

The oligosaccharidic component of the glycoconjugates in lichen planus, granuloma annulare, seborrheic keratosis and palmoplantar keratoderma: lectin histochemical study

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Summary. It is well known that cell surface glycoconjugates play an important role in cell proliferation, adhesion and differentiation. The aim of this investigation was to define the changes of the glycoconjugate saccharidic moieties in the epidermis and derma of patients affected by several skin pathologies such as seborrheic keratosis, lichen planus, granuloma annulare and palmoplantaris keratoderma.

Bioptical specimens from skin lesions as well as from normal skin were fixed in Carnoy's fluid and routinely processed. The sections were treated with HRP-lectins (PNA, DBA, SBA, WGA, ConA, LTA and UEAI). Cytochemical controls were performed for specificity of lectin-sugar reaction. Some sections were pre-treated with neuraminidase prior to staining with HRP lectins. In comparison with normal human skin, epidermal lectin binding pattern in the considered diseases showed considerable qualitative and quantitative variations. In general, in all the considered pathologies, a lack and/or a decrease in lectin binding at the epidermal layers was observed; among the various diseases, differences in cellular localisation of the sugar residues were also noted. In such respect, an exception was represented by seborrheic keratosis, where the cells of the basal layer showed PNA reactivity, which was absent in the basal layer of the normal skin. Although seborrheic keratosis and lichen planus have been studied by others authors, our findings are not in total accordance concerning lectin binding; this is probably due to the different fixatives employed. Our findings seem to reveal significant changes in keratinocyte glycoconjugate oligosaccharides in the previously mentioned diseases, providing clues to their pathogenesis.

Key words: Lichen planus, Granuloma annulare, Seborrheic keratosis, Palmoplantar keratoderma, Lectin histochemistry

Introduction

The oligosaccharidic component of the glycoconjugates in lichen planus and seborrheic keratosis has been the subject of study by various authors (Prime et al., 1985; Toto and Nadimi, 1987; Gao et al., 1992). The results reported in the literature, however, are not always in agreement.

Other dermatological pathologies such as palmoplantar keratoderma and granuloma annulare, to our knowledge, have never been studied regarding the distribution of the sugar residues, especially those present at the cell surface, which play an important role in cell recognition and in cell-to-cell adhesion for determination and maintenance of normal epithelium (Gheri et al., 1993, 1994). Variations in these sugar residues are accompanied by pathological alterations in the epidermis and derma.

The aim of the present research was to examine the distribution of the oligosaccharidic component of the glycoconjugates in lichen planus, granuloma annulare, seborrheic keratosis and palmoplantar keratoderma.

Materials and methods

Tissue collection and preparation

Bioptical specimens of skin pathologies in numbers of 5 for lichen planus, 4 for granuloma annulare, 6 for seborrheic keratosis and 4 for palmoplantar keratoderma were obtained; punch biopsies of normal skin were also obtained from 4 normal healthy volunteers. All the patients gave a written declaration of informed consent to the study according to the human subjects institutional review committee guidelines. The specimens were fixed in several mixtures such as: 4% formaldehyde solution; buffered formaldehyde solution; Helly fluid; 6% mercury dichloride in a 1% sodium acetate solution containing 0.1% glutaraldehyde and Carnoy's fluid. The samples were processed routinely and 5 μ m-thick paraffin sections were obtained. Sections from tissue

fixed in buffered glutaraldehyde-mercuric chloride were treated with Lugol's solution prior to staining. Some specimens were stained with haematoxylin-eosin for general observations.

Lectin histochemistry

After hydration, sections were treated with 0.3% hydrogen peroxide for 10 min (to inhibit the endogenous peroxidase), rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1M phosphate-buffered saline (PBS) pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-conjugated lectin) dissolved in phosphate-buffered saline (0.1M PBS pH 7.2, 0.1M NaCl, 0.1mM CaCl₂, MgCl₂ and MnCl₂) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as follows: DBA (*Dolichos biflorus*, binding specificity α -D-GalNAc) 25 μ g/ml; PNA (*Arachis hypogea*, binding specificity D-Gal (β 1 \rightarrow 3)-D-GalNAc) 25 μ g/ml; SBA (*Glycine max* binding specificity α/β -D-GalNAc>D-Gal) 20 μ g/ml; WGA (*Triticum vulgare* binding specificity (α -D-GlcNAc)_n and sialic acid) 20 μ g/ml; ConA (*Canavalia ensiformis* binding specificity α -D-Man> α -D-Glc) 50 μ g/ml; LTA (*Lotus tetragonolobus* binding specificity α -L-Fuc) 25 μ g/ml; and UEA I (*Ulex europaeus* binding specificity α -L-Fuc) 25 μ g/ml. Staining of the sites containing bound lectin-HRP was obtained by incubating the slides with PBS (pH 7.0), containing 3,3'-diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water; dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount. Controls for lectin staining including: 1) substitution of unconjugated lectins for lectin-HRP conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; and 4) exposure of sections to 10-20 μ g/ml of each lectin-HRP conjugate containing 0.1M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-D-mannopyranoside.

Sialidase digestion

In some experiments, sialic acid was removed by pre-treating the sections for 18 hr at 37 °C in a solution of sodium acetate buffer 0.25M, pH 5.5, containing 0.1 unit/ml Sialidase (neuraminidase Type X from *Clostridium perfringens* (Sigma Chemical. Co., St. Louis, MO)), 5.0mM CaCl₂ and 154mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

Results

With the goal of finding the most suitable fixative, we performed preliminary tests with fixatives commonly used in lectin histochemistry (formaldehyde solution, buffered formaldehyde solution, Helly fluid, 6% mercury dichloride in a 1% sodium acetate solution containing 0.1% glutaraldehyde, Carnoy's fluid). In our experience the best fixative, concerning both the best preservation of sugars together with optimum preservation of the morphological aspects of the structure, was Carnoy's fluid and therefore the following results refer to samples fixed with Carnoy's fluid.

Distribution of the oligosaccharidic component of the glycoconjugates in normal human skin (Table 1)

PNA

Cytoplasm and cell surface of the cells of the lucidum layer and most superficial cells of the granular layer were strongly reactive with this lectin, while the cytoplasm of the cells of the spinous and basal layers showed weaker reactivity. The cell surface of the cells of the most internal portions of the granular, spinous and basal layers were strongly reactive, except along the dermal side of the basal layer (Fig.1).

SBA

Moderate reactivity of the horny layer and a strong reactivity of the cell surface and cytoplasm of the lucidum and granular layers were present at the epidermal level with this lectin. The surface of the cells of the spinous and of the basal layers was strongly reactive (Fig. 2).

DBA

The surface and the cytoplasm of some cells of the basal layer showed strong reactivity.

Table 1. Lectin reactivity in normal skin.

	LECTINS						
	PNA	SBA	DBA	WGA	Con A	LTA	UEA I
<i>Epidermis</i>							
Horny layer		++		++	++	++	+++
Lucidum layer	+++	+++		+++	+++	++	+++
Granular layer	+++	+++		+++	+++	++	+
Spinous layer	+++	+++		+++	+++	++	+
Basal layer	+++	+++	+++	+++	+++	++	+
<i>Dermis</i>							
Collagenous fibers	+			+++	++		+
Vessels		+++	++	+++	+++		+++

+: weak reactivity; ++: moderate reactivity; +++ strong reactivity.

Lectin study of some skin pathologies

WGA

Moderate reactivity was present at the horny layer whereas all other epidermal layers showed strong cytoplasmic reactivity. At the most superficial portion of the granular layer reactivity was also observed at the cell surface.

Con A

Moderate reactivity in the horny layer and strong reaction of the cytoplasm of the cells of all the other epidermal layers occurred with this lectin (Fig. 3).

LTA

With this lectin moderate cytoplasmic reaction was



Fig. 1. HRP-PNA. Normal skin. The cytoplasm and the surface of the cells of the lucidum and granular layers strongly react. Intense reactivity of the surface of the cells of the spinous and basal layers is observable. x 560

Table 2. Changes in lectin reactivity in patients affected by lichen planus with respect to the normal subject (compared with Table 1).

	LECTINS						
	PNA	SBA	DBA	WGA	Con A	LTA	UEA I
<i>Epidermis</i>							
Horny layer				↓↓	↓↓		
Lucidum layer	-	↓↓		↓↓	↓↓		
Granular layer	-	↓↓		↓↓	↓↓		
Spinous layer				↓↓	↓↓		
Basal layer		↓↓		↓↓	↓↓		
<i>Dermis</i>							
Collagenous fibers							
Vessels		↓↓					
Infiltrate							

-: loss of reactivity; ↓↓: marked decreased in reactivity

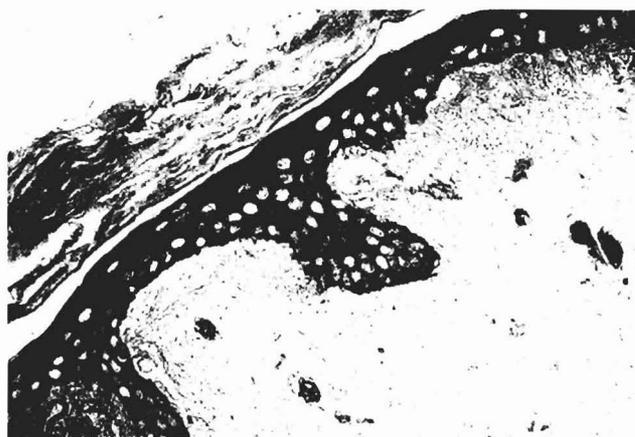


Fig. 2. HRP-SBA. Normal skin. Strong reactivity is observable at the cells of the lucidum, granular, spinous and basal layers. Within the derma the endothelium of the capillaries is strongly reactive. x 560

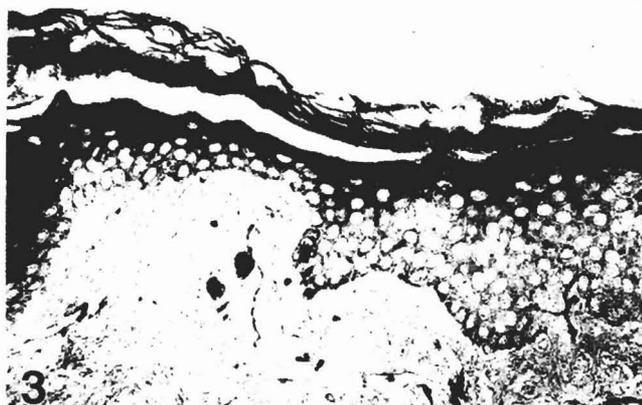


Fig. 3. HRP-Con A. Normal skin. The cells of all the epidermal layers are strongly reactive. Within the derma, the endothelium of the capillaries shows intense reactivity. x 560



Fig. 4. HRP-UEAI. Normal skin. Strong reactivity is observable at the horny, lucidum and most superficial part of the spinous layers. The cells of the other layers show weak reactivity. Within the derma the endothelium of the capillaries is reactive. x 560

present in all epidermal layers. Intense reactive cells, probably macrophages were seen in the derma.

UEA I

The cell surface and the cytoplasm of the cells of the horny and lucidum layers were strongly reactive whereas the surface and the cytoplasm of the cells of the granular, spinous and basal layers were weakly reactive (Fig. 4).

Variation in the oligosaccharidic component of the glycoconjugates in the skin of patients affected by lichen planus with respect to the normal skin (Table 2)

PNA

Loss of reactivity of the cell cytoplasm in the

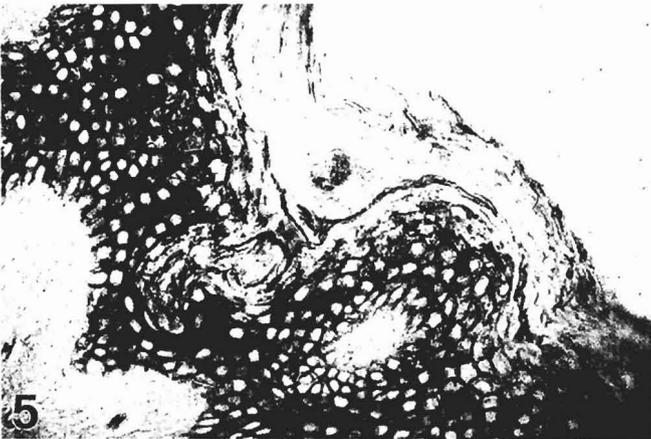


Fig. 5. HRP-PNA. Lichen Planus. In comparison with the normal skin, a loss of reactivity of the cytoplasm of the cells of the lucidum and granular layers is observable. x 560

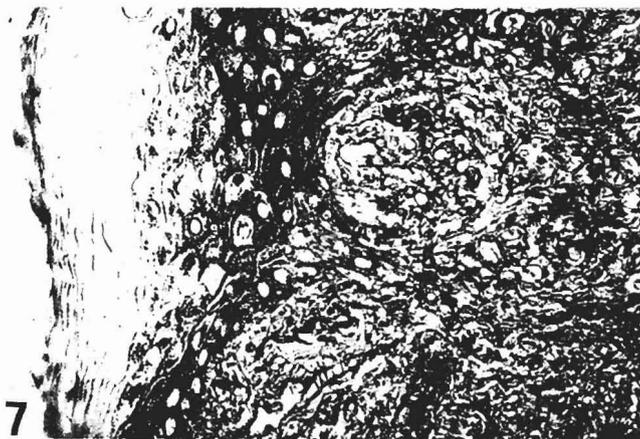


Fig. 7. HRP-ConA. Lichen Planus. Compared with the normal skin, a strong decrease in reactivity is observable in all the epithelial layers. x 560

lucidum and granular layers was observed compared to the normal skin (Fig. 5).

SBA

The cytoplasm and the surface of the cells of the lucidum, granular and basal layers showed a strong reduction in reactivity with this lectin. The basement membrane inconstantly reacted (Fig. 6).

WGA and ConA

A strong reduction in cytoplasmic and surface cell reactivity was seen in all the epidermal layers (Fig. 7).

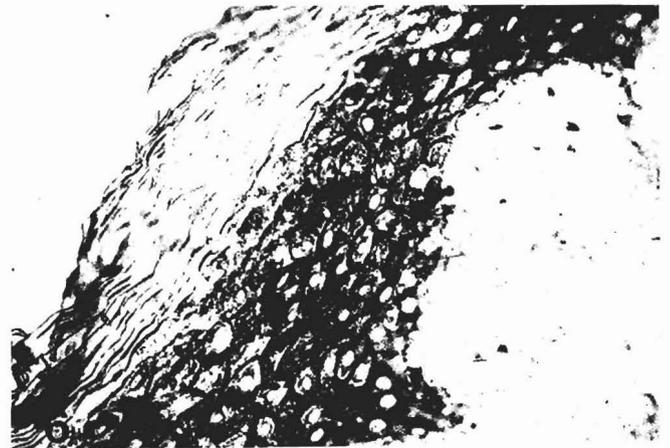


Fig. 6. HRP-SBA. Lichen Planus. In comparison with the normal skin the cytoplasm and the surface of the cells of the lucidum, granular and basal layers show a strong decrease in reactivity. Within the derma the reactivity of the endothelium of the capillaries is very weak. x 560

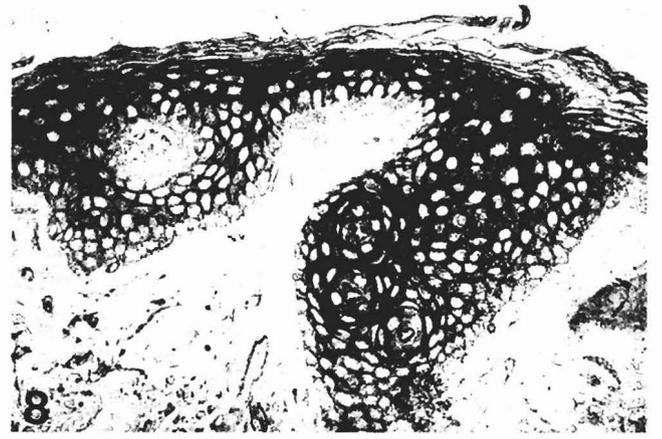


Fig. 8. HRP-SBA. Granuloma Annulare. In comparison to the normal skin, loss of reactivity is observable at the cytoplasm of the cells of the spinous and basal layers. Decrease in reactivity is also observable at the endothelium of the capillaries. x 560

Lectin study of some skin pathologies

Variation of the oligosaccharidic component of the glycoconjugates in the skin of patients affected by granuloma annulare with respect to the normal skin (Table 3)

SBA

Loss of reactivity of the cell cytoplasm in the spinous and basal layers was observed. In the basal layer reactivity was absent at the basal portion of the cell surface (Fig. 8).

WGA

A slight reduction in reactivity to this lectin was observed in all the epidermal layers.

Con A

A strong reduction in reactivity to this lectin was

evident in the cell cytoplasm of all epidermal layers. The cytoplasm of macrophages and lymphocytes was particularly reactive.

UEA I

The lucidum and granular layers and the most superficial portion of the spinous layer showed a strong reduction in reactivity of cell surface and cytoplasm.

Variation of the oligosaccharidic component of the glycoconjugates in the skin of patients affected by seborrheic keratosis with respect to the normal skin (Table 4)

PNA

The cytoplasm and the cell surface of the cells of the spinous layer showed a decreased reactivity. Furthermore, there was an inconstant reactivity of the basal

Table 3. Changes in lectin reactivity in patients affected by granuloma annulare with respect to the normal subject (compared with Table 1).

	LECTINS						
	PNA	SBA	DBA	WGA	Con A	LTA	UEA I
<i>Epidermis</i>							
Horny layer				↓	↓↓		
Lucidum layer				↓	↓↓		↓↓
Granular layer				↓	↓↓		↓↓
Spinous layer		-		↓	↓↓		↓↓
Basal layer		-		↓	↓↓		
<i>Dermis</i>							
Collagenous fibers				↓↓			
Vessels		↓↓		↓			
Infiltrate					+		

+: appearance of reactivity; -: loss of reactivity; ↓: moderate decrease in reactivity; ↓↓: marked decreased in reactivity

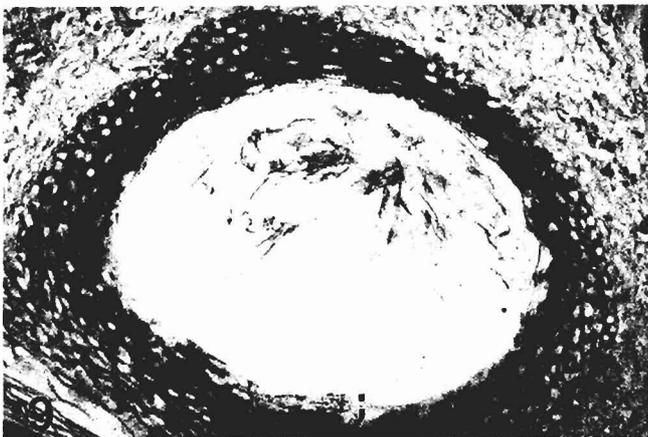


Fig. 9. HRP-SBA. Seborrheic Keratosis. In comparison with the normal skin, loss of reactivity of all the epidermal layers is evident. The pericyclic cells show prevalently cytoplasmic reactivity. x 560

Table 4. Changes in lectin reactivity in patients affected by seborrheic keratosis with respect to the normal subject (compared with Table 1).

	LECTINS						
	PNA	SBA	DBA	WGA	Con A	LTA	UEA I
<i>Epidermis</i>							
Horny layer		-		↓↓	↓↓		-
Lucidum layer	↓↓	-		↓↓	↓↓		-
Granular layer	↓↓	-		↓↓	↓↓		-
Spinous layer	↓↓	-		↓↓	↓↓		-
Basal layer	+	-		↓↓	↓↓		
<i>Dermis</i>							
Collagenous fibers							
Vessels							
Pericyclic cells	+	+					

+: appearance of reactivity; -: loss of reactivity; ↓↓: marked decreased in reactivity

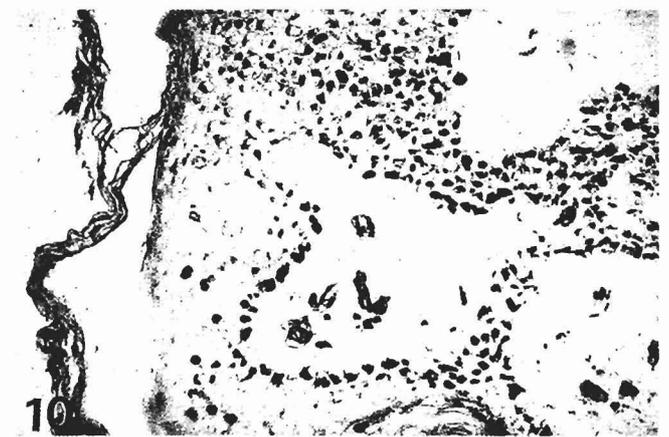


Fig. 10. HRP-UEA I. Seborrheic Keratosis. All the epidermal layers, compared to the normal skin, show a strong decrease in reactivity. Numerous melanocytes are seen. x 560

surface of the cells of the basal layer, which was completely absent in the skin of the healthy subjects. Cells surrounding cystic formations were reactive.

SBA

A loss of reactivity in all epidermal layers was observed. The cells surrounding the cystic formations showed cytoplasmic reactivity (Fig. 9).

WGA, ConA and UEAI

All the epidermal layers showed reduced reactivity to these lectins. The cytoplasm of the cells of the basal layer reacted with ConA (Fig. 10).

Variation of the oligosaccharidic component of the glycoconjugates in skin of patients affected by palmoplantar keratoderma with respect to the normal skin (Table 5)

PNA and SBA

A strong reduction in cytoplasmic and cell surface reactivity was present in all the layers. (Fig. 11).

Con A

A marked reduction in cytoplasmic reactivity in all the layers, and especially in the lucidum and granular ones, was seen. In the granular layer a marked reactivity of the cell surface appeared (Fig. 12).

Cytochemical controls

When the sections were stained with lectins in the presence of the haptenic sugars pertinent to each lectin, the above-described positive reactions completely

disappeared or were strongly reduced. No staining was evident in the sections which had been exposed to substrate medium only or to the unconjugated lectins. Pre-treatment of sections with periodic acid abolished the affinity of the histological sites to the lectins.

Discussion

From an analysis of the scarce literature on the pathologies considered in the present investigation it was not possible to reach a consistent interpretation of the variations in lectin reactivity in comparison to that of the normal skin.

If we compare our results with those of Gao et al. (1992) regarding seborrheic keratosis, some differences in reactivity of some lectins in some epidermal sites emerge. In certain cases the authors describe not well

Table 5. Changes in lectin reactivity in patients affected by keratosis palmaris et plantaris with respect to the normal subject (compared with Table 1).

	LECTINS					
	PNA	SBA	DBA	WGA	Con A	LTA UEA I
<i>Epidermis</i>						
Horny layer		↓↓			↓↓	
Lucidum layer	↓↓	↓↓			↓↓, +	
Granular layer	↓↓	↓↓			↓↓, +	
Spinous layer	↓↓	↓↓			↓↓	
Basal layer	↓↓	↓↓			↓↓	
<i>Dermis</i>						
Collagenous fibers						
Vessels						
Infiltrate						

+: appearance of reactivity at the cell surface; ↓↓: marked decreased in cytoplasmic reactivity



Fig. 11. HRP-PNA. Palmoplantar Keratoderma. In comparison with the normal skin, a strong decrease in reactivity at the cytoplasm of the cells of all the epidermal layers is shown. x 560

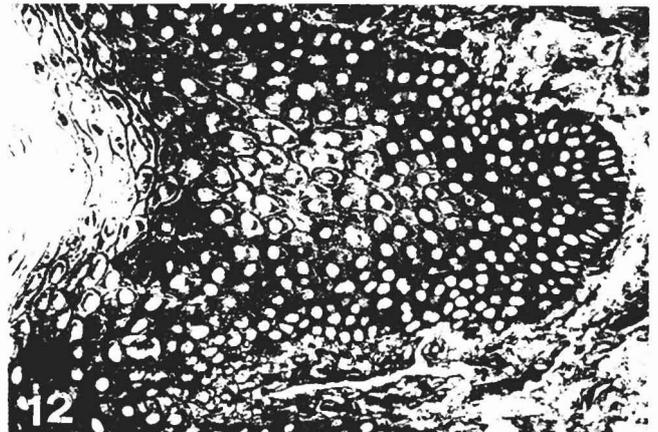


Fig. 12. HRP-ConA. Palmoplantar Keratoderma. In comparison with the normal skin, the cytoplasm of the cells of all the epidermal layers, and in particular that of the cells of the lucidum and granular ones, shows a strong decrease in reactivity. At the granular layer, appearance of reactivity at the cell surface is observable. x 560

identified "regions" showing weak reactivity with Con A, PNA, SBA and WGA lectins.

In the above-mentioned pathology, our results mainly indicate a uniform reactivity, when present, with the lectins used. In particular, in our patients, the basal layer cells were characterised by the presence of D-galactose ($\beta 1 \rightarrow 3$)-N-acetyl-D-galactosamine, reactive to PNA.

The different fixative fluid we used could explain the differences between our observations and those of Gao et al. (1992). As mentioned above, our group tested several types of fixative with Carnoy's fluid yielding the best morphological preservation of the tissues and of the saccharidic component. This is demonstrated by the good results obtained on embryonic fragments, including skin (Gheri et al., 1997), even taking into account the fragility of the samples and the scarcity of the oligosaccharidic components that it is possible to identify in them (Gheri et al., 1990, 1991, 1992).

Furthermore, in seborrheic keratosis, in comparison with the normal skin, we noted a loss of reactivity with UEA I, which reacted with terminal sugar residues of α -L-fucose.

In acanthotic seborrheic keratosis, cystic formations were constantly seen and characterised from a histopathological point of view (Lever and Schaumburg-Lever 1990). In this epidermal pathology there is a notable difference in the distribution of sugar residues compared to normal subjects. One interesting finding is that the oligosaccharidic material in the cells surrounding the cystic formations was similar to that found in normal epidermis. Since horny material is evident inside the cysts, it is possible to hypothesise a return to an apparently normal evolution of the epithelial cells which surround these neoformations.

Although Gao et al. (1992) observed no reactivity in the basal layer of the lichen planus with DBA, we always noted the presence of α/β -N-acetyl-galactosamine, evidenced by this lectin in the basal layer, as usually observed in the skin of normal subjects.

The same authors stated that SBA and WGA reactivity at the cytoplasmic granules of epidermal cells was a characteristic feature of lichen planus, whereas we noticed reduced reactivity of these lectins both in the cytoplasm and on the cell surface in all epidermal layers. There was also a loss of D-galactose ($\beta 1 \rightarrow 3$)-N-acetyl-D-galactosamine, evidenced with PNA, at the cytoplasmic level of the cells of the lucidum and granular layers and marked reduction in α -D-mannose, evidenced with Con A, in all epidermal layers.

Granuloma annulare is characterised by focal collagen degeneration with inflammation and reactive fibrosis (Lever and Schaumburg-Lever 1990). The affected areas are characterised by large zones of complete degeneration and by more restricted areas of incomplete degeneration comprised of foci in which some collagen fibres appear normal while others are in various degrees of degeneration. However, both types of histopathological alterations are usually present. The

degenerated zones appear to be surrounded by inflammatory cells and an infiltrate containing lymphoid cells and fibroblasts. In the cases we examined, focal degeneration was rarely evident. On the other hand, it is commonly accepted that in granuloma annulare the epidermis displays, from a histopathological point of view, fully normal characteristics, at least with the usual staining methods (Lever and Schaumburg-Lever 1990).

Regarding the distribution of sugar residues of glycoconjugates, we were able to observe in granuloma annulare significant differences in comparison with the normal epidermis. These differences were evidenced by a loss of α/β -N-acetyl-D-galactosamine at the cytoplasm of the cells of the spinous and basal layers, and in reduced α -D-glucosamine cytoplasmic and cell surface reactivity of the cells of all epidermal layers. There was also reduced D-mannose detection at the cell cytoplasm of all epidermal layers and of α -L-fucose at the surface and cytoplasm of cells in the lucidum, granular and most superficial part of the spinous layers.

The loss in reactivity with SBA at the basal portion of cells of the basal layer (possibly representing the basement membrane) should be emphasised. The absence of α/β -N-acetyl-D-galactosamine in this site could reflect an alteration in the exchanges of nutritive material, diffusing from underlying dermis to the epidermis.

The decrease in oligosaccharidic material in granuloma annulare, particularly at the surface of the cells, associated with nuclear pyknosis, being the nuclear material reactive with WGA and Con A, could indicate heavy damage at the epidermis. Our histochemical observations indicate significant modifications in the epidermis, besides the well-known modifications of the derma.

In palmoplantar keratoderma there is marked decrease in α -D-mannose, D-galactose ($\beta \rightarrow 3$)-N-acetyl-D-galactosamine and of α/β -N-acetyl-D-galactosamine, respectively reacting with Con A, PNA and SBA in all epidermal layers, and especially in lucidum and the most superficial granular layer cells. The appearance of Con A reactivity at the surface of the cells of the lucidum and granular layers is particularly significant, indicating the acquisition or loss of cell-to-cell recognition potential, of adhesion or of abnormal alteration in the exchange of information among epithelial cells. On the other hand is well known that sugar residues on the cell surface, as are present in epithelia during embryonic morphogenesis (Gheri et al., 1993, 1994), carry out an important function in cell-to-cell recognition, in their adhesion and building of epithelial structures.

It should be remembered that the appearance of reactivity in the above-mentioned sites of palmoplantar keratoderma could indicate a significant alteration in the epidermis, thus serving as a marker for this pathology.

Apart from our histochemical observations of abnormal alterations in the oligosaccharidic component, we noted that for each pathology, a standard experimental protocol should be established to find out

the best fixative fluid to be used. In our opinion this is the only way that allows a comparison among the results of different research groups.

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