Alterations of Glucosylceramide-β-Glucosidase Levels in the Skin of Patients with Psoriasis Vulgaris

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Hydrolysis of glucosylceramides by the enzyme glucosylceramide-β-glucosidase (GlcCer’ase) results in ceramide, a critical component of the intercellular lamellae that mediates the epidermal permeability barrier. A disturbance of ceramide formation is supposed to influence the transepidermal water loss in common skin diseases like atopic eczema or psoriasis. The aim of this study was to investigate whether GlcCer’ase levels were altered in the skin of subjects with psoriasis vulgaris. Skin punch biopsies were taken from lesional and non-lesional psoriatic skin and GlcCer’ase was evaluated both at the RNA and at the protein level. Normal skin from surgical patients provided the baseline GlcCer’ase expression in healthy subjects. Our results show that GlcCer’ase mRNA expression was decreased in psoriatic non-lesional skin compared to normal controls in all cases. Interestingly, in lesional psoriatic skin the level of GlcCer’ase was increased compared to non-lesional skin in all cases. For the immunohistochemical analysis, we used a newly synthesized monoclonal antibody anti-human GBC (GlcCer’ase-GST fusion protein). The results confirmed that GlcCer’ase, mainly present in the upper epidermis, was decreased in psoriatic skin compared to normal control and was increased in lesional compared to non-lesional psoriatic skin. Our findings support the concept that alteration in water permeability barrier in lesional psoriatic skin can serve as a trigger for the upregulation of the expression of enzymes like GlcCer’ase with consequent stimulation of ceramide generation.

Key words: epidermal lipids/glucosylceramide-β-glucosidase/psoriasis vulgaris

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The epidermal permeability to water and to water soluble material is mediated by a system of lamellar sheets of lipids localized in the intercellular space of the stratum corneum (SC) as described best by the “brick and mortar” model (Elias, 1983). These lamellar sheets, enriched with ceramides (Cer),1 cholesterol and free fatty acids, serve as a barrier against excess transepidermal water loss (TEWL), (Elias, 1983; Lampe et al, 1983a; Grubauer et al, 1989b). Especially Cer have shown to have a distinctive role in epidermal permeability barrier homeostasis (Hellman et al, 1991). SC Cer comprise an heterogeneous family of at least eight structural groups that differ from each other in the architecture of the polar sphingoid head group, in the length of the non-polar fatty acid chains and ω-hydroxylation of constituent amide-linked fatty acid (Wertz et al, 1985; Vietzke et al, 2001; Ponec et al, 2003). Cer[EOS] and Cer[EOH] containing linoleic acid ω-esterified to N-acyl fatty acids are key constituents of the extracellular lamellar membrane organization. In addition to this layer, easily removed by organic solvents, a portion of the ω-hydroxycacids pool forms a monolayer covalently bound to the cornified envelope, which can be analyzed after exhaustive extraction of free epidermal lipids and release through mild alkaline hydrolysis (Wertz et al, 1989; Behne et al, 2000). The resulting corneocyte lipid envelope is thought to serve as a scaffold upon which extracellular lamellar bilayer structures form in the SC, being of great relevance for epidermal barrier function (Downing, 1992).

In addition to a de novo sphingolipid synthesis, catalyzed by the enzyme serine palmitoyltransferase (Hollera et al, 1991), there are two main pathways for Cer generation. One is represented by the degradation of GlcCers, catalyzed by glucosylceramide-β-glucosidase (GlcCer’ase), and the other by the hydrolysis of sphingomyelin (SM) catalyzed by sphingomyelinase (SMase). Both the early activation of SMase and the later degradation of GlcCers are essential for skin barrier repair (Hollera et al, 1993, 1994a; Jensen

Abbreviations: AE, atopic eczema; Cer, ceramides; GBC, GlcCer’ase-GST fusion protein; GlcCer, glucosylceramide; GlcCer’ase, glucosylceramide-β-glucosidase; SC, stratum corneum; TEWL, transepidermal water loss

1The following Cer code used (Motta et al, 1993; Robson et al, 1994) contains: Cer EOS (or Cer 1): ester-linked fatty acids (E), ω-hydroxy fatty acids (O), and sphingosines (S). Cer NS (or Cer 2): non-hydroxy fatty acids (N) and sphingosines (S). Cer NP (or Cer 3): non-hydroxy fatty acids (N) and phytosphingosines (P). Cer EOH (or Cer 4): ester-linked fatty acids (E), ω-hydroxy fatty acids (O), and 6-hydroxy sphingosines (H). Cer AS (or Cer 5): ω-hydroxy fatty acids (A) and sphingosines (S). Cer AP (or Cer 6): ω-hydroxy fatty acids (A) and phytosphingosines (P). Cer AH (or Cer 7): ω-hydroxy fatty acids (A) and hydroxy sphingosines (H).
Epidermal sphingomyelins have been shown to be important precursors of Cer[NS] and Cer[AS], but other Cer species, including the ω-hydroxy fatty acid-containing Cer do not derive from sphingomyelins, but rather from GlcCers (Uchida et al., 2000; Hamanaka et al., 2002). GlcCer'ase enzyme activity has been described in the upper mest differentiated epidermal layers and accounts for the hydrolysis of acylGlcCers and GlcCers and the accumulation of Cer in the SC (Lampe et al., 1983b; Holleran et al., 1992; Takagi et al., 1999). The degradation of protein-bound GlcCer, the precursor of protein-bound Cer, by GlcCer'ase requires the presence of a cofactor, saposin C, and also of negatively charged lysosomal lipids (Doering et al., 1999). Deficiency of GlcCer'ase as genetically determined in Gaucher's disease (or induced in the Gaucher mouse model) or inhibition of its activity in the epidermis decreases the amount of SC Cers and creates an incompetent epidermal permeability barrier function (Holleran et al., 1993, 1994b; Doering et al., 1999), demonstrating the importance of the hydrolysis of GlcCer in skin barrier homeostasis. A significant increase in GlcCer'ase was demonstrated following skin barrier disruption and increased TEWL in hairless mice; furthermore, the addition of a GlcCer'ase inhibitor delayed permeability barrier recovery (Holleran et al., 1994a).

Alteration of Cer composition, including epidermal protein-bound Cer, has been reported in several skin diseases characterized by an abnormal SC and water permeability barrier function, like atopic eczema (AE) and psoriasis (Wertz et al., 1989; Melnik, 1991; Motta et al., 1993, 1994b; Bleck et al., 1999; Macheleidt et al., 2002).

There is evidence, that an abnormal expression of enzymes or cofactors involved in Cer biosynthesis or degradation may play a role in both AE and psoriasis (Murata et al., 1996; Cui et al., 1997; Alessandrini et al., 2001). In our previous study evaluating prosaposin (the precursor of the mature saposins, key cofactors for sphingolipid and glycosphingolipid degradation) and SMase levels in lesional and non-lesional skin of psoriatic patients, we found decreased levels of prosaposin in both lesional and non-lesional skin and of SMase in lesional skin of patients with psoriasis vulgaris (Alessandrini et al., 2001). In order to ascertain whether the alteration of skin barrier function seen in psoriatic disease could be also related to disturbances in the GlcCer'ase pathway of Cer generation, we asked whether the expression of GlcCer'ase would be compromised in the skin of patients with psoriasis vulgaris. We quantified the RT-PCR products and evaluated the protein expression of GlcCer'ase in normal skin and in lesional and non-lesional psoriatic epidermis.

Results

The mRNA expression of GlcCer'ase is decreased in psoriatic epidermis; its level is higher in lesional compared with non-lesional psoriatic skin The separation of the GlcCer'ase and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) RT-PCR products by gel electrophoresis and ethidium bromide staining showed a decrease in the mRNA expression of GlcCer'ase in psoriatic epidermis compared with normal control skin (Fig 1) as shown also by the semi-quantitative assessment of the GlcCer'ase PCR bands normalized to GAPDH (Fig 2). Although interindividual differences in GlcCer'ase mRNA expression were observed, the level of GlcCer'ase mRNA expression in psoriatic lesional skin was higher compared with non-lesional skin in all cases (Figs 1 and 2). Furthermore, the elevation of the mean GlcCer'ase mRNA expression in lesional skin was more pronounced than in non-lesional skin (Fig 2b). The results are consistent with the hypothesis that the decrease in GlcCer'ase expression is a primary event in the pathogenesis of psoriasis vulgaris.
expression in lesional compared with non-lesional psoriatic skin was statistically significant (Fig 2b; *p < 0.05).

Three samples obtained by simply scraping off scales from psoriatic lesions resulted in non-significant PCR bands (data not shown).

**Western immunoblotting** The separation of aliquots of human Cerezyme protein and purified GlcCer’ase-GST fusion protein by SDS-PAGE and binding to the monoclonal antibody GlcCer’ase-GST fusion protein (GBC)-1A5 (rat IgM, κ) showed one band at 58 kDa for human Cerezyme and one band at 61 kDa for the GlcCer’ase-GST fusion protein (Fig 3). The results were reproducible.

The protein level of GlcCer’ase is decreased in psoriatic skin and higher in psoriatic lesional versus non-lesional skin All samples investigated showed a very similar pattern of immunoreactivity. In control skin, GlcCer’ase protein was localized in the outer layers of human epidermis, the upper stratum granulosum, and SC (Fig 4a). In non-lesional psoriatic epidermis GlcCer’ase immunoreactivity was lower compared with control and localized only in few spots in the transition zone between granular layer and SC (Fig 4c). In lesional psoriatic epidermis GlcCer’ase immunoreactivity was increased compared to non-lesional, both in the higher stratum granulosum and in the lower SC (Fig 4d). The negative control showed no staining (Fig 4b) and the positive control always showed a typical stain for cytokeratin (data not shown).

**Discussion**

The results of this study show decreased levels of GlcCer’ase in the skin of subjects with *psoriasis vulgaris* compared with normal controls. Interestingly, lesional psoriatic skin showed an increased level of GlcCer’ase compared with non-lesional in all cases, both at the RNA and at the protein level.

For the immunohistochemical study a newly synthesized monoclonal antibody anti-human GBC was used. Its binding capacity was confirmed by western Immunoblotting and by immunohistochemistry. The results obtained by immunohistochemistry on normal control skin were in fact comparable to previous studies on the localization of GlcCer’ase activity and protein expression in human and mouse skin (Holleran et al, 1992; Takagi et al, 1999). In order to exclude the influence of the parakeratosis of the psoriatic SC in our analysis, we evaluated samples obtained by scraping off psoriatic lesions. We obtained no significant GAPDH and GlcCer’ase PCR bands, demonstrating that parakeratotic nuclei do not contain sufficient RNA to
influence the results. This is in accordance with the finding of solely condensed DNA and RNA in the parakeratotic cells in psoriasis, presumably without metabolic function (Suzuki et al, 1980).

Ceramide concentration in the epidermis depends on the rate of de novo Cer synthesis (Holleran et al, 1991) as well as on the availability of enzymes involved in Cer generation from pre-existing precursors (Holleran et al, 1993, 1994a; Jensen et al, 1999). Important signals for the upregulation of skin lipid enzymes occur upon permeability barrier requirements (Grubauer et al, 1989a; Holleran et al, 1994a; Harris et al, 1997; Stachowitz et al, 2002). Inhibition of sphingolipid synthesis produces a delay in barrier recovery in response to epidermal irritants, confirming the role of sphingolipids for the maintenance of the epidermal permeability barrier to water and water-soluble material (Grubauer et al, 1989a; Holleran et al, 1991).

Abnormal skin lipid profiles can be associated with deficient barrier function in common skin diseases like atopic eczema and psoriasis, both of which exhibit hyperproliferation, impaired epidermal differentiation, and barrier function (Melnik et al, 1991; Motta et al, 1994b). Most of the barrier lipids are unbound and thus free extractable with organic solvents (Vietzke et al, 2001). The human epidermis contains also covalently bound Cer and fatty acids, which are of critical importance in the formation of the cornocyte lipid envelope (Wertz et al, 1989). The alteration in covalently bound lipids was analyzed in lesional and non-lesional skin of AE subjects. The authors found a significant reduction in protein-bound ω-OH-Cer in both lesional and non-lesional epidermis, compared with healthy controls, accompanied by an increase of protein-bound ω-OH-FA (Macheleidt et al, 2002). Also psoriatic scales showed to differ from normal SC with augmented level of bound linoleic acid and alterations in the covalently bound lipids (Wertz et al, 1989). A detailed analysis of Cer composition of psoriatic scales revealed decreased linoleic acid-esterified Cer (Cer[EOS]), and Cer-containing phytosphingosine (Cer[NP] and Cer[AP]) compared with normal control skin, and increased Cer containing sphingosine (Cer[NS] and Cer[AS]), (Motta et al, 1993, 1994a). The authors speculated that the relative decrease in phytosphingosine-containing Cer and increase in sphingo-sine-containing Cer in the psoriatic scale could derive from deranged water bioavailability, caused by the excess water loss occurring in psoriatic scales, compared to normal SC. The decrease in Cer[EOS], which seems to have a critical function in the molecular arrangement of the SC lamellar lipid bilayer formation and assembly (Bouwstra et al, 1998), can cause alterations in the molecular organization of the SC in psoriasis (Motta et al, 1993; Ghadially et al, 1996).

Studies on patients with psoriasis vulgaris showed that TEWL and water content in non-lesional skin were very similar to normal control skin and only water retention capacity differed slightly from control (Berardesca et al, 1990). On the other hand, the increase in TEWL in lesional psoriatic epidermis has been well documented (Tagami and Yoshikuni, 1985; Serup and Blchmann, 1987). Also hydration by bound water in the psoriatic lesions appears to be inferior to control skin (Takenouchi et al, 1986). An ultrastructural analysis of the SC of psoriatic lesions has revealed extremely narrow intercellular spaces between a large number of parakeratotic cornocytes containing few pathological epidermal lipids lamellae (Fartasch, 1997).

An abnormal expression of enzymes or cofactors involved in Cer biosynthesis or degradation may play a role both in AE and in psoriasis (Murata et al, 1996; Cui et al, 1997; Alessandrini et al, 2001). According to two aforementioned studies, the level of prosaposin, the precursor of mature saposins, was decreased in both atopic and in psoriatic skin. Prosaposin-deficient mice, which lack the activator protein for GlcCer’ase, accumulate GlcCer in the SC and present abnormal lamellar lipid bilayers and thickened stratum lucidum with evidence of scaling (Doering et al, 1999). Also in the Gaucher mouse and in the GlcCer’ase inhibitor-treated models, the skin samples show abnormal lipid bilayers in the epidermis with loosely packed, unprocessed lamellar body-derived sheets, similarly to the Type II Gaucher neonate characterized by very low levels of GlcCer’ase (Holleran et al, 1994b). On the other hand, the importance of sphingomyelinase-dependent Cer production has been demonstrated both in a mouse model as well as in patients with Niemann–Pick disease following acute barrier disruption (Jensen et al, 1999; Schmuth et al, 2000). Therefore, for the recovery of barrier function following acute disruption and in skin diseases with disturbed water barrier, e.g., atopic eczema or psoriasis, both pathways of Cer generation may be required. Nevertheless, it was shown that Cer[EOS] are only produced by GlcCer’ase and not by sphingomyelinase (Uchida et al, 2000), therefore the pathway of Cer generation by GlcCer’ase seems critical. Galactocerebrosides appear to be potent stimulators of GlcCer’ase activity in the epidermis (Hara et al, 1998). A recent work performed by the same group showed that topical application of a compound, which mimics β-galactosylceramide and increases GlcCer’ase activity in keratinocytes, significantly reduced the TEWL, improving skin barrier function, in a UVB-induced barrier disruption mouse model (Fukunaga et al, 2003).

GlcCer’ase activity in atopic and aged dry skin was evaluated by analyzing SC sheets obtained by tape stripping. The authors found no difference in GlcCer’ase activity between non-lesional atopic epidermis/aged dry skin and control (Jin et al, 1994). We did not measure the enzyme activity, but we observed a clear decrease of GlcCer’ase mRNA and protein level in the skin of psoriatic subjects compared with normal controls. The decrease of GlcCer’-ase affects the postsecretory processing of GlcCer to Cer. Considering the studies performed by Berardesca et al (1990), this decrease should have no affect on the water retention capacity in psoriatic non-lesional skin. Interestingly, we observed an increase in GlcCer’ase level in lesional psoriatic epidermis compared to non-lesional. This increase suggests a compensatory capacity of psoriatic lesional skin to try and generate Cer in response, e.g., to water barrier requirements. It was shown that the epidermis of active plaque psoriasis generates a large number of lamellar bodies, which remain in the corneocyte cytosol and do not form functional intercellular bilayer structures (Ghadially et al, 1996). We can then speculate that the unbalanced generation of Cer species with different molecular structure and physical properties can affect the coherence of the SC in psoriasis patients.
Apart from the importance of Cer as structural component of SC lipids, there is an additional aspect in Cer function that should be taken into account. The balance of Cer/GlcCer appears to be critical for maintaining cellular homeostasis. Although Cer are involved in the regulation of keratinocyte proliferation and differentiation (Gelien et al, 1997), GlcCer accumulation by inhibition of GlcCer’ase stimulates epidermal proliferation in hairless mouse epidermis, resulting in epidermal hyperplasia (Marsh et al, 1995). Epidermal hyperplasia has been shown to be related to permeability barrier dysfunction (Proksch et al, 1993), which in turn has been suggested to induce the recruitment of inflammatory cells in the epidermis and contribute to the inflammatory status of this disorder (Nickoloff and Naidu, 1994). Alterations in the balance of GlcCer/Cer and permeability barrier requirements in psoriasis could therefore affect keratinocyte proliferation, SC desquamation and inflammation, all aspects being relevant in the pathogenesis of psoriasis.

Materials and Methods

Subjects and materials Fresh skin tissue biopsies were obtained from the lesional and non-lesional skin of five male subjects with psoriasis vulgaris (age ranging from 25 to 39 y). Two additional biopsies from the lesional skin of two psoriatic subjects were used for immunohistochemical analysis. The study was conducted according to the Declaration of Helsinki Principles. The ethical committee of the Technical University of Munich approved the study, and volunteers were enrolled in the study after providing written informed consent. Normal control skin (n = 5) was obtained from non-lesional areas of surgical tumor resections from non-inflammatory skin diseases (basalioma, keratoma). All specimens underwent a histopathological examination after routine hematoxylin & eosin (H&E) staining, which revealed that all samples were typical examples of “active plaque” type psoriasis.

All chemicals were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany), unless otherwise specified.

Isolation of mRNA The mRNA was isolated from the specimens obtained from both the lesional and non-lesional areas of five male subjects with psoriasis vulgaris and from three normal control subjects. Three additional samples were obtained from the same psoriatic subjects by simply scraping off the psoriatic lesions. RNA isolation was performed as previously described (Alessandrini et al, 2001).

Desoxyribonuclease (DNase) digestion To avoid DNA contamination, the mRNA was digested with 10U DNase (Roche Diagnostics GmbH) with the addition of 10 U RNAse Inhibitor (Roche Diagnostics GmbH, Petersberg, Germany) followed by phenol/chloroform extraction and ethanol precipitation.

RT-PCR Reverse transcription was performed as previously described (Alessandrini et al, 2001). For each experiment, primer concentrations, RNA amounts, and PCR cycles were titrated to establish standard conditions. The validity of quantitative comparisons of PCR products was insured by using a number of PCR cycles mapped to, within the linear curve. For PCR, 1–3 |L of the sample was incubated in 20 mM Tris-HCl, pH 8.4, 50 mM KCI, 1.5 mM MgCl2, 200 |L M NTP mix, and 0.2 pmol of GlcCer’ase and GAPDH primers (see below). The thermal profile used on a Hybaid Limited TouchDown Thermal Cycler (Hybaid, Middlesex, UK) consisted on denaturation at 94 °C for 45 s, annealing at 54 °C for 30 s and an extension temperature of 72°C for 1 min for 29 cycles. By optimized conditions, the PCR runs were repeated on the same RNA samples to assure the reproducibility of the data.

Oligonucleotides used for PCR The oligonucleotides used were chosen overlapping introns, to avoid the amplification of genomic DNA. GAPDH was used as internal control. GlcCer’ase (forward primer: 5’-ATGGCTCTGCTTGTGTC-3’; reverse primer: 5’-GCA-CAAGCCGATTGTC-3’), (GenBank, accession no. D-13286), and GAPDH (forward primer: 5’-GGTGAAAGGTCGGAGATTACAAGGA-3’, reverse primer: 5’-GAGGGATCTCGCCCTCGGAAGA-3’), (GenBank, accession no. M33197).

Quantification of RT-PCR—products and statistics PCR reactions were separated by gel electrophoresis on 2% agarose gels stained with 0.4 mg per mL ethidiumbromide. The gels were visualized over an on-line UV light source transilluminator (Gel Doc 1000 Video documentation System, Bio-Rad, Hercules, California). The PCR products of the expected size (392 bp for GlcCer’ase, and 430 bp for GAPDH) were then manually defined and the bands intensity was quantified using Quantity One quantitation software (Bio-Rad). The intensity of the PCR bands of GlcCer’ase were expressed normalized to GAPDH. For statistical analysis, a t test was used to compare GlcCer’ase mRNA expression (in % of GAPDH) of psoriatic non-lesional to lesional skin. A p value below 0.05 was considered to be significant.

Cloning of recombinant GlcCer’ase protein DNA coding for human GlcCer’ase (nucleotides 721–1422 of the human GlcCer’ase coding sequence, GenBank, accession no. D-13286) was amplified from human skin cDNA by PCR (forward primer: 5’-ATGAATTCCGGCCGGTGGAGACATCTACCACCAGACCTGG-3’; reverse primer: 5’-TATGCGAATCTAATGTTGATGATGCGCGCTTGGCCGACGCCAGCACCAGTATGAACACCTGG-3’). The PCR products were cloned into the EcoRI/XhoI sites of pGEX4BamHI (Leenders et al, 1996), a modified pGEX-2T vector (Amersham Biosciences) for the bacterial expression of GST-fusion protein (GBC).

GBC protein expression and purification The recombinant plasmid was transformed into Escherichia coli BL21 DE3 codon plus RIL (Stratagene). After bacteria were grown at 37 °C to an OD600 of approximately 0.8 in LB medium supplied with ampicillin (50 |g per mL), protein expression was induced by addition of IPTG (0.25 mM final). Bacteria were harvested after overnight induction by centrifugation, resuspended in PBS and lysed by four freeze–thaw cycles in the presence of lysozyme. After centrifugation for 15 min at 17,500 x g at 4 °C the supernatants were again centrifuged for 20 min at the same conditions. GST-fusion protein was bound from the supernatant to GT-sepharose (Pharmacia) and eluted by Tris HCl/glutathione after several washing steps as described (Leenders et al, 1996). The eluates were directly used for generation of antibodies, or SDS-PAGE.

Production of monoclonal antibodies against the C-term of GBC Lou/C rats were immunized subcutaneously and intraperitoneally with a mixture of GBC-GST fusion protein (50 |g), 5 nmol CPG oligonucleotide (ODN 2006, TIB Molbiol, Berlin, Germany), 500 |g PBS and 500 |L IFA. After a 4-wk interval a final boost without adjuvant was given 3 d before fusion of the rat spleen cells with the murine myeloma cell line P3X63-Ag8.653 (Kremmer et al, 1995). Hybridoma supernatants were tested in an ELISA using bacterial extracts from E. coli expressing either the GBC fusion protein or an irrelevant GST fusion protein. Monoclonal antibodies reacting with GlcCer’ase-GST fusion protein and not with an irrelevant GST fusion protein were analyzed by western immunoblotting.

Western immunoblotting For western immunoblotting both purified GlcCer’ase-GST fusion protein (GBC, 6 |g) and human Cerezyme protein (4 |g) were used. Cerezyme is a recombinant form of human GlcCer’ase used for medical purposes (e.g.: to replace GlcCer’ase in Gaucher disease patients) and was generously donated by Dr Paul (Genzyme Therapeutics, England). Aliquots of the two proteins were separated by SDS-PAGE (8% total acrylamide concentration). Molecular weight standards were from Bio-Rad (Munich, Germany). Proteins were transferred from gels to
PVDF membranes (Pall) by semi-dry blotting (Trans-Blot SD Semi-Dry Transfer Cell; Bio-Rad) according to the manufacturer’s protocol.

Non-specific binding of primary antibodies was reduced by pre-incubating the membranes after blotting with 5% skimmed milk powder in PBS for 30 min. A solution of monoclonal anti-GBC antibody 1:1000 in PBS/5% skimmed milk powder was incubated overnight at 4°C. After washing, the membranes were incubated for 2 h at room temperature with a peroxidase-labeled goat anti-rat IgG 1:100 in PBS/0.5% skimmed milk powder, Dianova). Peroxidase was detected using the ECL system (Amersham Biosciences, UK). By optimized conditions, the western Immunoblots were repeated to assure the reproducibility of the data.

**Immunohistochemistry**

For the immunohistochemical study, all biopsies taken from lesional and non-lesional skin of three psoriatic subjects (see above, subjects and materials), two additional biopsies from lesional skin of psoriatic subjects and normal control skin (n = 5) were snap frozen in liquid nitrogen for cryostat sectioning. Frozen tissue samples were submerged in Optimum Cutting Temperature embedding compound (OCT, Tissue-Tek, Miles Laboratory, Elkhart, Indiana) and cryostat sections (4 µm) were prepared on positively charged slides and allowed to air-dry. Tissue fixation was obtained with fresh 2% paraformaldehyde in phosphate-buffered saline (PBS), for 5 min, and methanol, for 10 min at 4°C. The tissue sections were further processed for indirect immunohistochemistry with an ABC-based method (Vector Laboratories, Burlingame, California) previously described (Alessandrini et al., 2001). Briefly, after blocking both endogenous biotin and peroxidases, the sections were incubated overnight at 4°C either with a monoclonal specific antibody anti-GBC (GBC-1A5, dilution factor 1:50, see Results, Western Immunoblotting), or a non-specific purified rat specific antibody anti-GBC (GBC-1A5, dilution factor 1:50, see Results, Western Immunoblotting), or a monoclonal anti-cytokeratin PAN antibody (10 isotype control (Vector Laboratories) or a monoclonal antibody 1:1000 in PBS/0.5% skimmed milk powder, Dianova). Peroxidase was detected using the ECL system (Amersham Biosciences, UK). By optimized conditions, the western Immunoblots were repeated to assure the reproducibility of the data.

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