Glycophenotype of Psoriatic Skin

(galectins / epidermis / psoriasis / glycobiology)

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Abstract: Psoriasis is considered an auto-immune disease with consequential keratinocyte hyperproliferation resulting in specific architecture of psoriatic skin. This process is associated with phenotypical keratinocyte changes including an altered carbohydrate expression pattern studied by labelled plant lectins. Expression of endogenous lectins and their reactive glycoligands are differentiation-dependent in squamous epithelia including epidermis. However, no data are available on psoriatic skin, although this disease represents an important medical problem. We investigated the expression of galectin-1, -3, -7 and the presence of their glycoligands in the psoriatic skin and compared the results with the normal skin samples. The results were correlated to expression patterns of cytokeratin 10 and cytokeratin peptide 37 as markers of keratinocyte differentiation as well as to the expression of proliferation marker Ki67. Contrary to normal epidermis, the psoriatic epithelium expressed no galectin-3 and no glycoligands for galectin-1. Strong expression of galectin-3/galectin-3-reactive glycoligands in capillaries of psoriatic dermis represents one of the most important findings demonstrating the activation of endothelium in the course of the disease. The keratin expression pattern was not affected in psoriatic skin compared with normal epidermis. In conclusion, the altered galectin expression and binding pattern in psoriatic skin indicates the modified process of keratinocyte maturation in hyperactivated psoriatic epithelium. The enhanced expression of galectin-3/galectin-3-reactive glycoligands in dermal capillaries of psoriatic skin can be important for rearrangement of the capillary network and migration of inflammatory cells to psoriatic skin.

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Abbreviations: gal-1 – galectin-1, gal-3 – galectin-3, gal-7 – galectin-7, GL – glycoligand, Kp37 – keratin peptide 37, K10 – keratin 10.

Introduction

Psoriasis is considered an auto-immune disease presumably triggered by an unknown antigen. The activation of antigen-presenting Langerhans cells leads to T-cell stimulation and the cascade of inflammatory changes resulting in cytokine release, activation and hyperproliferation of keratinocytes, dermal vascular expansion and increase of neuropeptergic nerve fibre density in the epidermis (Krueger et al., 2005). This process corresponds well with the major histopathological changes (epidermal hyperplasia, lymphocyte-rich perivascular infiltrate, elongated and prominent capillaries) and the clinical picture (well-defined red scaly plaques) of psoriatic lesions. Epidermal hyperproliferation results in the epidermal thickening and enlargement of the dermo-epidermal interface area (Krueger et al., 2005). In normal skin the proliferative compartment including stem and transit amplifying cells is confined to the basal layer. However, in psoriatic tissue the increase of cell density pushes the transit amplifying cells to the suprabasal layers (Iizuka et al., 2004). The keratinization process also differs in lesional skin, including expression morphological changes in keratohyaline granules, or loss of the granular layer (Krueger et al., 2005; Schön and Boehncke, 2005). Cell surface carbohydrate abnormalities were described in psoriatic epidermis as well using incorporation studies and plant lectins (King et al. 1981; Bell and Skerrow, 1985; Kariniemi and Virtanen, 1989).

Lectins are plant or animal sugar-binding proteins different from immunoglobulins and enzymes. Galectins are an animal lectin family with binding specificity for β-galactosides and polylactosamine chains. They perform a variety of functions in extracellular, cytoplasmic and nuclear localizations. The role of these

proteins in intercellular and cell-matrix interactions, cell migration, immunomodulation, cell growth and apoptosis was described. Galectin-1 (gal-1) and galectin-3 (gal-3) are also implicated in the pre-mRNA splicing. The prototype gal-1 and galectin-7 (gal-7) consist of a single carbohydrate recognition domain in gal-1 naturally non-covalently bonded into dimers, whereas gal-3 is a chimeric structure containing an N-terminus and a carbohydrate recognition domain in the C-terminus. Self-association of N-termini to multivalent lectin complexes increases gal-3 affinity to its glycoligands (Liu et al., 1996). The multivalent cross-linking of carbohydrates seems to be essential in many of galectin functions. Gal-1 and gal-3 distribution in mammals shows some tissue specificity and is developmentally regulated (Barondes et al., 1994; Gabius, 1997; Kaltner and Sierstorfer, 1998; Kaltner et al., 2002; Lahm et al., 2004; Smetana et al., 2006). Gal-7 was first identified in human epidermis and can be considered as a marker of all subtypes of keratinocytes (Magnaldo et al., 1998), where expression of this type of galectin seems to be connected with the process of epithelium stratification (Chovanec et al., 2005).

It is generally accepted that the distribution of endogenous lectins as well as carbohydrate residues as their ligands in the epidermis and other squamous epithelia under normal and pathologic conditions is linked to the process of differentiation and its alterations are connected to developmental, autoimmune or malignant changes of the skin (Gabius et al., 1990; Tsubura et al., 1992; Gillenwater et al., 1996; Plzák et al., 2001; Betka et al., 2003; Pasmatzi et al., 2005). Some studies used plant lectin histochemistry to confirm the modification of carbohydrate expression in psoriatic epidermis (Bell and Skerrow, 1985; Reano et al., 1982; Kariniemi, 1989). However, the data focusing on the family of galectins are still missing, although this knowledge may help understand the changes in psoriatic epidermis because of the high biological efficiency of galectins mentioned above.

Material and Methods

Tissue samples

Three samples of normal human skin were obtained from the Department of Aesthetic Surgery (Charles University, 3rd Faculty of Medicine). Nine samples of lesional psoriatic skin were obtained from the Department of Dermatovenerology (Charles University, 1st Faculty of Medicine) after informed consent of patients. All samples were cryoprotected by Tissue-Tek (Sakura, Zoeterwoude, the Netherlands) and frozen in liquid nitrogen. They were cut into 7-μm thin sections using Cryocut-E (Reichert-Jung, Vienna, Austria) and mounted onto the surface of poly-L-lysine (Sigma-Aldrich, Prague, Czech Republic) treated supporting glass.

Immunohistochemical and lectin analysis

Frozen specimens were fixed in 2 % (w/v) paraformaldehyde in PBS (pH 7.2). Non-specific binding of secondary antibody was blocked by preincubation with normal swine serum (DAKO, Brno, Czech Republic) diluted in PBS for 30 min. The double-labelling procedure at the single-cell level was performed for simultaneous lectin and immunohistochemical analysis of specimens. The cytokeratin profile of squamous epithelia was analysed using monoclonal antibody against keratin peptide 37 (Kp37) (basal keratinocytes) obtained from Sigma-Aldrich, and keratin 10 (K10) (differentiated keratinocytes) obtained from DAKO. The proliferation activity of keratinocytes was detected by Ki67 monoclonal antibody (DAKO). Gal-3 was detected by the A1D6 monoclonal antibody against gal-3 (Liu et al., 1996). Rabbit polyclonal antibodies tested for the absence of cross-reactivity against other galectins were used to detect gal-1 and -7 expression (André et al., 1999; Nagy et al., 2003). The galectin-reactive glycoligands were detected using biotinylated galectins-1, -3, -7 (Gabius, 2001) as probes in a concentration of 20 μg/ml in PBS (pH 7.2). In addition to the whole biotinylated molecule of gal-3, we also employed a truncated version of this galectin (Agrwall et al., 1993) enabling visualization of gal-3 and its glycoligands at the single-cell level (Smetana et al., 2006).

Swine anti-mouse immunoglobulins labelled by FITC (ALSEVA, Prague, Czech Republic) or goat anti-mouse immunoglobulins labelled by TRITC (Sigma-Aldrich) and ExtrAvidin-TRITC (Sigma-Aldrich) were employed for visualization of immunohistochemical and lectin histochemical reaction. Control of the specificity of immunohistochemical reaction was performed by replacements of the first step antibody by irrelevant antibody to exclude non-specific binding of the antibody, for example via Fc receptors. The omission of biotinylated galectin or competitive inhibition by lactose was employed to test the specificity of lectin histochemistry. Nuclei were counterstained by DAPI (Sigma-Aldrich). The specimens were mounted to Vectashield (Vector Laboratories, Burlingame, CA). The analysis of specimens and data storage were performed using an Optiphot-2 fluorescence microscope equipped by specific filterblock for FITC, TRITC and DAPI (Nikon, Prague, Czech Republic), by a CCD camera (COHU) and computer-assisted image analysis system LUCIA 3.2 (Laboratory Imaging, Prague, Czech Republic).

Results

The main results are summarized in the table (Table 1). No remarkable difference between normal and psoriatic skin was observerd in the expression of both studied keratins, i.e. Kp37 and K10. While Kp37 was observed only basally, K10 was expressed in suprabasal keratinocytes (Fig. 1). Proliferating keratinocytes exhibiting

Table 1. Phenotypic characteristic of normal and psoriatic epidermis

Marker	Normal epidermis			Psoriatic lesion		
	Basal	Suprabasal	Corneal	Basal	Suprabasal	Corneal
Keratin p37	+	–	–	+	–	–
Keratin 10	–	+	++	–	+	++
Ki67	+	–	–	+	– */+	–
Gal-1	–	–	–	–	–	–
Gal-1-reactive glycoligands	+	+	+	–	–	–
Gal-3	– */+	+*/++	+*/++	–	–	–
Gal-3-reactive glycoligands	–	+	+	–	– /+*	++
Gal-7	+	+	++	+	+	++
Gal-7-reactive glycoligands	–	–	–	–	–	–

– negative, + positive, ++ strongly positive, * prevailed expression

nuclear signal for Ki67 were located strictly basally in normal epidermis. The same pattern of proliferating cells was also detected in psoriatic skin, with the exception of sites where the suprabasal cells with Ki67-positive nuclei were also found (Fig. 1).

The cytoplasm of normal as well as psoriatic keratinocytes was positive for gal-7, where the maximal signal was detected in the corneal layer (Fig. 2). No expression of gal-1 was detected in both, i.e. normal and affected epidermal cells. The high signal for the presence of this type of galectin was observed in extracellular matrix of both normal and psoriatic dermis (Fig. 2). A strong difference between normal and psoriatic skin was observed for the expression of gal-3. This type of galectin is absent in psoriatic epidermis and expressed predominantly suprabasally in normal epithelium (Fig. 2). No signs of nuclear expression of the studied lectins were observed in normal and psoriatic skin.

To compare the expression of galectins with the expression of glycoligands for these galectins we detected these sites using labelled galectins as probes. No glycoligands for gal-1 were detected in the psoriatic

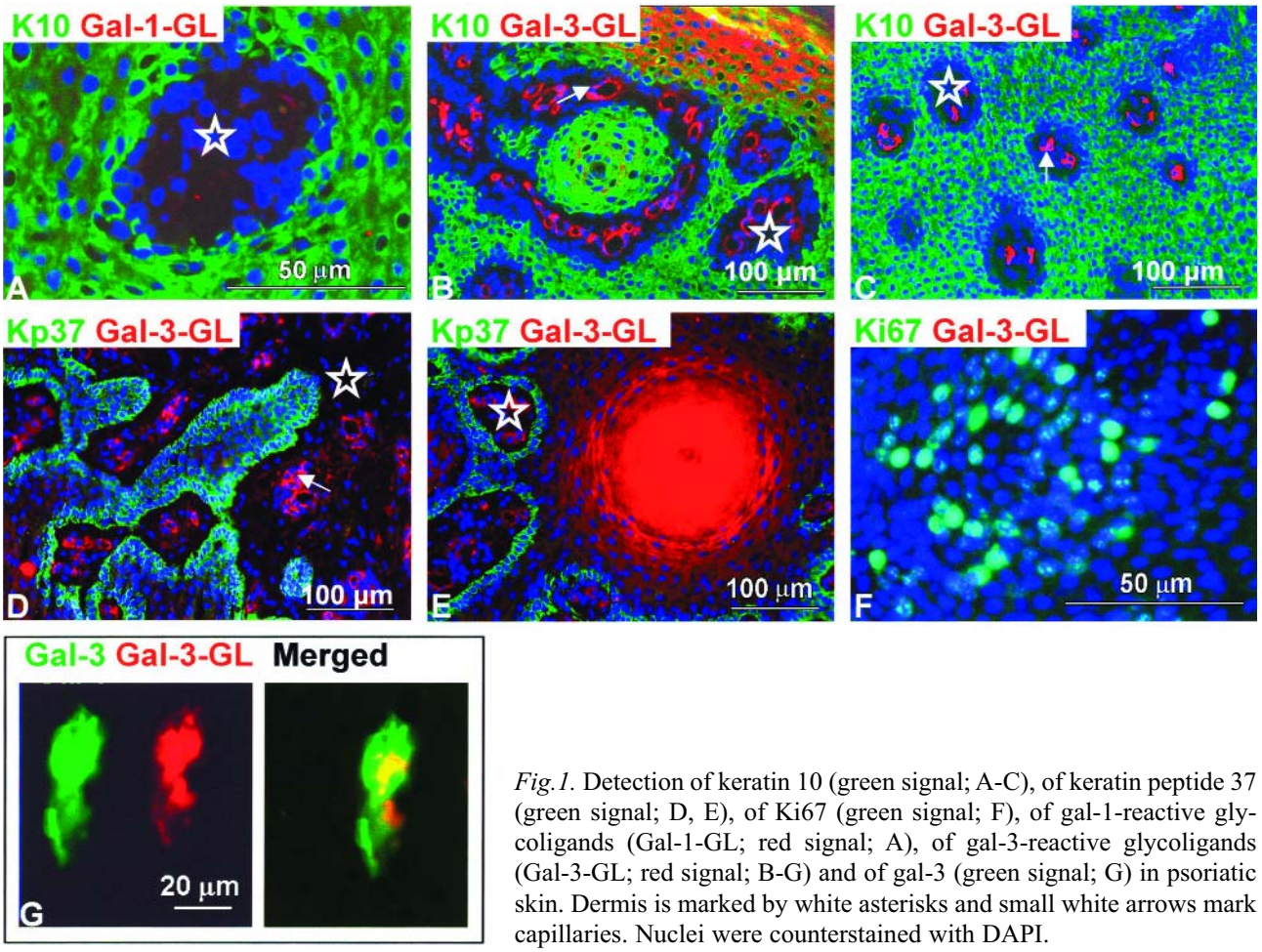

Fig.1. Detection of keratin 10 (green signal; A-C), of keratin peptide 37 (green signal; D, E), of Ki67 (green signal; F), of gal-1-reactive glycoligands (Gal-1-GL; red signal; A), of gal-3-reactive glycoligands (Gal-3-GL; red signal; B-G) and of gal-3 (green signal; G) in psoriatic skin. Dermis is marked by white asterisks and small white arrows mark capillaries. Nuclei were counterstained with DAPI.

epidermis (Fig. 1), which contrasted with binding of this galectin to the cytoplasm of keratinocytes of all layers of the normal epidermis. Gal-3-reactive glycoligands were localized mainly in the upper spinous and granular layer, with the strongest positivity of hyperkeratotic corneal layer in normal as well as in psoriatic skin (Fig. 1). Interestingly, a strong signal of gal-3 binding was revealed in the endothelia of dermal capillaries of 6/9 of psoriatic samples. Employment of a truncated version of gal-3 has also shown that these glycoligands in dermal capillaries are co-expressed with gal-3 itself (Fig. 1). No glycoligands for galectin-7 were observed in normal and psoriatic epithelium (not shown). The results of the expression of all studied markers in normal epidermis are not shown in the figures in this study because they were identical with the results published earlier.

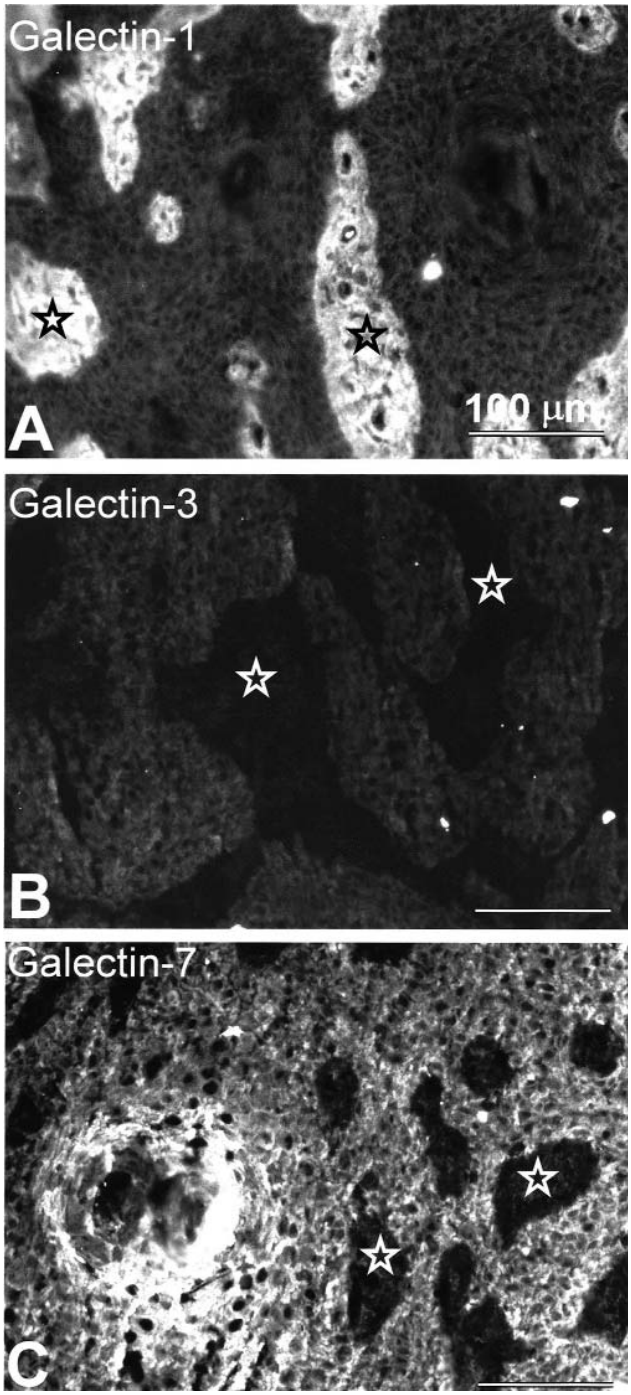

Fig.2. Galectin-1 (A), -3 (B) and -7 (C) expression in psoriatic skin. Dermis is marked by asterisks.

Discussion

No striking differences between normal and psoriatic epidermis were found in the expression of both types of keratins, where Kp37 was expressed basally and K10 suprabasally. It indicates that psoriatic epidermis has a normal differentiation pattern concerning the position of proliferating and terminally differentiated cells (Mommers et al., 2000). Suprabasal binding of gal-3 in psoriatic skin is in agreement with this observation and interpretation because in our previous studies we observed that expression of gal-3-reactive glycoligands is also connected with differentiation of squamous epithelia under normal conditions and in cancer (Plzák et al., 2001; Holíková et al., 2002; Plzák et al., 2004). Rare suprabasal occurrence of keratinocytes with Ki67-positive nuclei in psoriatic skin can be explained by the hyperproliferative character of this disease, but it is important that these cells were not recognized by galectin-3. It is in agreement with our previous observations (see Plzák et al., 2005) where proliferating cells of squamous epithelia and related tumours were not recognized by gal-3. In context of these data, it is not surprising that both the normal and psoriatic epidermis express gal-7, galectin typical for squamous epithelia whose expression is related to the programme of stratification of the epithelium that is not affected by the disease (Magnaldo et al., 1998; Chovanec et al., 2005). However, the psoriatic epidermis is not identical with normal epithelium from the point of view of galectin expression. The affected epithelium is devoid of gal-3 expression. This galectin was found in normal epithelium predominantly in suprabasal postproliferative layers (Konstantinov et al., 1994). Similarly, psoriatic keratinocytes were not recognized by gal-1, probe reactive for the cytoplasm of all layers of normal squamous epithelia (Holíková et al., 2002). The explanation of this difference is problematic, but it is possible that the differences in kinetics of cell proliferation in psoriatic epidermis is in the background of this feature. An important difference in galectin/galectin-reactive glycoligand expression between normal and psoriatic skin was found in dermal capillaries, where these strongly express gal-3 and gal-3-reactive glycoligands in contrast to normal epidermis. This phenomenon can be explained by changes in the dermal capillary network structure and function so characteristic for this disease (Creamer et al., 2002; Seely et al, 2003), where it is of note that gal-3 positively influences formation of capillaries under *in vitro* conditions (Nangia-Makker et al., 2000). Upregulation of endothelial gal-3 was observed

during activation of endothelium in the course of metastasizing (Glinsky et al., 2001; Glynskii et al., 2004), when galectin positively influenced adhesion of tumour cells to the endothelium. Therefore, the expression of gal-3 in the endothelium of psoriatic capillaries can be important for the dynamics of the disease, because it can influence both the changes of structure of dermal capillaries and migration of leukocytes from vessels to the dermis (Hughes, 2001; Sato et al., 2002), which is important for further progression of psoriasis.

In conclusion, differences between normal and psoriatic skin in galectin/galectin-reactive glycoligand expression in the epidermis and dermal capillaries were found. Further research is necessary to better understand their influence on the disease.

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