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INVESTIGATIVE REPORT

Matrix Metalloproteinase-19 is Expressed by Keratinocytes in Psoriasis

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Keratinocyte hyperproliferation, inflammatory infiltrates, neoangiogenesis and alterations in cytokine levels are hallmarks of psoriatic skin. Matrix metalloproteinases (MMPs) have been associated with the remodeling of the extracellular matrix during inflammation, neovascularization, and malignant transformation. We have previously shown that particularly MMP-12 is abundantly expressed by macrophages and MMP-9 in macrophages and neutrophils of psoriatic lesions. In this work the expression of two novel metalloproteinases, MMP-19 and MMP-28, was investigated in psoriatic lesional and non-lesional skin. MMP-19 protein was detected by immunohistochemistry in 28/29 samples in keratinocytes in the same regions as Ki67 (marker of proliferating keratinocytes) and p63 (marker of keratinocyte stem cells). Immunosignaling was also seen in endothelial cells and fibroblasts. Furthermore, MMP-19 mRNA was upregulated in psoriatic keratinocytes and skin as assessed by quantitative real-time polymerase chain reaction. In lichen planus and lichenoid chronic dermatitis, MMP-19 staining was found in keratinocytes in areas where the basement membrane was abnormal. MMP-28 was not detected in psoriatic or non-lesional skin. Our results suggest that keratinocytes as well as the previously reported cell types (smooth muscle, endothelial and macrophages) can express MMP-19 in psoriasis and lichen planus. Upregulation of MMP-19 in keratinocytes may be influenced by changes in the architecture of the basement membrane zone. ***Key words: hyperproliferation; epilysin; lichen planus; p63.***

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Human matrix metalloproteinases (MMPs) comprise a family of 23 structurally related zinc-dependent endopeptidases, which together can degrade all the major components of the extracellular matrix during normal and pathological conditions (1, 2). Furthermore, MMPs participate in cell-surface proteolysis, leading to the release of growth factors and angiogenic factors and generation of bioactive extracellular matrix fragments (3). We have previously demonstrated that particularly MMP-12 is abundantly expressed in macrophages and MMP-9 in macrophages and neutrophils of psoriatic lesions (4). In keratinocytes, tissue inhibitors of metalloproteinases (TIMPs), particularly TIMP-1 and to a lesser extent TIMP-3, were also expressed – perhaps reflecting the antiangiogenic properties of TIMPs.

Psoriatic lesions are characterized by keratinocyte hyperproliferation, neoangiogenesis, inflammation and discontinuities in the basement membrane zone (4–6). MMP-2 and TIMP-2 have been shown to be upregulated in keratinocytes in psoriasis along with basement membrane disruptions (6). Production of active MMP-1 by fibroblasts is also elevated in psoriatic plaques compared to non-lesional skin or normal skin (7). Synthetic MMP-inhibitors have even been proposed as a new treatment strategy for psoriatic lesions (8).

Based on their structure, the newly identified members of the MMP family, MMP-19 and MMP-28, have been proposed to belong to a new MMP subfamily, called "other MMPs" (9, 10). Human MMP-19, originally cloned from liver and mammary gland and isolated as an autoantigen from the synovium of a patient suffering from rheumatoid arthritis (11), is widely expressed in tissues, including placenta, lung, pancreas, ovary, spleen and intestine by Northern hybridization (9). MMP-19 protein is also detected in the capillary endothelial cells in acute inflammation of synovium together with vascular endothelial growth factor 2, vascular endothelial growth factor receptor 2, and $\alpha v\beta 3$ integrin (12), suggesting a functional role of MMP-19 in angiogenesis. *In vitro* MMP-19 is capable of cleaving many important basement membrane components such as type IV collagen, laminin-1, nidogen and fibronectin, as well as the extracellular matrix components tenascin-C and type I gelatin, and two components of cartilage, i.e. aggrecan and cartilage oligomeric protein. However, the role of MMP-19 in skin biology has not been elucidated.

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The most recently cloned human MMP, MMP-28 (epilysin), was cloned from testis, keratinocyte, and lung cDNA libraries (13, 14). MMP-28 is expressed at high levels particularly in testis by Northern analysis and in injured epidermis by immunohistochemistry (13, 15). The *in vivo* substrates and activators of MMP-28 are still unknown.

The purpose of this study was to investigate the expression of the structurally related MMP-19 and MMP-28 in psoriasis and in another T-cell-mediated disorder, lichen planus. Our results suggest that MMP-19 expression is associated with keratinocyte hyperproliferation in psoriasis and remodeling of the basement membrane in lichen planus.

MATERIAL AND METHODS

Patients

Twenty-nine patients affected with psoriasis (duration of disease from newly diagnosed to 41 years) were included in this study and biopsies taken from the center of psoriatic lesions were obtained from different parts of the body. Biopsies from 14 of the patients were also taken from the normal-looking skin approximately 10 cm away from the psoriasis lesion. Seventeen of the patients were untreated, 8 used topical corticosteroids, 3 topical calcipotriol, and 1 topical corticosteroids + calcipotriol. The formalin-fixed, paraffin-embedded specimens were obtained from the Departments of Dermatology, University of Helsinki, Turku and Oulu, and the Central Hospital of Päijät-Häme. The study protocol was approved by the Ethics Committees of Helsinki, Turku and Oulu University Central Hospitals and Central Hospital of Päijät-Häme. Formalin-fixed, paraffin-embedded specimens of lichen planus ($n=5$), lichenoid chronic dermatitis (=neurodermatitis) ($n=3$), and normal skin ($n=6$) were obtained from the Department of Dermatopathology, University of Helsinki. The punch biopsies for TaqMan® analysis were taken from the center of untreated psoriatic lesions and from the normal-looking skin approximately 10 cm away from the lesion and frozen in liquid nitrogen. The diagnoses were confirmed by an experienced dermatopathologist (A-LK).

Immunohistochemistry

Immunostaining was performed using the avidin-biotin-peroxidase complex technique (Vectastain ABC Kit; Vector laboratories, Inc., Burlingame, CA for MMP-19, type IV collagen, and MMP-9; and StreptABComplex/HRP Duet (Mouse/Rabbit) Kit, DAKO, A/S Glostrup, Denmark, no. K0492 for p63, Ki67, and MMP-28). Diaminobenzidine (DAB) or aminoethylcarbazole (AEC) were used as chromogenic substrates. Sections were pretreated with trypsin (10 mg/ml; type IV collagen, MMP-9) or by antigen retrieval (Ki67; MMP-28). The antibodies included monoclonal anti-MMP-9 (dilution 1:400, DB-2211, Diabor, Finland) (16), polyclonal anti-MMP-19 (dilution 1:60, RDI-MMP19abR, Research Diagnostics Inc., Flanders, NJ), polyclonal anti-Ki67 (dilution 1:200, A047, DAKO, A/S Glostrup DK) as a hyperproliferation marker (17), monoclonal anti-type IV collagen (dilution 1:75, M785, DAKO, A/S Glostrup DK) as a marker of intact basement membrane, and monoclonal anti-p63 (dilution 1:250, MS-1081-P1, Neomarkers, Fremont, CA, USA) as a marker for dividing keratinocytes (stem cell marker) (18). Type IV collagen, Ki67 and p63 immunohistochemistry was performed on sections serial to those used for MMP-19. Polyclonal MMP-28 antibodies were a kind gift from Dr Jouko Lohi (13). The tissues were counterstained with hematoxylin. Controls for MMP-19 were performed using normal rabbit immunoglobulin (Fig. 1*E*).

Cell cultures

Normal human keratinocytes were isolated from normal adult skin obtained from reductive mammoplasties (19). Subcutaneous fat and deep dermis were removed, and the remaining tissue was incubated overnight at 0.25% trypsin in solution A (Gibco BRL, Life Technologies). Following the incubation, keratinocytes were scraped off from the epidermis with a scalpel and suspended in keratinocyte growth medium (KGM, Gibco BRL, Life Technologies), supplemented with 5 ng/ml epidermal growth factor and 50 μg/ml bovine pituitary extract (supplied by the vendor), and containing 2% decalcified fetal calf serum. Keratinocytes were maintained in KGM supplemented with epidermal growth factor and bovine pituitary extract until confluency.

Psoriatic lesional and non-lesional keratinocyte cell lines were established from psoriatic plaque lesions and normal-looking skin nearby, at Oulu University Hospital. Cell lines were cultured in calcium-free keratinocyte basal media-2 (KBM-2, Clonetics, BioWhittaker, Inc., Walkersville, Maryland, USA), as previously described (20), containing 1% penicillin-streptomycin and supplemented with bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, transferrin, epinephrine, gentamicin sulfate, and amphotericin B (all from Clonetics). Cells were grown on cell culture dishes until confluency.

Polymerase chain reaction (PCR) primers and probes

PCR primers and minor groove binding (MGB) probe (CCCGTGGACTACCTG) for MMP-19 were designed using the computer program, Primer Express (Applied Biosystems, Foster City, CA). Primers used for amplification were: forward 5′-GCTTCCTACTCCCCATGACAGT-3′, and reverse 5′-GGCTTCTGTAGGTACCCATATTGT-3′. The fluorogenic probe contained a reporter dye (FAM) covalently attached at the 5′end and a quencher dye (TAMRA) covalently attached at the 3′ end. The fluorogenic probe was purified by HPLC. Pre-developed TaqMan® assay reagents for endogenous control human GAPDH labeled with VIC™ reporter dye (Applied Biosystems) was used for amplification of the control gene.

RNA analysis

Punch biopsies from lesional and non-lesional skin that had been maintained in liquid nitrogen were crushed with mortar and pestle. Total cellular RNA was isolated from punch biopsies as well as from psoriatic, non-lesional and normal keratinocytes using the RNAeasy Miniprep-Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions. RNA was then reverse transcribed to cDNA with TaqMan® Reverse Transcription Reagents (Applied Biosystems) and used as a template in a PCR. Real-time quantitative PCR reactions were performed with the ABI PRISM® 7700 Sequence Detector System (Applied Biosystems) (19). Reaction conditions were programmed on a power Macintosh 7200, linked directly to the sequence detector. PCR amplifications were done in a total volume of 20 μl, containing 5 μl cDNA sample, 10 μl TaqMan® Universal PCR Master Mix (Applied Biosystems), 200 nM of each primer and 200 nM of fluorogenic probe. The predeveloped GAPDH endogenous control reagents were used as a control gene from the same samples. MicroAmp® Optical 96-Well Reaction Plates and Optical Caps (Applied Biosystems) were used in reactions.

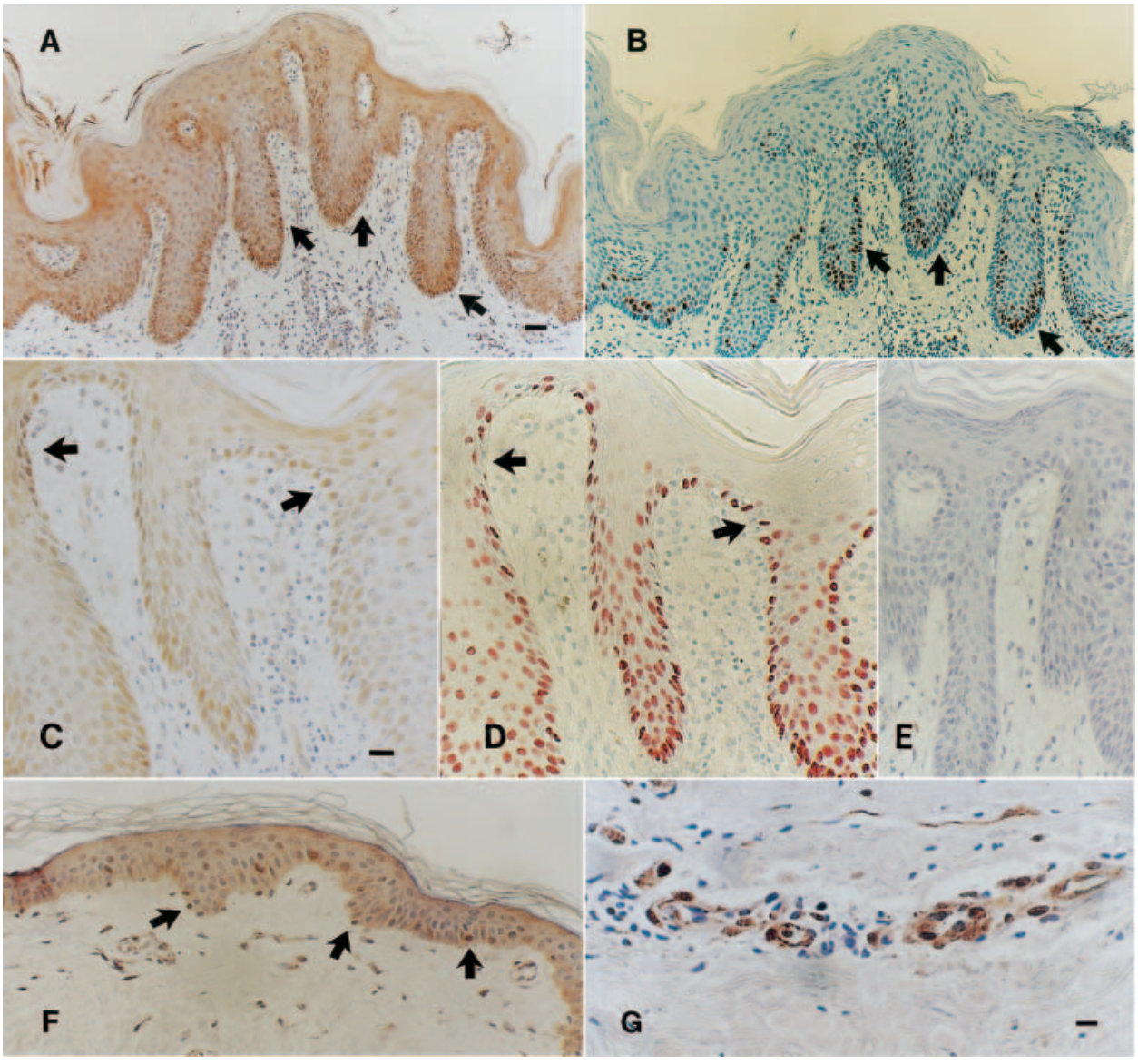

Fig. 1. Matrix metalloproteinase-19 (MMP-19) protein is detected in basal and suprabasal keratinocytes in psoriasis. (*A*) MMP-19 staining is seen in the basal keratinocytes above the dermal papillae and at rete ridges in an untreated psoriatic lesion. (*B*) Ki67 immunostaining in the parallel section is intense adjacent to MMP-19-positive cells in the epidermis. Arrows depict corresponding spots. (*C*) MMP-19 staining showing positive basal and suprabasal keratinocytes (untreated psoriatic lesion). (*D*) In the parallel section, p63-positive cells are either adjacent to or the same as MMP-19-positive cells. Arrows depict corresponding spots. (*E*) The negative normal rabbit IgG immune control. (*F*) Only scattered cells in the normal epidermis were MMP-19-positive, histologically most likely representing melanocytes (arrows). (*G*) Staining is also seen in smooth muscle of capillaries, in endothelial cells, and stromal histiocyte- and fibroblast-type cells. Counterstaining was done with hematoxylin. *Scale bars*: 50 μm (*A*, *B*), 25 μm (*C-F*), 12.5 μm (*G*).

Reaction conditions were the following: 2 min at 50°C and 10 min at 94°C, followed by a total of 40 cycles of 15 s at 94°C and 1 min at 60°C. The normal keratinocyte and psoriatic keratinocyte reactions were also done by conventional PCR reactions with the same primers but without fluorogenic probes. The PCR consisted of 39 cycles with an initial 10 min denaturing temperature of 95°C followed by 15 s of denaturing, 1 min of annealing and elongation (60°C) and 2 min of final elongation, to produce a product of 99 bp. Twenty microliter aliquots of the products were run in a 2% low melting-point agarose gel in the presence of 5 ng ethidium bromide per ml and visualized under ultraviolet light.

RESULTS

MMP-19 in psoriatic lesional skin

Expression of MMP-19 protein was studied by immunohistochemistry, using polyclonal antibodies, which recognized both the latent and activated forms. MMP-19 staining was detected in the majority of psoriatic samples (28/29) in the basal keratinocytes both above the dermal papillae and at rete ridges (Fig. 1*A*). Ki67 immunostaining for proliferative keratinocytes was

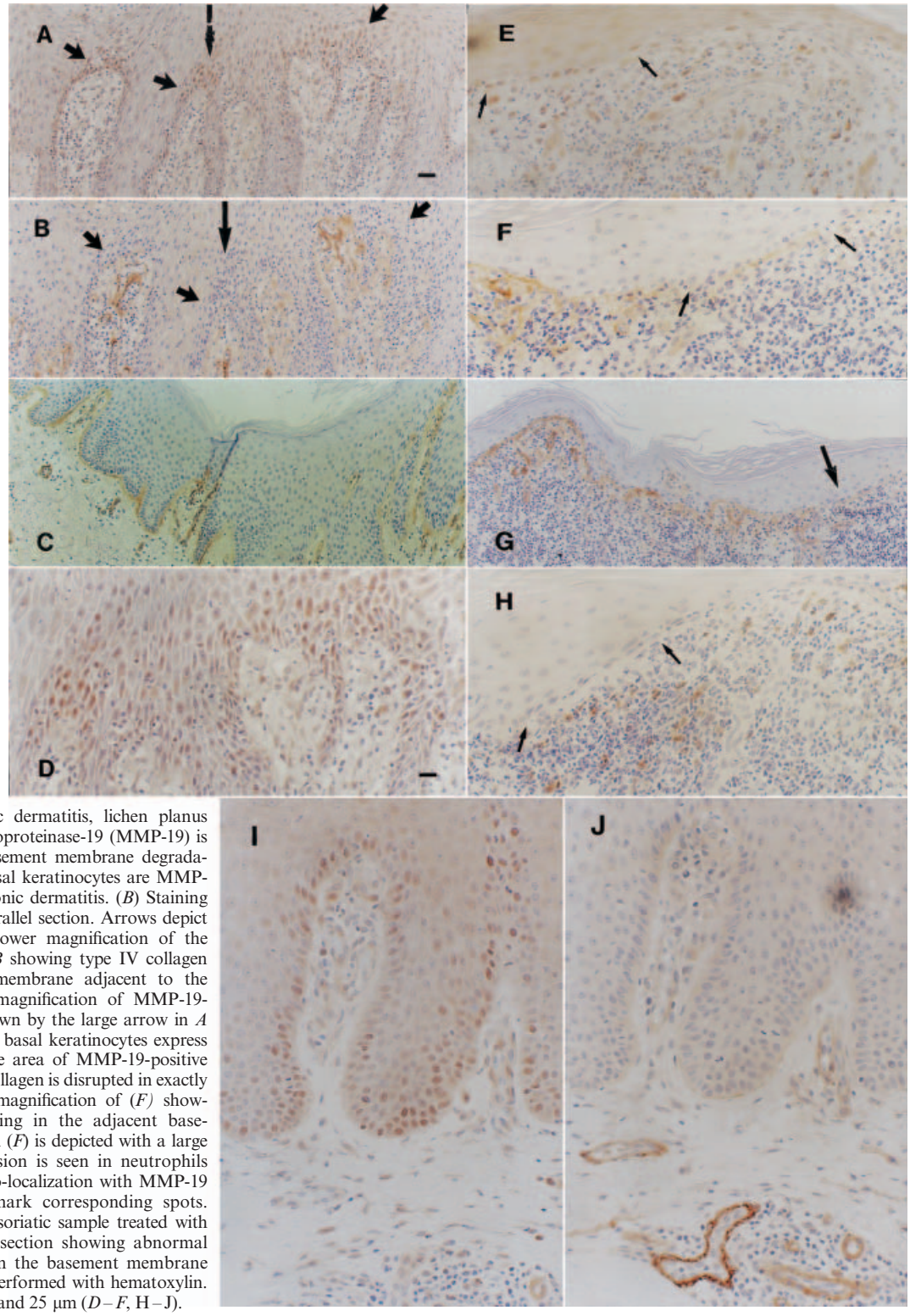

Fig. 2. In lichenoid chronic dermatitis, lichen planus and psoriasis, matrix metalloproteinase-19 (MMP-19) is detected in the areas of basement membrane degradation. (*A*) Basal and suprabasal keratinocytes are MMP-19 positive in lichenoid chronic dermatitis. (*B*) Staining for type IV collagen in a parallel section. Arrows depict corresponding spots. (*C*) Lower magnification of the same sample as in *A* and *B* showing type IV collagen staining in the basement membrane adjacent to the affected area. (*D*) Higher magnification of MMP-19-positive cells in the area shown by the large arrow in *A* and *B*. (*E*) In lichen planus, basal keratinocytes express MMP-19. Arrows depict the area of MMP-19-positive keratinocytes. (*F*) Type IV collagen is disrupted in exactly the same area. (*G*) Lower magnification of (*F*) showing type IV collagen staining in the adjacent basement membrane. The area in (*F*) is depicted with a large arrow. (*H*) MMP-9 expression is seen in neutrophils in the dermis showing no co-localization with MMP-19 immunostaining. Arrows mark corresponding spots. (*I*) MMP-19 staining in a psoriatic sample treated with calcipotriol. (*J*) A parallel section showing abnormal type IV collagen staining in the basement membrane zone. Counterstaining was performed with hematoxylin. Scale bars: 50 μm (*A* – *C*, *G*) and 25 μm (*D* – *F*, H – J).

particularly intense adjacent to MMP-19 positivity mainly in the rete ridges, but not all proliferating cells were MMP-19 positive judged histologically from the parallel sections (Figs 1*A* and *B*). Stem-cell marker p63-positive cells were basal cells lining the dermal papillae or individual cells inside the rete ridges (Fig. 1*D*). Histologically, a subpopulation of these cells seemed to be either adjacent to or the same as MMP-19-positive cells (Figs 1*C* and *D*). Immunosignal for MMP-19 was also seen in endothelial, histiocyte- and fibroblast-type cells (Fig. 1*G*) as well as in smooth muscle cells and sweat gland epithelial and myoepithelial cells (data not shown). Cells showing lymphocyte morphology did not show any staining.

MMP-19 in non-lesional psoriatic and normal skin

In the non-lesional skin ($n=14$), as in normal skin ($n=6$), MMP-19 was not detected in keratinocytes. Only scattered cells in the epidermis stained positively. In a histological examination they most likely represented melanocytes or Langerhans' cells (Fig. 1*F*). Smooth muscle cells, endothelial cells, sweat glands, and occasional histiocytes were MMP-19 positive, as in the lesional skin (Fig. 1*G*).

MMP-19 in lichen planus and lichenoid chronic dermatitis

To investigate whether keratinocyte hyperproliferation and acanthosis *per se* can induce MMP-19, three samples of lichenoid chronic dermatitis were stained. In one of the samples the keratinocytes were MMP-19 (Fig. 2*A*) positive. The same sample demonstrated disrupted basement membrane as assessed by type IV collagen immunostaining, (Figs 2*B–D*). This prompted us to do an MMP-19 immunoanalysis also on lichen planus, which is a T-cell-mediated disorder like psoriasis and is often characterized by substantial disruptions in the basement membrane zone (21). MMP-19 protein was upregulated in keratinocytes at the areas of basement membrane degradation in lichen planus (Figs 2*E–G*). Abnormal staining for type IV collagen was also detected adjacent to MMP-19-positive epidermis in psoriasis (Figs 2*I* and *J*).

MMP-19 is able to cleave proMMP-9, another MMP capable of degrading components of the basement membrane, causing conformational changes that expose the final activation site of MMP-9 to be activated by a second proteolysis (22). However, in our psoriasis or lichen planus specimens, MMP-9 expression was seen in neutrophils in the dermis (Fig. 2*H*) showing no co-localization with MMP-19 immunostaining (Fig. 2*E*).

Expression of MMP-19 is upregulated in psoriatic compared to non-lesional keratinocytes

To study the ability of keratinocytes to express MMP-19, we cultured normal, non-lesional and lesional keratinocytes and used quantitative real-time PCR for the analysis. MMP-19 expression by unstimulated normal and non-lesional keratinocytes was fairly low, detected around cycle 30–32, and similar in both cell lines (Fig. 3). In agreement with the immunostaining data, the expression of MMP-19 mRNA by lesional keratinocytes was induced almost 15-fold (Fig. 3). We also used the TaqMan® analysis to determine whether MMP-19 expression is induced *in vivo* in psoriatic human skin: a 3.5-fold induction of MMP-19 mRNA expression in untreated psoriatic skin compared with non-lesional skin was observed (data not shown).

To confirm the ability of psoriatic keratinocytes to express MMP-19, we also ran aliquots of RT-PCR products in agarose gel. Visualized under ultraviolet light, we observed the same 99 bp product in two lesional keratinocyte cell lines as in placenta, our positive control (data not shown).

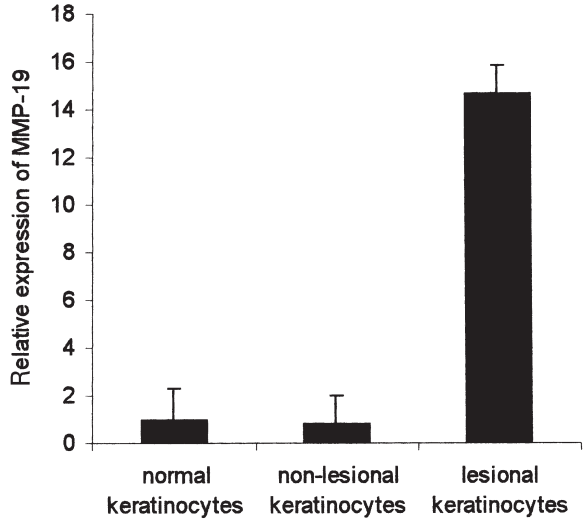

Fig. 3. Relative expression of matrix metalloproteinase-19 (MMP-19) in normal, non-lesional and psoriatic lesional keratinocytes assessed by quantitative, real-time polymerase chain reaction. Keratinocytes were cultured in keratinocyte growth medium or keratinocyte basal media-2. Total RNA was isolated and reverse transcribed to cDNA. TaqMan® was performed as described in the Material and Methods section using GAPDH as an endogenous control. The results are shown relative to mRNA levels from normal keratinocytes, assigned the value 1.

MMP-28 in psoriasis

MMP-28 expression could not be detected in psoriatic (10/10) or non-lesional skin (5/5) (data not shown). Wounds were used as positive controls in the same experiments (13).

DISCUSSION

MMPs have been implicated in the progression of tumors and specifically in extracellular matrix degradation and remodeling. The novel member of the MMP family, MMP-19, has previously been detected in endothelial and vascular smooth-muscle cells *in vivo* (12). Our results herein demonstrate that MMP-19 protein is expressed in the hyperproliferative, p63-positive area of the epidermis in psoriatic lesions (p63 is a marker of keratinocyte stem cells). However, it was not detected in lichenoid chronic dermatitis without evidence of basement membrane discontinuities. Interestingly, Djonov et al. (23) have recently shown that epithelial cells in benign tumors of the breast express MMP-19 more than normal breast tissue or its invasive carcinomas. MMP-19 staining in psoriasis was seen in basal and suprabasal keratinocytes, the strongest intensity concentrated in the rete ridges in accordance with the most prominent areas of hyperproliferation. In

non-lesional skin, we found MMP-19 only in scattered cells, which was similar to the findings in normal skin. This is in agreement with our previous work on some other MMPs (4), demonstrating that there does not seem to be any alteration in the phenotype of keratinocytes before the psoriatic lesion has developed. In psoriasis, MMP-19 was also expressed by the endothelial cells in concordance with previous findings in acutely inflamed synovium (12). Other stromal cells that, histologically, could be either fibroblasts or macrophages, were also positive. In fact, MMP-19 has recently been described in both macrophages (24) and fibroblasts *in vitro* (25).

Taking into account the *in vitro* substrates of MMP-19, this protease may also have a role in the remodeling of the basement membrane. In lichen planus, we found MMP-19 expression basally in areas of basement membrane disruption as determined by the disappearance of type IV collagen. Bordering the lesions, type IV collagen stained continuously again and MMP-19 expression was almost lost, representing the staining pattern of normal skin. MMP-9, which is also capable of degrading the basement membrane and which can involve MMP-19 in its activation process, was not expressed in the keratinocytes in the same area (Figs 2*E* and *H*). This same kind of staining pattern was found in lichenoid chronic dermatitis lesions, implying that MMP-19 expression in disrupted basement membranes may not only be associated with disease but may represent a more general phenomenon. MMP-19 expression in psoriasis was seen in keratinocytes bordering dermal papillae, a typical region for basement membrane discontinuities observed in this disease. In normal skin, fibroblasts and basal keratinocytes do not interact, but in psoriatic skin, cell–cell and cell–matrix interactions are altered (5, 6, 26). Moreover, psoriatic fibroblasts are able to induce hyperproliferation even in healthy keratinocytes (27, 28). Thus if MMP-19 contributes to basement membrane alterations in psoriasis, it could mediate keratinocyte hyperproliferation. However, we cannot exclude the other scenario, that MMP-19 is upregulated in keratinocytes in response to basement membrane degradation and in fact assists in basement membrane remodeling during repair.

Our present TaqMan® data indicate that in psoriatic keratinocytes, expression of MMP-19 is notably upregulated in agreement with our immunostaining data. *In vivo*, expression of MMP-19 in psoriatic skin is increased compared to non-lesional skin (3.5-fold), but less than *in vitro* (15-fold). This possibly reflects the fact that skin biopsies of normal and psoriatic skin contain MMP-19 producing dermal fibroblasts.

The cytokines inducing MMP-19 in keratinocytes are not currently known. Epidermal growth factor induces MMP-19 in smooth-muscle cells, and tumor necrosis factor-α as well as interleukin-1β induce the expression in endothelial cells (12). All these cytokines are overexpressed in psoriatic skin (see 29–31) and may be involved in stimulating MMP-19 expression in keratinocytes. All the known specific MMP inhibitors, TIMP-2, -3, -4, and to a lesser extent TIMP-1, are able to inhibit MMP-19 (32). Corticosteroid treatment was found to upregulate TIMP-1 in keratinocytes (4) and thus might also inhibit MMP-19 activity.

In this study, we did not find MMP-28 in psoriatic lesions, which implies that this protease is not induced by mere benign keratinocyte hyperproliferation. Previously, MMP-28 was found to be expressed in injured epidermis and it may well be that total disruption of the basement membrane is needed to upregulate it, as with so many other MMPs (15).

In summary, this is the first study showing MMP-19 expression by keratinocytes *in vivo*. In addition to its participation in the progression of rheumatoid arthritis and ovulation (24, 33), MMP-19 seems to undergo specific regulation in T-cell-mediated inflammatory skin disorders characterized by changes in the integrity of the basement membrane zone.

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