Altered Distribution of Heparan Sulfate Proteoglycans in Psoriasis

M. M. B. SEYGER, J. VAN DEN BORN, J. SCHALKWIJK, P. C. M. VAN DE KERKHOF, and E. M. G. J. DE JONG

Departments of Dermatology and Nephrology, University Hospital Nijmegen, The Netherlands

Heparan sulfate proteoglycans (HSPGs) are components of the basement membrane (BM) of various tissues. They consist of a core protein and negatively charged glycosaminoglycan side chains: the heparan sulfate (HS) moiety.

In psoriasis, uninvolved skin of psoriasis patients and in normal skin, the distribution of HSPGs was studied immunohistochemically by means of three different monoclonal antibodies: JM-72, directed against the HSPG-core protein, JM-13 against the sulfated domains of HS and JM-403 against the unmodified/low sulfated parts of HS.

In psoriasis JM-13 staining was consistently absent in the tips of the dermal papillae, whereas JM-13 showed a continuous staining in the BM of uninvolved and normal skin. JM-403 staining was present in BM of all specimens. In addition, a honeycomb-like staining was found in epidermis of normal skin and to a lesser extent in uninvolved skin, due to binding with plasma membrane-associated HS. In psoriasis this JM-403 staining of the epidermis was invariably absent. JM-72 showed a continuous staining of BM in all biopsies.

In conclusion, normal human skin and involved psoriatic skin show clear differences in expression of HS. These data may provide insight into the role of HSPGs in psoriasis, which remains further to be elucidated. Key words: dermo-epidermal junction; basement membrane; immunohistochemical; normal skin; uninvolved psoriasis.

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M. M. B. Seyger, Department of Dermatology, University Hospital Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands.

Psoriasis is a hyperproliferative skin disease, the aetiology of which is still unknown. In the epidermis of uninvolved skin of psoriasis patients, various changes have been described, but none of these have until now been shown to play the major role in the pathogenesis of psoriasis (1). Investigations of incipient psoriatic lesions and spreading psoriatic plaques have shown that even in very early lesions and in adjacent uninvolved skin, changes can be detected in the stroma. Using laser Doppler flowmetry, Macdonald Hull et al. showed increased blood flow that could be demonstrated before clinical lesions appeared (2). In addition, the endothelial marker enzyme alkaline phosphatase was distinctly increased in the tips of the dermal papillae, whereas JM-13 showed a continuous staining in the BM of uninvolved and normal skin. JM-403 staining was present in BM of all specimens. In addition, a honeycomb-like staining was found in epidermis of normal skin and to a lesser extent in uninvolved skin, due to binding with plasma membrane-associated HS. In psoriasis this JM-403 staining of the epidermis was invariably absent. JM-72 showed a continuous staining of BM in all biopsies.

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Permission to conduct this study was obtained from the local Ethics Committee of the University Hospital Nijmegen. Informed consent was given by all subjects in this study.

**Antibodies**

For immunohistochemical staining, three monoclonal antibodies against HSPG were used. mAb JM-72 (19), which is directed against the BM-associated HSPG-core protein, and two mAbs, designated as JM-13 (20) and JM-403 (21) against the HS-side chains. The production, characterisation and demonstration of specificity of these monoclonal antibodies were carried out by van den Born et al., as previously published (19-21). The two mAbs JM-13 and JM-403 recognise different epitopes along the HS polysaccharide chain: JM-13 binds to N-sulfated epitopes in HS and heparin (which is closely related to HS but much more sulfated); JM-403 preferentially binds to low-sulfated HS preparations. Moreover, the epitope is fully dependent on the presence of an N-unsulfated glucosamine residue, thus having a free amino group. These units are not found in hyaluronic acid, thereby excluding binding of JM-403 to hyaluronic acid (22). In conclusion: mAb JM-13 binds to an epitope in the highly sulfated domains of HS and heparin, whereas mAb JM-403 binds to unmodified/low sulfated regions of HS.

**Immunohistochemical staining**

For all three antibodies an indirect peroxidase technique was used. Control stainings were carried out using nonimmune mouse IgG. After thawing at room temperature for 30 min, slides were fixed again in acetone (0°C) for 10 min, washed in phosphate-buffered saline (PBS) and incubated for 1 h at room temperature with the primary antibodies [anti HSPG-core mAb JM-72 (ascites, 1:250 in PBS), anti HS mAb JM-403 (ascites, 1:250) and anti HS mAb JM-13 (undiluted culture supernatant)].

After having been washed twice in PBS the slides were incubated for 30 min with a solution of rabbit-anti-mouse-immunoglobulins (DAKOPATTS, Copenhagen, Denmark) conjugated with peroxidase, diluted 1:50 in phosphate buffer containing 5% human AB-serum. After two further washes in PBS and a preincubation with sodium acetate buffer (pH 4.9), slides were stained in a solution of 3-amin-9-ethyl-carbazole (AEC) in sodium acetate buffer containing 0.01% H2O2 for 10 min. Slides were counterstained with Mayer’s hematoxylin (Sigma, St Louis, U.S.A.) and mounted in glycerin gelatin (Sigma, St Louis, U.S.A.).

**Histological examination**

The distribution of the staining in dermis, epidermis and basal membrane was assessed by two independent investigators using a 3-point scale: 0 = staining not present, 1 = discontinuously distributed staining, 2 = continuously distributed staining. In addition, staining of BM of bloodvessels in the dermis was scored being either present or absent and the presence of cell membrane-associated staining was recorded as well.

**RESULTS**

All control stainings using nonimmune mouse IgG were negative.

**JM-13**

All 20 specimens of psoriatic skin showed a striking staining pattern, completely different from the staining pattern in normal and uninvolved skin. JM-13 staining was consistently absent in the tips of the dermal papillae. A clear, linear staining was present invariably in the lower parts of the dermal papillae (Fig. 1b).

The JM-13 staining in all 20 specimens of normal skin showed a continuous staining of the BM at the dermo-epidermal junction (Fig. 1a). The clinically uninvolved skin of psoriatic patients showed a pattern that was similar to that observed in healthy volunteers. In contrast to the antibodies JM-403 and JM-72, mAb JM-13 only stained the BM of the dermo-epidermal junction and not the BM of the bloodvessels.

**JM-403**

In all specimens from normal skin, JM-403 staining, directed against the unmodified/low sulfated parts of HS, displayed a strong honeycomb-like staining in the epidermis (Fig. 2a). The staining pattern suggested staining of membrane-bound structures or intercellular substances. This staining pattern was most pronounced in the stratum spinosum and stratum granulosum and was less prominent in the stratum corneum and basal layers. In uninvolved epidermis of psoriatic patients the cell membrane associated staining was seen more focally and was less conspicuous, whereas this staining pattern was invariably absent in the lesional skin of all psoriatic patients (Fig. 2b).

In the BM of skin and bloodvessels the JM-403 staining was bright, continuous and present in all specimens.

**JM-72**

Staining with the antibody JM-72, directed against the core protein of the HSPG, was positive in all specimens. In normal...
Fig. 2. Immunohistochemical staining of HS-side chains by mAb JM-403. (a) Normal skin. Note the honeycomb-like staining in the epidermis. (b) Psoriasis. Note the absence of honeycomb-like staining.

Skin, uninvolved psoriasis and psoriatic lesions the staining was restricted to the BM of the dermal-epidermal junction and the BMs of the blood vessels in the dermis. There was a strong and continuous linear staining (Fig. 3). No additional structures were stained.

DISCUSSION

At present little is known about the distribution of HSPGs in human skin. In normal human epidermis, Tammi et al. localised keratinocyte surface-associated HSPGs by means of electron microscopy (23), and recently, van den Born et al. described the expression of BM-associated HSPGs in normal human skin (19).

In this study we analysed the distribution of HSPGs in psoriatic skin, uninvolved psoriasis and normal skin by means of immunohistochemical staining. Three new monoclonal antibodies were used, two directed against the HS-side chains (JM-13 and JM-403) and one against the HSPG-core protein (JM-72) (19-21). mAb JM-72 gave a continuous, similar staining in all specimens, indicating that the core protein of HSPG is present in normal skin, uninvolved psoriasis and psoriatic skin without a difference in distribution. This also applies to the staining of the BM by the mAb JM-403, directed against the HS-side chains, which also gave a bright, continuous staining in all specimens.

However, staining with JM-13, directed against a different epitope on the HS-side chain, showed a clearly different pattern in all specimens. It was absent in the tips of the dermal papillae of all psoriasis specimens. The heterogeneity in staining of the HS-side chains by the two antibodies JM-13 and JM-403 can be explained by the different epitopes recognised. The epitope of JM-403 is located in the unmodified/low sulfated domains of HS. This is in contrast with the epitope of JM-13, which is located in the N-sulfated parts of HS-side chain.

Several mechanisms can explain the absence of JM-13 staining in the tips of the dermal papillae. This might be the result of decreased accessibility of HS due to epitope masking by cationic molecules or immune complexes, or alternatively the epitopes are lost due to enzymatic cleavage of HS. A third possibility could be a steric hindrance of BM components caused by changed molecular interactions. These three possibilities are discussed below.

In psoriasis, neutrophil polymorphonuclear leukocytes (PMNs) and plasma proteins move from the tip of the capillary loops in the dermal papillae into the epidermis (24, 25). It is striking to observe that in this part of the basal membrane, where the PMNs and plasma proteins pass, the JM-13 staining is absent. It is tempting to assume a connection between these events. Maybe the JM-13 epitope is blocked by plasma and/or PMN-derived cationic proteins, remaining the unmodified/low sulfated regions in HS accessible for JM-403 binding. This mechanism has been described in previous studies on SLE-nephritis, showing a binding of cross-reactive anti-nuclear antibodies to HS (26, 27). Recently, the adherence of leukocyte
expression in psoriasis have never been described before. These co-ordinates adhesive functions including leukocyte migration. The adhesion to heparan sulfate of this integrin, which acts a role in cell-cell interactions, such as PMNs, that may destroy the JM-13 epitope. Thirdly, many interactions between the HS-side chain and the BM components fibronectin, laminin and collagen type IV are known. Battaglia et al. describe the formation of ternary complexes between laminin, nidogen and proteoglycan, suggesting a key role for nidogen in basement membrane assembly (15, 29). It could thus be that one of these interactions is changed in the tips of the dermal papillae in such a way that the accessibility of the JM-13 epitope is altered by steric hindrance of BM components. A previous study already found a general loss of BM integrity in psoriatic lesions (30) and by electron microscopy, Cox (31) found foci of discontinuity in the basal lamina, especially in active lesions.

A second interesting finding was the honeycomb-like staining of the epidermis with mAb JM-403 in normal skin and non-lesional psoriatic skin and the remarkable absence of this staining in psoriasis. Most likely this is due to binding of the antibody to the keratinocyte plasma membrane-associated HSPGs. In literature, however, such crossreactivity has never been described (32, 33). Two possible candidates of cell membrane-associated HSPGs in skin which share homologies with BM-associated HSPGs are syndecan and CD44v3. The possibility of staining of hyaluronic acid by mAb JM-403 was ruled out, as the epitope of mAb JM-403 is fully dependent on the presence of an N-unsubstituted glucosamine residue, thus having a free amino group. These units are not found in hyaluronic acid (22).

Both CD44v3 and syndecan-1 are known to play an important role in cell-cell interactions, cell-ECM interactions and the binding of growth factors (34–36). These biological functions are important in the regulation of cell growth and migration. Absence of either syndecan-1 or CD44v3 in the epidermis of a psoriatic lesion could contribute to changes in proliferation, differentiation and migration in psoriasis.

In conclusion, an altered HSPG expression in psoriasis was described compared to normal skin, namely the absence of high sulfated HS-side chains (JM-13) in the tips of the dermal papillae in psoriatic patients and the different staining pattern with mAb JM-403 against low sulfated HS-side chains in psoriasis compared to uninvolved and normal skin. To the best of our knowledge, these changes in HSPG expression in psoriasis have never been described before. These data may provide insight into the role of HSPGs in psoriasis, which remains further to be elucidated.

REFERENCES


*Acta Derm Venereol (Stockh) 77*


