P-value of 0.048 (Nair et al. 2000). Moreover, the cluster 17 haplotype seems to be very rare (of 90 Finnish trios in our material, three individuals carried the cluster, unpublished). It is unlikely that such a rare haplotype would carry a major disease variant.

Veal et al. (2002) extended the refinement of PSORS1 and genotyped 171 parent-affected offspring trios using 59 of 119 high-frequency SNPs they had identified by resequencing a 220 kb region at chromosome 6p21. Family-based association analysis revealed two noncoding SNPs (7 and 9) lying 7 and 4 kb proximal to HLA-C with much greater associations than any previously described SNP. In haplotype-based analysis, overtransmission of chromosomes with the same unique SNP 7 and 9 markers was seen. These markers defined a 10 kb PSORS1 core risk haplotype with five alternative major

haplotype blocks. All but one rare haplotype of the overtransmitted haplotypes carried a HCR risk allele, and in three haplotype blocks the haplotype remained intact between SNPs 7 and 9 and the HCR haplotype. LD was strongest in a block containing HLA-C and HCR and decreased between this block and CDSN, which was seen in our Study I as well. The study by Veal et al. (2002) defines SNPs 7 and 9 candidates for psoriasis susceptibility. Although noncoding, they can be causal if located in a regulatory or promoter region of a gene.

Based on these results and previous studies, it seems that HLA-Cw*6, HCR*WWCC, CDSN*5, and other linked genes or alleles in PSORS1 may contribute to the development of psoriasis either alone or in interaction with each other (locus interaction). To refine "the psoriasis gene" demands either more extensive genetic studies, or better understanding of the function of the various susceptibility genes, to identify the biochemical pathways involved in the development of psoriasis.

8.3   Altered structure of HCR protein (I)

The COIL and PAIRCOIL programs were used to analyze the predicted secondary structures produced by the wildtype and WWCC susceptibility alleles of the HCR protein. These programs give the probability of coils (Lupas et al. 1991, Berger et al. 1995). Compared with the wild-type form, the probability of a coil decreased at the sites of three of the four non-conservative amino acids of the HCR*WWCC allele (I, Fig. 1). These changes may affect the antigenic properties of the HCR protein and thus contribute to the immunological response characteristic of psoriasis. No strong conclusions can, however, be made solely based on the structural predictions without experimental data.

8.4   HCR expression in lesional vs nonlesional and normal skin (I, II)

The basal keratinocytes were immunopositive with HCR polyclonal peptide antibodies in control and nonlesional skin (I, Fig. 2A-B). In psoriatic lesional skin, HCR expression was upregulated in basal and suprabasal keratinocytes of dermal papillae at sites where the epidermal layer is at its thinnest. Basal keratinocytes in rete ridges remained faintly stained (I, Fig. 2C; II, Fig. 1a-b). Immunostaining with the hyperproliferation marker Ki67, however, was strongest in rete ridges in parallel skin sections, in contrast to HCR staining (I, Fig. 2D). Based on the immunostaining profile, a hypothesis arose suggesting that when T cells gather into lesions they downregulate HCR expression in rete ridges by releasing IFN-γ, which might affect keratinocyte proliferation. The observed changes in HCR expression in psoriasis can be causal in the pathogenesis of psoriasis or result from changes already occurring in psoriatic skin.

8.5   HCR expression in hyperproliferative skin disorders and cancers (II)

We did HCR immunoanalyses in samples of chronic eczema, chronic skin ulcers, lichenoid chronic dermatitis, mycosis fungoides, ichtyosis vulgaris, pityriasis rubra pilaris, and lichen

planus. No overexpression of HCR was detected, nor could we demonstrate any specific features of expression (II, Fig. 1d-g). This suggests that HCR expression is not affected solely by keratinocyte hyperproliferation and acanthosis, but may be associated with keratinocyte differentiation or antigenicity as well.

Bowen's disease, BCCs, extramammary Paget's disease, grade I and II SCCs did not demonstrate HCR immunostaining. In 3/6 more dedifferentiated grade III SCCs, however, there was positive cytoplasmic staining in cancer cells either at the edge or in the middle of the tumour. In psoriasis, it is assumed that keratinocytes are senescent in the mid and upper layers of the epidermis, which would explain why psoriasis plaques rarely evolve into skin cancers in spite of chronic inflammation (Nickoloff 2001).

While 2/2 samples of SCC of the lung showed no HCR expression, staining was evident in 4/4 adenocarcinomas of the lung. Ductal adenocarcinomas of the breast (2/2) showed cytoplasmic HCR staining as well (II, Fig. 2c). As in psoriasis, Ki67 was positive in adjacent, but not the same cells (II, Fig. 2b, d). This suggested difference in adenocarcinoma vs. SCC HCR expression prompted us to perform immunohistochemistry also in esophageal adeno- (n = 3) and squamous cell carcinomas (n = 6). These remained negative, as did six colon carcinomas and normal colon epithelium (n = 2) studied. Immunostaining in cancer cells in the epithelium of mucinous ovarial carcinoma (1/1) was detected while 2/2 serous ovarial carcinomas did not stain.

8.6   HCR expression in lesional, nonlesional, and normal keratinocytes (II)

The psoriatic lesional keratinocytes showed intense immunostaining in the nuclei, with fainter staining also in the cytoplasm (II, Fig. 3c). Perinuclear staining was evident in normal human keratinocytes (II, Fig. 3a) while nonlesional keratinocytes stained positively in both the cytoplasm and the nucleus (II, Fig. 3b). HCR protein may thus act in both the nucleus and cytoplasm but further studies are needed to verify these findings. In transfected COS cells, HCR (renamed StAR-binding protein, SBP) localizes to cytoplasmic organelles such as endosomes and mitochondria (Sugawara et al. 2003). This recent study suggests that SBP could have a role in the synthesis of steroid hormones via influencing cholesterol trafficking in the mitochondria.

The normal and psoriatic keratinocytes stained most intensely in areas where the cells did not grow in confluency (II, Fig. 3a-d). Abnormal adhesion was also suggested *in vivo* in psoriatic skin, as HCR immunopositive keratinocytes showed no membraneous β-catenin staining (II, Fig. 1b-c). Moreover, $\alpha 3\beta 1$ integrin (VLA-3) influencing cell-cell interactions and interacting with laminin, type I collagen, and fibronectin, and ICAM-1 attaching to immunocytes show similar expression patterns to HCR in psoriasis (Kellner et al. 1991). Calcium concentration did not affect the staining pattern of the normal keratinocytes, suggesting no role for HCR in keratinocyte differentiation.

8.7   Quantitative HCR mRNA expression in primary human keratinocytes (II)

We cultured normal keratinocytes, stimulated them with different cytokines, growth factors, and anti-psoriatic agents, and measured HCR mRNA levels by quantitative real-time PCR in order to verify the regulation of HCR. A two-fold downregulation of HCR mRNA

expression was observed when stimulated with two IFN-γ products from two different companies for 24 hours (II, Fig. 4). The result was independent of the IFN-γ (1 ng/ml from Promega and 10 U from Roche) or control gene (human GAPDH: P = 0.00749 and P = 0.00016 respectively; human β-actin: P = 0.00285 and P = 0.00097 respectively) (II, Fig. 4). There was no effect when normal keratinocytes were stimulated for either 24 or 48 hours with TNF-α, EGF, PMA, TGF-α or β, KGF, bFGF, VEGF, IGF-1, LPS, IL-1β, IL-10, 1,25-$(OH)_2$ D3 (calcitriol), dexamethasone, or retinoic acid. Stromelysin-2 (induced by EGF) and ISG12 (induced by IFN-γ) were used as positive controls (Rechardt et al. 2000, Gjermandsen et al. 2000).

The amount of Ca^{2+} in culture medium did not affect HCR expression levels, in agreement with the immunoanalyses, nor did the different densities of seeded normal keratinocytes have any influence on the HCR mRNA expression.

Interestingly, of all tested cytokines, IFN-γ, one of the key cytokines in the pathobiology of psoriasis, downregulated HCR expression. This might contribute to the anti-hyperproliferative role of HCR. We studied primary keratinocytes, however, and lesional keratinocytes might have given a different result. We have continued our investigations by developing a transgenic mouse model overexpressing HCR under the keratin-14 promoter. Currently we are attempting to grow and stimulate keratinocytes of the transgenic epidermis in order to verify our TaqMan data and to define the location of the HCR protein more precisely by immunoelectronmicroscopy. By transfection experiments, we are examining the effect of HCR overexpression on proliferation of cultured normal and transformed cells. Additionally, we screened an Affymetrix array to compare the wild type mice with risk haplotype mice (HCR*WWCC) and non-risk haplotype mice (human non-HCR*WWCC) and to determine the biochemical consequences of HCR overexpression in basal keratinocytes under the keratin 14 promoter.

8.8 Expression of various MMPs in psoriasis (III, IV)

MMP-12 mRNA was expressed in 19/21 psoriatic samples either in the migrating macrophages of the epidermis (14/21) or in the inflammatory infiltrates of the superficial dermis (8/21) by *in situ* hybridization (III, Fig 1d). The positive cells were CD68 immunopositive as well, and we assumed them to most likely be macrophages (III, 1e-f). Keratinocytes expressed no MMP-12, which means that psoriatic hyperproliferation alone is not enough to induce MMP-12 production in keratinocytes, but rather an oncogenic stimulus is needed (Kerkelä et al. 2000).

IL-1β, VEGF, GM-CSF, and T cell contact stimulate MMP-12 expression in macrophages in cell culture (Kumar et al. 1996, Feinberg et al. 2000, Wu et al. 2000), and uPa (urokinase-type plasminogen activator), which is also upregulated in psoriasis, is capable of activating MMP-12 (Spiers et al. 1994). MMP-12, in turn, stimulates the release of TNF-α from the proTNF-α fusion protein (Chandler et al. 1996) and releases growth factors from the matrix, perhaps modifying the cytokine profile in lesions. BM passaging by macrophages, which occurs in psoriasis, may be facilitated by MMP-12, but the MMP-12 upregulation may equally well be a host-response effect because of MMP-12 anti-angiogenicity.

MMP-9 protein was detected in macrophages (24/27) and in neutrophils and neutrophil abscesses (20/27) of psoriatic lesions by immunohistochemistry (III, Fig 2a-d). MMP-9 may be induced by elevated TNF-α and IL-1β levels (Saren et al. 1996). Only in three

samples was positive immunostaining detected in basal keratinocytes. This is in accordance with the *in vitro* data of Buisson-Legendre et al. (2000) demonstrating downregulated MMP-9 protein levels in psoriatic keratinocyte cell cultures compared to normal keratinocytes. On the other hand, a previous report on early psoriasis lesions had shown MMP-9 expression in keratinocytes (Feliciani et al. 1997) and another study showed MMP-9 to be upregulated in the culture fluid of psoriatic lesional skin (Varani et al. 1998).

MMP-3 mRNA was identified basally in 4/21 lesions with strong epithelial hyperproliferation (III, Fig. 1a-b). In 3/3 samples, intracellular laminin-5 immunostaining was detected basally and suprabasally in the tips of the dermal papillae (III, Fig. 1c) as a hallmark of BM degradation (Airola et al. 1997, Kähäri and Saarialho-Kere 1997). This is in accordance with the discontinuities of laminin-1 in psoriasis (Mondello et al. 1996) and our previous data on MMP-3 in wound repair and dermatitis herpetiformis (Saarialho-Kere et al. 1994, Airola et al. 1997). BM alterations accompanied by epidermal overexpression of MMP-2 (72-kDa gelatinase) and its inhibitor TIMP-2 in psoriatic keratinocytes have been reported as well (Fleischmajer et al. 2000).

Stromal cells expressed **MMP-1** mRNA in 3/13 samples, but in only one sample was signal detected in the epidermal compartment basally. In psoriatic arthritis, however, patients and their siblings (also unaffected) are reported to have elevated MMP-1 serum levels, suggesting a genetic difference (Myers et al. 2004). No **MMP-10** (7/7)**, MMP-13** (7/7), or **MMP-28** (10/10) mRNA was detected in any of the biopsies. **MMP-7** was only detected in sweat glands (5/7) in agreement with previous data (Saarialho-Kere et al. 1995). In the intestine MMP-7 cleaves defensins, significant proteins also in psoriatic lesions, but in psoriasis they seem not to be activated by MMP-7. MMP-13 is upregulated by keratinocyte transformation and our results for psoriasis are consistent with this (Johansson et al. 1999). MMP-28 is expressed in injured epidermis, and it is likely that the BM must be totally disrupted to induce MMP-28 expression (Saarialho-Kere et al. 2002). Upregulation of MMP-28 occurs by stimulation with TNF-α in primary keratinocytes, suggesting that MMP-28 expression in psoriasis could be enhanced (Saarialho-Kere et al. 2002).

None of the analyzed MMPs was detected in nonlesional skin, although many other characteristics of keratinocytes are evident even in nonlesional skin (Pellegrini et al. 1992). The treatment or duration of the psoriasis seemed to have no effect either on our findings (III, Table I). Though T cells are capable of secreting several matrix metalloproteinases such as MMPs -2, -9, -10 and -13 (Willmroth et al. 1998, Leppert et al. 1995), we detected no signal for any investigated MMPs in cells with typical lymphocyte morphology.

8.9   *MMP-19 expression in lesional vs nonlesional and normal skin (IV)*

MMP-19 protein was not upregulated as assessed by immunostaining with polyclonal peptide antibodies in keratinocytes in nonlesional skin (n = 14) nor in normal skin (n = 6). In 28/29 psoriatic lesions the basal and suprabasal keratinocytes were, however, MMP-19 immunopositive. Ki67 immunopositivity was detected in keratinocytes adjacent to MMP-19 positive cells mainly in the rete ridges, while the stem cell marker p63 positive cells tended also to line the tips of the dermal papillae (IV, Fig. 1A-D). Many of these cells seemed to be either adjacent or the same as MMP-19 positive cells. Type IV collagen immunostaining

was disrupted adjacent to MMP-19 positive epidermis bordering dermal papillae, making alterations in BM evident (Fig. 2I-J).

If MMP-19 contributes to BM alterations in psoriasis, it could mediate keratinocyte hyperproliferation through fibroblast-keratinocyte interactions. On the other hand, MMP-19 may only be upregulated in keratinocytes in response to BM degradation. EGF, TNF-α, and IL-1β, cytokines upregulated in psoriasis, may stimulate MMP-19 expression in keratinocytes as they induce MMP-19 in other tissues (Kolb 1999). Hieta et al. (2003) have recently shown that dermal fibroblasts produce MMP-19 mRNA and pro-MMP-19 *in vitro*, especially when stimulated by TNF-α. The signalling pathways mediating the induction of MMP-19 included mitogen-activated ERK1/2 and stress-activated JNK and p38. In primary keratinocytes, MMP-19 was induced by TNF-α and PMA (Impola et al. 2003).

In accordance with our results, Sadowski et al. (2003a) later showed that MMP-19 is found in suprabasal and spinous epidermal layers in psoriasis. In their studies, however, MMP-19 was expressed by the basal keratinocytes of normal skin as well. The discrepancy may be due to different antibodies. In HaCat cells, constitutive expression was downregulated at high calcium concentrations and restored with anti-E-cadherin antibodies, indicating that calcium regulation occurred via E-cadherin-mediated cell-cell contacts (Sadowski et al. 2003b). They also demonstrated that MMP-19 degraded IGF binding protein-3, augmenting signalling through the IGF-1 receptor and resulting in increased proliferation and migration. Previously, Mauch et al. (2002) has shown that MMP19 production can be upregulated by adhesion.

Djonov et al. (2001) have shown that epithelial cells of benign mammary gland tumors express MMP-19, but transformation to a malignant phenotype downregulates its expression. In addition, the recent findings of our group confirm that proliferating epithelium expresses MMP-19 in wounds, oral verrucous hyperplasia and carcinoma, and in BCC and SCC, but that the expression is downregulated in cancer cell nests (Impola et al. 2003, Impola et al. 2004).

Endothelial, histiocyte, and fibroblast type cells (IV, Fig. 2G), smooth muscle cells, and sweat gland epithelial and myoepithelial cells were immunopositive as well, as described in granulating wounds and chronic venous ulcers (Hieta et al. 2003) and in other situations (Kolb et al. 1999, Djonov et al. 2001, Mauch et al. 2002).

8.10 MMP-19 expression in lichen planus and lichenoid chronic dermatitis (IV)

Keratinocytes expressed MMP-19 in one of three samples of lichenoid chronic dermatitis. This sample demonstrated disrupted BM as assessed by type IV collagen immunostaining (IV, Fig. 2A-D). Lichen planus, characterized by substantial disruptions in the BM zone as well (Giannelli et al. 1996), showed MMP-19 staining in keratinocytes at the areas of basement membrane degradation (IV, Fig. 2E-G). Disrupted BM areas seem to express MMP-19 generally with no association to a specific disease. The BM can also be degraded by MMP-9, which is needed in the activation process of MMP-19, but MMP-9 was not expressed in the keratinocytes in the same area (IV, Fig. 2E, H).

8.11 MMP-19 expression in lesional, nonlesional, and normal keratinocytes (IV)

We cultured normal, nonlesional, and lesional keratinocytes and analyzed their MMP-19 mRNA levels by quantitative real time PCR. MMP-19 expression in unstimulated normal and nonlesional keratinocytes was low in both cell lines (IV, Fig. 3). In lesional keratinocytes, however, the expression of MMP-19 mRNA was induced almost 15-fold (IV, Fig 3) verifying our *in vivo* data. A lesser induction of 3.5-fold was observed *in vivo* in untreated psoriatic skin compared with nonlesional skin, possibly reflecting the influence of fibroblasts producing MMP-19 both in normal and psoriatic skin. We also ran aliquots of RT-PCR products in agarose gels and found the same 99 bp product in two lesional keratinocyte cell lines as in our positive control placenta.

8.12 Expression of MMP-21, MMP-26, and MMP-8 in psoriasis (unpublished results)

Using two different non-commercial antibodies, we generally detected no **MMP-26** in our psoriatic samples, suggesting that mere hyperproliferation is not enough to induce MMP-26 expression (unpublished results). This is also the case with **MMP-21** (Ahokas et al. 2003).

MMP-8 was expressed in 12/20 psoriatic samples, mainly in the neutrophils of the upper dermis but in areas of neutrophil accumulation in the epidermis (8/8) as well. In two samples, positive immunostaining was detected also in neutrophils invading the epidermis, resembling the findings of MMP-9 in psoriasis (**Figure 6**). The different treatments seemed to have no effect (untreated n = 10, corticosteroid n = 3, calcipotriol n = 3, corticosteroid + calcipotriol n = 1, UVB n = 1, acithretin + PUVA n = 2). No positive cells with fibroblast morphology were found, and the keratinocytes generally remained negative.
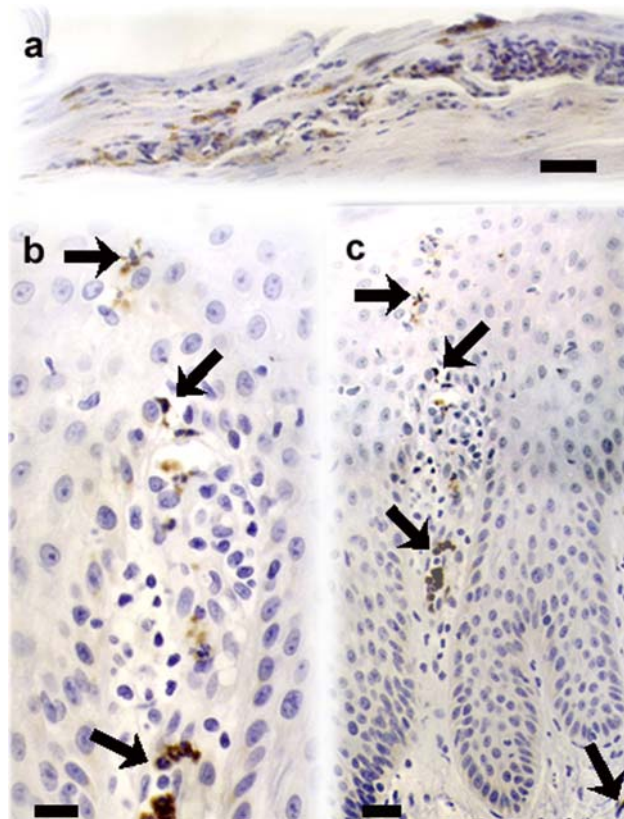
Figure 6. MMP-8 is expressed in neutrophils of psoriatic lesions. a) Accumulation of immunopositive neutrophils in the epidermis, b) Neutrophils are detected in the papillary dermis and invading the epidermis, c) Lower magnification of b showing dermal neutrophils as well. Arrows depict corresponding neutrophils expressing MMP-8. *Scale bars*: a) and b) 25 μm, c) 50 μm.

8.13 Expression of TIMP-1 and TIMP-3 mRNAs in psoriasis (III)

We detected **TIMP-1** in inflammatory infiltrates and endothelial cells of dermal papillae (22/29) with the most abundant signal in psoriatic samples treated with topical corticosteroids (III, Table 1, Fig. 3a-d). Corticosteroid treatment influenced epithelial TIMP-1 expression as well (III, Fig. 3c; Table 1). Occasional fibroblasts express TIMP-1 in normal skin (Saarialho-Kere et al. 1992). TIMP-1 is upregulated in psoriatic keratinocyte cultures as well (Buisson-Legendre et al. 2000).

TIMP-3 expression was lower in 8/16 psoriatic lesional samples perivascularly (III, Fig. 3e-f) and occasional signal was also detected in nonlesional skin in the perivascular stromal cells and in hair follicles, in agreement with Airola et al. (1998). Our TIMP data may be explained by the anti-angiogenic properties of TIMPs as a host response (Nagase & Woessner 1999). Suprabasal TIMP-2 together with suprabasal MMP-2 have been shown to be overexpressed in both lesional and nonlesional skin, suggesting involvement in BM alterations or alterations in cell-cell and cell-matrix relations (Fleischmajer et al. 2000).

8.14 Expression of IFI27 mRNA (V)

In Northern analyses, we could not detect IFI27 mRNA in fibroblasts but detected remarkably variable amounts of IFI27 in many keratinocyte cell lines. Keratinocytes treated with TNF-α showed approximately two-fold increased IFI27 levels (V, Fig. 1a).

TNF-α, IFN-γ, and TGF-β1 upregulated IFI27 expression in primary keratinocyte cultures measured with quantitative Taqman RT-PCR even more, 3 to 17-fold (V, Fig 1b). Upregulation was more pronounced when the cells were stimulated with TNF-α for 48 hours (16-fold) than for 24 hours (8-fold). TGF-β1 is supposed to inhibit keratinocyte proliferation *in vitro* (Symington 1989) and controversial data exists on TNF-α and IFN-γ in the context of mitogenic activity, but many cytokines have different effects on keratinocytes *in vivo* and in culture (Turksen et al. 1992). TNF-α did not upregulate IFI27 in SV40 transformed human keratinocytes. We did observe, however, upregulation of IFI27 in keratinocytes immortalized with E6/E7 viral proteins. The different results may reflect the differences between primary keratinocytes and immortalized SV40 transformed cells (Celis and Olsen 1994). Retinoic acid and vitamin D downregulated IFI27 expression (expression level 26-44% of the untreated control) (V, Fig. 1c), suggesting that with healing lesions during oral or topical therapy, IFI27 expression ceases in keratinocytes. IFI27 pathways may possibly, in fact, be activated with novel TNF-α antagonist drugs.

In agreement with previous gene array data (Bowcock et al. 2001), *in situ* hybridization revealed abundant expression of IFI27 mRNA in psoriatic skin (V, Fig. 2a-b,e). The level of IFI27 signal was roughly equal in all untreated and treated lesions. **Normal skin** did not express IFI27 - in accordance with previous RT-PCR findings (Rasmussen et al. 1993) - but IFI27 was expressed to a lower extent in **non-lesional skin** (V, Fig. 2d-e). *In situ* hybridization is, however, a qualitative, not a quantitative technique, and the exact amount of expression cannot be measured. Rasmussen et al. (1993) have shown IFI27 expression in other tissues such as colon, stomach, and lung.

Normally healing wounds from pinch graft sites of upper thigh (Vaalamo et al. 1996) express IFI27 mRNA in proliferating keratinocytes. The number of IFI27 positive cells increased from 1-day to 9-day wounds (V, Fig 2g-l) and seemed to be temporally and spatially regulated. Proliferating keratinocytes in psoriasis are reminiscent of proliferative keratinocytes in normal re-epithelialization, and IP-10 (IFN-γ-inducible protein 10) and MIG (monokine induced by IFN-γ), two proteins reported to be upregulated in psoriasis (Engelhardt et al. 1998, Gillitzer and Goebeler 2001), are also regulated spatially and temporally. Migrating cells that stained strongly with laminin-5 (Rechardt et al. 2000) were not positive for IFI27 (V, Fig. 2g-l).

Basal cell cancers lacked IFI27 mRNA, although 9/13 **SCCs** of various degrees of differentiation were strongly positive (V, Fig. 2m), possibly reflecting their histopathological differences and higher proliferation index of SCCs (al-Sader et al. 1996). Novel TNF-α antagonist drugs might thus also slow down the growth of SCC through inhibition of IFI27. Recently it has been shown that breast carcinoma cells, different cancer cell lines, and cervical cytobrush with neoplastic lesions expressed ISG12 and a new IFN-inducible splice variant, ISG12-S (Smidt 2003).

Epidermal staining was demonstrated in other hypertrophic benign skin disorders **chronic eczema** (3/3), **lichen planus** (3/3), and **lichenoid chronic dermatitis** (3/4) (V, Fig. 2f). In lichen planus and eczema, IFN-γ is overexpressed, which may well induce IFI27 mRNA expression (Fayyazi et al. 1999, Carroll et al. 1997). **Palmoplantar pustulosis**, **pityriasis**

rubra pilaris, and **chronic venous ulcers** were devoid of IFI27 mRNA. The different findings in chronic ulcers (duration > three months) vs acute wounds may reflect different cytokine profiles, such as levels of activeTNF-α and IFN-γ, and inflammatory cells of these skin disorders (Loots et al. 1998, Hübner et al. 1996).

9 Summary and Conclusions

This study defines the association of a specific HCR*WWCC haplotype with psoriasis in different populations and investigates the role of HCR protein in the pathogenesis of psoriasis. The risk haplotype was found to change the predicted secondary structure of the HCR protein, possibly affecting the biochemical or antigenic properties of the HCR protein. The association of HCR*WWCC with the psoriatic phenotype could not be dissected from the association of CDSN or HLA-CW*6, because of the strong LD between these three genes in all the populations studied. To further separate the associations of these individual genes, either very large family cohorts studied with common markers and statistical methods or several thousands of cases and controls, depending on the contribution of the single locus and frequency of the associated allele, would be required. Recently, a large collaborative study combining the patient material of different groups worldwide has been established and indeed, haplotype mapping should reduce the number of markers needed in order to define "the psoriasis gene".

At this point, functional studies rather than further genetic dissection of the identified candidate genes may give clues for the biochemical pathways involved in the pathogenesis of psoriasis. In this study, HCR was expressed in psoriatic lesions differently than in normal skin or in other hyperproliferative skin disorders. The protein localized basally and suprabasally above the dermal tips in contrast to Ki67, the hyperproliferation marker, which showed most intense staining in the rete ridges. β-catenin staining was absent in the very same area, suggesting that the adhesive properties of the cells expressing HCR were abnormal. In accordance with this, cultured primary keratinocytes stained most intensely when they were not growing in confluence. Both *in vivo* and *in vitro* the HCR protein could be found in either the cytoplasm or the nucleus or both. The expression of HCR was not confined solely to psoriasis, but the protein was encountered in lung and breast adenocarcinomas as well. Again, Ki67 positivity was confined to adjacent cells, suggesting that the HCR positive cells were not proliferating. We attempted to illustrate the function of HCR as well by studying its regulation in primary keratinocytes, stimulating them with different cytokines and measuring the HCR mRNA levels with quantitative real-time TaqMan PCR. Of all the tested cytokines and anti-psoriatic drugs, only IFN-γ seemed to downregulate HCR mRNA expression. IFN-γ is a crucial cytokine in the pathobiology of psoriasis, but its actual effect on keratinocyte hyperproliferation is somewhat controversial. It seems to be antiproliferative in cultured primary keratinocytes, but induces hyperproliferation in psoriatic keratinocytes. Taking into account our immunohistochemical findings, we concluded that HCR may regulate the proliferation of keratinocytes, but further studies using transfection experiments, a mouse model, and yeast-two hybrid systems are needed to determine the precise function of this putative candidate gene in the pathogenesis of psoriasis.

MMPs have rarely been studied in the context of psoriasis. We demonstrated here that MMP-12 was upregulated in psoriatic lesions in macrophages of the inflammatory infiltrate either in the epidermal compartment or in the superficial dermis. This is in accordance with findings of MMP-12 aiding macrophage migration through BM, because invasion of activated macrophages to the epidermis occurs in psoriatic lesions as well. MMP-12 may further stimulate the disease process by releasing TNF-α from its precursor form. MMP-19 was upregulated in the basal and suprabasal keratinocytes of hyperproliferative areas in psoriasis, possibly propagating the disease, and in endothelial and fibroblast- or macrophage-like cells. In psoriatic lesional keratinocytes, MMP-19 was upregulated 15-

fold compared to normal or nonlesional keratinocytes, supporting our *in vivo* data. Moreover, recent studies have suggested the MMP-19 protein in psoriatic lesions to be in processed, i.e. active, form. MMP-19 may contribute to BM disruptions either as a causative agent or as a response to disruption - this was further implicated in samples of lichen planus and lichenoid chronic dermatitis. When the BM is disrupted, fibroblasts grow in contact with keratinocytes, and can induce their hyperproliferation. MMP-8 expression was very similar to MMP-9 expression, showing intensely stained neutrophils especially in Munro's microabscesses. MMP-3 was found in basal psoriatic keratinocytes in a few samples with prominent hyperproliferation and positive laminin-5 staining indicating disruptions of the BM zone, similar to the process in which MMP-3 is functioning during wound healing. The recently cloned MMPs -21, -26, and -28 were not upregulated in our psoriatic samples, implicating that mere keratinocyte hyperproliferation is not enough to induce their expression. TIMP-3 mRNA was found perivascularly and TIMP-1 mRNA abundantly in both the epidermal and dermal compartments possibly reflecting the anti-angiogenic properties of the TIMPs. In this study, different MMPs were expressed in psoriatic lesions, encouraging the investigations of targeted MMP inhibitors as anti-psoriatic drugs. Keratinocytes were, however, rarely positive for MMPs, suggesting that epithelial expression of MMPs is generally induced only by malignant transformation or wound repair.

IFN-γ is an important cytokine in the pathogenetic network of psoriasis and IFI27, an interferon inducible gene, is upregulated in psoriasis. Here we show that IFI27 mRNA occurred abundantly in the psoriatic epidermis but also in other hyperproliferative skin disorders – such as lichen planus – and SCCs. Its expression seemed to be spatially and temporally regulated as seen in acute wounds where the expression increased progressively. A low level of expression could be found in nonlesional psoriatic skin, which is in accordance with results on integrins being already altered in nonlesional skin. IFN-γ, TNF-α, and TGF-β1 upregulated IFI27 mRNA expression of primary cultured keratinocytes, possibly affecting the proliferation status of the keratinocytes. In conclusion, IFI27 may be a novel marker of epithelial proliferation and cancer.

Psoriasis is phenotypically and genetically a heterogeneous disease with multiple variations in disease history, antigens, superantigens, environmental factors, and genetic susceptibility. Defining the psoriasis specific genes and biological pathways hopefully aids in developing diagnostic and prognostic indicators, and targeted therapies.

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12 Original publications