Acta Derm Venereol (Stockh) 1994; 74: 269–272

Expression of Thrombospondin-1 (TSP1) and Its Receptor (CD36) in Healthy and Diseased Human Skin

ÁGNES BÉGÁNY[1], MIKLÓS SIMON JR[2], NIKOLAUS DEHMEL[2] and JÁNOS HUNYADI[1]

[1]Department of Dermatology, University Medical School Debrecen, Debrecen, Hungary, and [2]Department of Dermatology, University of Erlangen-Nürnberg, Erlangen, Germany

In the present study, an analysis was made of the expression pattern of thrombospondin-1 (TSP1) and its receptor (CD36) in skin biopsies obtained from healthy volunteers and from patients with lichen planus, lupus erythematosus, cutaneous T-cell lymphoma and psoriasis vulgaris. Using monoclonal antibodies against TSP1 in biopsies from the healthy volunteers and from both clinically involved and uninvolved skin of the patients, a specific peroxidase-positive reaction was detected around the sweat glands in the dermis. In all cases investigated, the CD36-positive lesional keratinocytes remained TSP1-negative. These findings favour the hypothesis that CD36-positive keratinocytes might have some functional relevance via oxidized low-density lipoprotein and/or collagen fibrils, without any connection with TSP1. ***Key words: skin immune system; adhesion molecules; immunohistochemistry; human keratinocytes; sweat glands.***

(Accepted January 4, 1994.)

Acta Derm Venereol (Stockh) 1994; 74: 269–272.

Á. Bégány, Department of Dermatology, University Medical School Debrecen, Nagyerdei krt. 98, H-4012 Debrecen, Hungary.

During the last few years, it has been amply documented that human keratinocytes (HKs) can synthesize and express cell surface moieties characteristic of effector and/or accessory cells of the immune system. Accordingly, HKs react specifically with monoclonal antibodies (MABs) against CD16, and under certain circumstances they may also become HLA-DR, HLA-DQ, CD21, CD54, CD11a and CD18-positive cells (1–6).

The MAB against CD36 (OKM5) recognizes an 88-kD antigen on the cell membrane, which is present on 60–80% of peripheral blood monocytes and platelets (7). In skin specimens from healthy subjects, CD36 was found only on vascular and perivascular structures and in some cases on cells of the acrosyringium (8). In contrast, a net-like intercellular CD36-positive pattern is characteristic of the upper part of the stratum spinosum and granulosum in the delayed-type hypersensitivity reaction, and in skin lesions of patients with different dermatoses, such as lichen planus (LP), lupus erythematosus (LE), cutaneous T-cell lymphoma (CTCL) and psoriasis vulgaris (PV) (9–12). Recent data suggest that the antigen detected by OKM5 serves as the membrane-binding site for thrombospondin-1 (TSP1) (13). In addition, CD36 serves as receptor for both the oxidized low-density lipoprotein (14) and collagen type I fibrils (15).

Three genes encoding for three distinct TSPs (TSP1, TSP2 and TSP3) have recently been described (16). TSPs are platelet α-granule glycoproteins expressed on the surface of thrombocytes only after being activated. Besides the thrombocytes, several other cells can produce and secrete TSPs (16–18).

Thrombin, basic fibroblast growth factor and transforming growth factor-β are the most important known signals for stimulation of TSP production (17, 18). TSPs are important proteins involved in platelet aggregation (19, 20). Further, as adhesion molecules, TSPs can mediate intercellular binding, either by receptor-molecular-receptor interactions or by linking with other adhesive proteins (21).

Recently, several studies have become available concerning the TSP1 receptor CD36 expression of HKs in healthy and diseased skin (8–12). However, data regarding TSP expression by HKs are rather limited (22). We have therefore investigated the expression of TSP1 on HKs in skin specimens obtained from healthy volunteers and from the lesional skin of patients with PV, LE, LP or CTCL, using MABs against TSP1 (P10 and P12), immunohistochemically. Our results with P10, P12 and OKM5 MABs show that TSP1 and CD36 are never co-expressed around the sweat glands and acrosyringium. Moreover, in contrast with the TSP1 receptor CD36, TSP1 is not detectable on the surface of HKs in the clinically involved skin of our patients.

MATERIAL AND METHODS

Patients

Investigations were carried out on surgical skin specimens from healthy volunteers ($n = 5$), who underwent plastic surgery, and on punch biopsy specimens (3 mm) of involved and uninvolved skin from patients with PV ($n = 4$), LE ($n = 4$), LP ($n = 2$) or CTCL ($n = 2$). None of the patients had been involved in any systemic treatment or had used topical corticosteroids during the last 3 weeks before the biopsy. Each specimen was immediately frozen in liquid nitrogen.

Immunohistochemistry

4-μm sections were cut on a cryostat, and the reactivities with the MABs P10 and P12 (Dianova, Hamburg, Germany) and OKM5 (CD36) (Ortho Diagnostics, Raritan, N. J., USA) were visualized by means of the multistep immunoperoxidase method described by Poppema et al. (23). Briefly, cryostat sections were frozen at −70°C for at least 24–48 h. After thawing they were immediately washed in phosphate-buffered saline (PBS), pH 7.2, and incubated with the MABs for 30 min at room temperature. The sections were subsequently washed three times in PBS and incubated with peroxidase-conjugated rabbit-antimouse antibody (Dakopatts, Glostrup, Denmark), diluted 1:10 with human AB serum diluted 1:1 with PBS (SPBS). This was followed by PBS washing and by a 30-min incubation with peroxidase-conjugated swine-antirabbit antibody (Dakopatts), diluted 1:10 with SPBS. After three washes with PBS, the specific peroxidase activity was revealed by using aminoethyl carbazole (AEC) as substrate, followed by a 10-min rinse in distilled water. Finally, counter-staining was performed with hemalum. Two kinds of negative controls were included: first, omission of the primary MAB and, second, substitution of the primary antibody with irrelevant antibodies (mouse MAB IgG1; Becton Dickinson).

RESULTS

Using the MABs P10 and P12, we were able to demonstrate TSP1 in the skin of the healthy volunteers and in the clinically

 ISSN 0001-5555

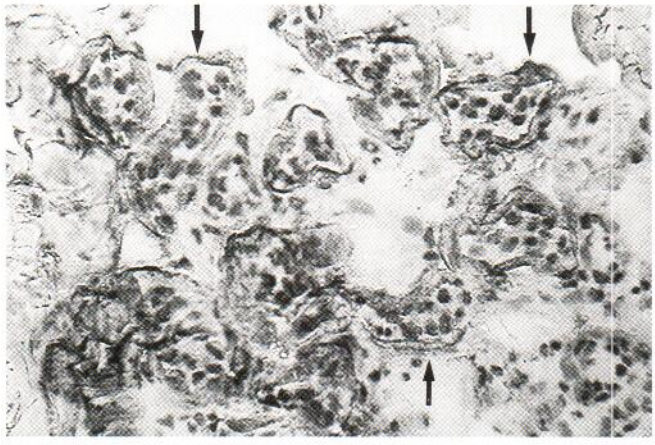

Fig. 1. TSP1-positive sweat glands (*arrows*) in healthy skin (AEC × 250).

involved and uninvolved skin of our patients with PV, LE, LP or CTCL around the sweat glands (Fig. 1). In all specimens investigated, the dermo-epidermal junction showed a negative TSP1 staining.

A net-like intercellular CD36-positive pattern was detected in the upper part of the stratum spinosum and granulosum; moreover, CD36-positive monocytes/macrophages were also seen in the dermal inflammatory infiltrate in the involved skin of patients with PV, LE, LP or CTCL. In all cases investigated, the same CD36-positive epidermal region failed to give a specific reaction when the MABs against TSP1 P10 and P12 were used (Fig. 2a, b).

DISCUSSION

TSPs are adhesive 450 kD glycoproteins of platelets and nucleated cells. TSPs are made up of several protease-resistant domains, including NH_2-terminal, COOH-terminal, procollagen homology domains, and type I, type II and type III repeats (16). The type II repeat shows homology with the epidermal growth factor (16). TSP1 is a heparin-binding protein. It contains binding sites for three cellular receptors: the sequence Val-Thr-Cyst-Gly, which binds to CD36 (7, 24), the sequence Arg-Gly-Asp, which binds to CD51/CD61 (the αV/β3 integrin) (25) and the COOH-terminal domain, which binds a M_r 105,000/80,000 receptor (26). Not all of these interactions occur in all cells, and it seems that separate cell surface receptors are used in a cooperative manner to bind TSPs. It is possible that TSP1, TSP2 and TSP3 are regulated in a cell type-specific fashion (16). TSPs become incorporated into the extracellular matrix after synthesis and secretion by a wide variety of cells, including osteoblasts, endothelial cells, fibroblasts, smooth muscle cells and macrophages (26).

Recent data indicate that HKs can also synthesize and secrete TSP in vitro. TSP produced by HKs yielded specific granular staining within the cytoplasm of the cultured cells (27). Treatment of cultured HKs with γ-interferon (γ-IFN) inhibits TSP production (28). TSP is believed to be a potent adhesion factor for keratinocytes. Antibodies against the heparin-binding domain of the TSP molecule significantly inhibit TSP binding to HKs. Accordingly, it is postulated that HKs bind TSP in a receptor-like manner (29).

The expression of the receptor for TSP1 with the MAB OKM5 (CD36) is well documented in cells of different origins. Thrombocytes, monocytes, macrophages and melanocytes (30) express this cell-surface moiety continuously, while HKs exhibit CD36 only under certain circumstances. The HKs in healthy human skin are CD36-negative, whereas in different cytokine-mediated dermatoses the lesional HKs are consequently CD36-positive (7–12, 30). It seems that the expression of CD36 on lesional HKs correlates with the presence of activated T-lymphocytes in the dermis (9, 10, 12). Activated T-cells release a variety of lymphokines, including γ-IFN, interleukin-2 (IL-2), IL-3, IL-4, IL-5, IL-6 and IL-10. Lymphokines produced by T-cells, particularly γ-IFN, can induce the appearance of immunologically active molecules on the surface of HKs, such as HLA-DR, CD54 (ICAM-1) and CD16 (31–33). Moreover, CD36 is presumed to belong among those molecules which can be upregulated by γ-IFN on normal HKs (9, 10, 12).

Cultured normal HKs do not exhibit the other TSP1-binding receptor, αV/β3, although αV/β1 is present on their surface (34). Data concerning the expression of the third TSP1 receptor, M_r 80,000/105,000, on HKs are not available yet.

Using rabbit anti-human platelet thrombospondin serum, Wight et al. observed strong specific staining at the dermo-

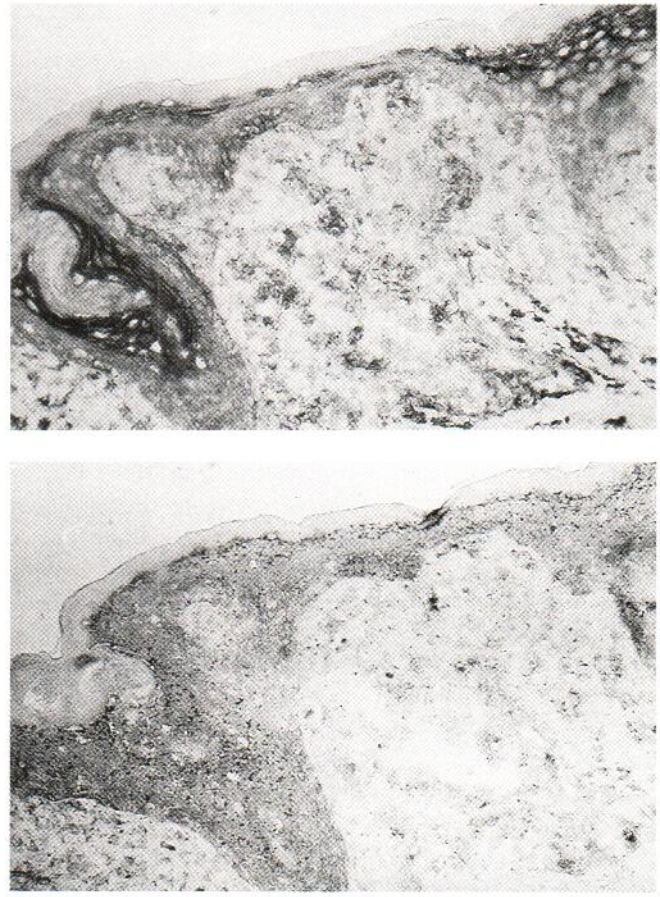

Fig. 2. (*a*) OKM5 (CD36)-positive membraneous staining of HKs in LP. Note OKM5-positive infiltrating cells as well (AEC × 150). (*b*) Absence of TSP1-positive HKs in LP (AEC × 150).

epidermal junction and at the base of the epithelial cells of sweat glands in healthy human skin (22). In contrast, with the MABs P10 and P12 we were able to demonstrate TSP1 only around the sweat glands (Fig. 1). The dermo-epidermal junction in all specimens obtained from healthy volunteers and from the lesional skin of patients with PV, LE, LP or CTCL remained negative.

Previous observations (9, 10, 12) detected a net-like intercellular CD36-positive pattern in the upper part of the stratum spinosum and granulosum in the involved skin of patients with PV, LE, LP or CTCL. In all cases investigated, the same CD36-positive epidermal region failed to give a specific reaction when the MABs against TSP1 P10 or P12 were used (Fig. 2a, b). However, in some samples darkly stained unspecific granules were occasionally seen around the nuclei of epithelial cells (Fig. 2b). Our data suggest that HKs of healthy individuals and those of involved and uninvolved skin in patients with cytokine-mediated dermatoses do not exhibit TSP1 in vivo.

The present study does not deal with the question of whether TSP2 and TSP3 or other TSP receptors are expressed in normal or diseased human skin. It is possible that a complex set of interactions between cell surface receptors and TSPs is required to fulfil their role in the skin. The absence of the co-expression of CD36 and TSP1 on HKs of the lesional skin in patients with cytokine-mediated dermatoses points to the possibility that the expression of CD36 might have some functional relevance, via oxidized low-density lipoprotein and/or collagen type fibrils (14, 15), without any connection with TSP1.

ACKNOWLEDGEMENTS

The excellent technical assistance of Miss A. Krause, Miss W. Leisgang and Mrs. B. Simon is gratefully acknowledged. This work was supported by grant Si 291/4–2 from the Deutsche Forschungsgemeinschaft.

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