Lectin-binding Sites in Squamous Cell Carcinomas and Kerato-acanthomas

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The lectin-binding pattern of malignant and benign human epidermal tumours was studied. Twelve squamous cell carcinomas and ten kerato-acanthomas were investigated, using fluorochrome-conjugated lectins. Lectins used were *Canavalia ensiformis* agglutinin (Con-A), *Helix pomatia* agglutinin (HPA), *Phaseolus vulgaris* agglutinin (PHA-L), and *Ricinus communis* agglutinin (RCA-120). Two types of fluorescence pattern, Intercellular 'Pemphigus-like' and Peritumoral, were noted. Both allowed classification of these tumours on the basis of their lectin-binding reactivity. Moreover, the expression of receptors for these lectins made it possible to distinguish between low-grade and well-differentiated squamous cell carcinomas. *Key words: Epidermal tumours; Glycoconjugates.* (Received November 12, 1987.)

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Kerato-acanthomas (KA) are benign keratinocyte tumours, whereas squamous carcinomas (SCC) are concers capable of metastasizing (1). The clinical aspects of both types of neoplasia are often comparable, and diagnosis requires histological examination. However, SCC can masquerade as kerato-acanthomas, not only clinically but also histologically (1).

Cell surface glycoconjugates are believed to play an important role in the collective behaviour of cells (2) and are widely involved in cell differentiation.

Lectins have been chosen because they are able to bind to cell-associated glycoconjugates containing specific carbohydrate subunits (3). By using such an approach, the presence of oligosaccharides in the membrane of the keratinocyte has been studied in normal skin (4–6), in inflammatory dermatoses such as psoriasis (7, 8), lichen planus (9), pemphigus vulgaris (10), in some epidermal tumours such as basal cell carcinomas (11), in Paget's disease (12) and in malignant melanomas (13).

These investigations have demonstrated that the distribution of receptors for lectins could be of use as a marker in the study of epidermal differentiation under normal and pathological conditions.

The purpose of this study was to investigate the distribution of lectin-binding sites in SCC at different stages of malignancy and to compare binding characteristics with those obtained in KAs that could be used to distinguish between malignant and benign skin tumours.

MATERIAL AND METHODS

Skin samples

Twelve cases of squamous cell carcinomas located on the lip, ear, or back of the hand, and 10 cases of identically situated kerato-acanthomas were studied.

Biopsy specimens were immediately divided into two parts, one snap-frozen for fluorescence examination, and the other fixed in 10% formalin according to conventional histopathological techniques for routine histology.

Lectins

Fluorescein isothiocyanate (FITC) or rhodamine isothiocyanate (RITC) conjugated lectins were employed. *Canavalia ensiformis* (CON-A) specific to α-D-mannosyl and α-D-glucosyl oligosaccharide; *Phaseolus vulgaris* (PHA-L) specific to *N*-acetyl-D-galactosaminyl oligosaccharide; *Helix pomatia* (HPA) specific to α-*N*-acetyl-galactosaminyl oligosaccharide; and *Ricinus communis* 120 (RCA-120) specific to β-D-galactosyl oligosaccharide, were purchased as fluorochrome conjugates from Sigma Chemical Company, St. Louis, Mo. USA.

Each conjugate was diluted to 15–50 μg protein/ml with phosphate-buffered solution (PBS) (Bio-Merieux, France) to pH 7.2. CON-A was diluted with 0.05 M Tris, 0.01 M $CaCl_2$, 0.01 M $MnCl_2$ and 0.15 M NaCl, to pH 8.

Fluorescence microscopy

Samples were set in O.C.T. freezing compound (Lab. Tek., Miles Lab, Naperville, Ill., USA), exposing a cross-section of skin. Four-micron sections were cut on a cryostat at −25°C, mounted on glass slides and allowed to dry in air. The sections were fixed in acetone at −20°C for 20 min, then flooded with the appropriate fluorochrome–lectin conjugate for 45 min at room temperature. They were then washed three times for a total of 30 min and mounted under coverslips in a modified polyvinyl alcohol medium.

In order to obtain meaningful results, the activity of each lectin preparation was checked against normal human epidermis and was found to be identical with that previously described (4).

A competition method was used for controls. Each fluorochrome–lectin conjugate was preincubated in 0.2 mol/l of its specific sugar: 1-0-methyl-D-glucose with (CON-A), *N*-acetyl-galactosamine with (HPA and PHA-L) and D-galactose with (RCA-120) for 2 h at 4°C before use. Slides were viewed under a Nikon Labophot fluorescence microscope.

Conventional histology studies performed in parallel established the diagnosis in each case and provided a graded classification according to the criteria given published by Lever & Schaumburg-Lever (1).

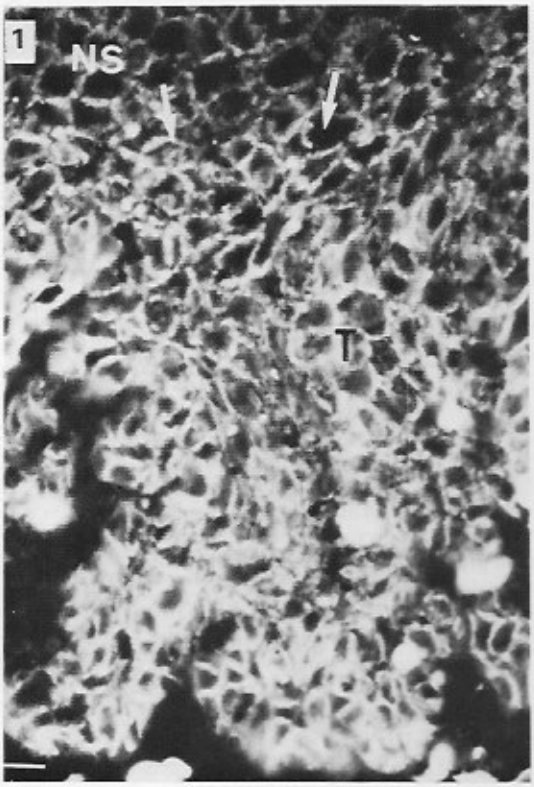
RESULTS

The results of the fluorescence experiments were both invariably concordant and highly reproducible. Specificity controls were consistently negative. The results of the normal skin and the tumours are summarized in Table I.

Table I. *Lectin binding pattern in normal skin and tumours*

Cells	CON-A	HPA	PHA-L	RCA-120
Normal skin				
Basal cells	++	−	+	+++
Spinous cells	++	++	++	++
Granular cells	++	+++	++	+
B.M.Z.	−	−	+	++
Tumour cells				
SCC I	ICS ++	ICS +	ICS +/++	PT +
SCC II	PT +	ICS +	ICS +	PT +
SCC III	PT +	N	ICS +	PT +
KA	ICS ++	ICS++	ICS ++	ICS +

B.M.Z., basal membrane zone; SCC I, squamous cell carcinomas, grade I; SCC II, Squamous cell carcinomas, grade II; SCC III, Squamous cell carcinomas, grade III; KA, kerato-acanthomas; ICS, intercellular staining; PT, peritumoral pattern.
Intensity of fluorescence = None: −; Low: +; Mild: ++; Intense: +++. N,—Neither ICS nor PT was observed.



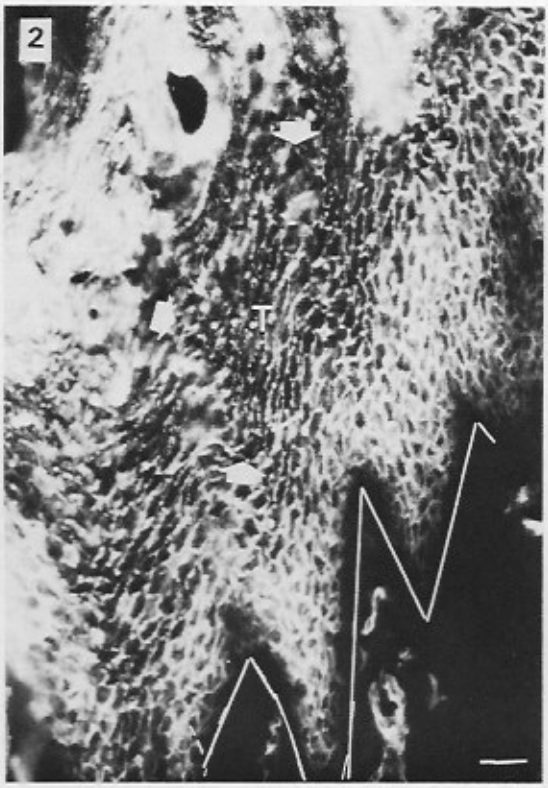


Fig. 1. Squamous cell carcinoma grade I reacted with Con-A. *NS*, Normal skin; *T*, tumour. *Arrows*, Margins of tumour.

Fig. 2. Kerato-acanthoma reacted with HPA. *T*, tumour; *Arrows*, Margins of tumour. Dermo-epidermal junction.

Two staining patterns were encountered: a 'Pemphigus-like' intercellular staining (ICS) and a 'peritumoral' (PT) pattern.

CON-A

This lectin reacts selectively with α-D-mannosyl/α-D-glucosyl residues.

In grade I SCC, a strong intercellular staining of epithelial cells was observed; no peritumoral pattern was found (Fig. 1).

In grades II and III SCC, no intercellular labelling could be observed, though a peritumoral pattern was seen as a broad band of varying width, with occasional discontinuities.

Kerato-acanthomas showed an intense intercellular staining without reaction at the tumour periphery.

HPA

This lectin reacts specifically with α-*N*-acetyl-galactosaminyl oligosaccharide.

In grade I SCC the surfaces of tumoral cells expressed a ICS pattern, without peritumoral staining.

In grade II SCC the cell membranes exhibited an ICS pattern, peritumoral labelling being absent.

In grade III SCC, neither labelling pattern—ICS or PT—was observed.

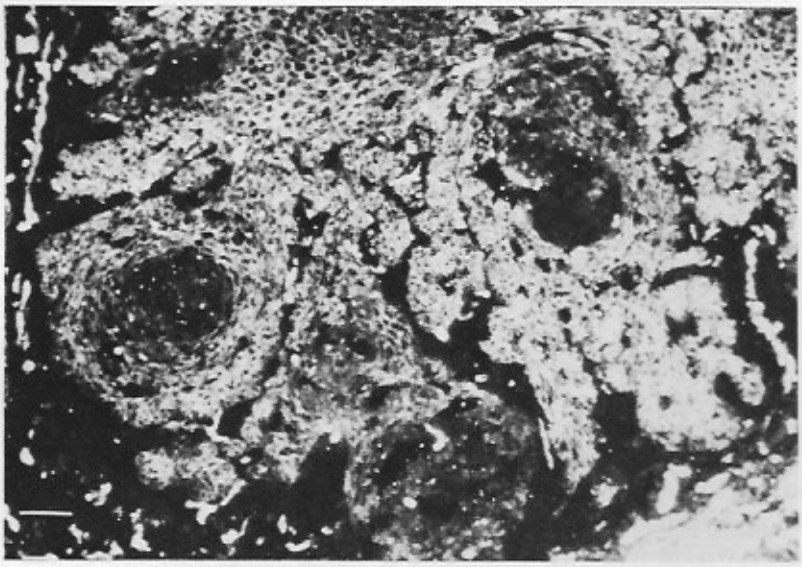


Fig. 3. Squamous cell carcinoma grade II reacted with PHA-L. Intercellular pattern in tumour, does not observe peritumoral pattern.

Kerato-acanthomas exhibited an ICS pattern similar to SCC but of greater intensity. Cells attached to the basement membrane were negative, as were basal cells in normal epidermis. No PT pattern was seen (Fig. 2).

PHA-L

This lectin reacts selectively with *N*-acetyl-D-galactosaminyl residues.

In grade I SCC, the surface of proliferative keratinocytes expressed an ICS pattern.

In grades II and III, SCC expressed the same labelling as in grade I though with weaker intensity (Fig. 3).

The staining of KA was identical with that obtained for grade I SCC.

No PT pattern was observed in any of the SCC or KA studied.

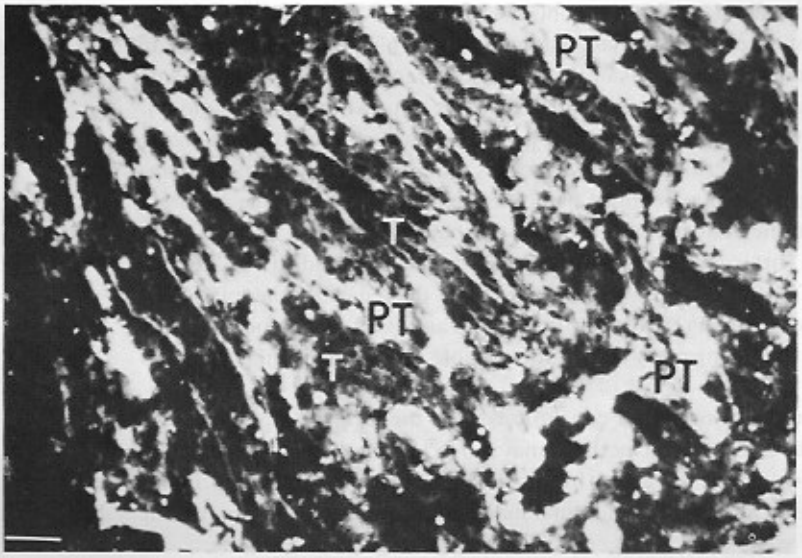


Fig. 4. Squamous cell carcinoma grade III reacted with RCA-120. *T*, tumour cells; *PT*, peritumoral pattern.

RCA-120

This lectin reacts with the sugar β-D-galactosyl.

The epithelial cells of low grade or well-differentiated (grades I, II and III) SCC did not express this oligosaccharide. Staining occurred at the tumour margins in all SCC studied (Fig. 4). By contrast, in KA, epithelial cell surfaces were stained by RCA-120, but no labelling was seen at the tumour margins.

DISCUSSION

The distribution of receptors for lectins in normal human epidermis is well documented. The lectins CON-A, PHA-L and RCA-120 label the whole epidermis, whereas HPA identifies only its specific saccharide at the surface of suprabasal cells (4, 6).

Malignant transformation affects the cell membrane and associated cell coat. A main feature in SCC was the dramatic loss in binding of RCA-120 at the surface of epithelial cells in all SCC studied—even in the low-grade tumours (grade I); this loss had previously been reported in oral epithelial tumours (14). The reduced reaction for terminal D-galactopyranosyl residues therefore appears to occur in the first phases of malignant evolution.

However, the lack of staining of lectins in cross tissue sections may have several explanations, such as the absence of surface receptors due to incomplete synthesis, their enzymatic digestion, or their masking by other surface components (14). We can only speculate that the inflammatory infiltrate may be responsible for a putative enzymatic digestion of the glycocalix.

A staining of the basement membrane zone and of the stroma surrounding the tumours indicates alterations in the normal appearance of glycoconjugates, Fibronectin, a glycoprotein containing mannose and -D-galactose residues, is involved in wound repair, inflammatory phenomena and tumoral processes (15), although some doubt whether fibronectin is synthesized by keratinocytes, or by fibroblasts or derives from the plasma. Moreover, collagen degradation occurs in these areas (11). This may partly account for the peritumoral staining of SCC with CON-A and RCA-120.

In contrast, KA demonstrated a lectin-binding profile similar to that found in the normal epidermis. In addition, no peritumoral staining was noticed when using RCA-120 and CON-A.

These findings indicate that, in contrast to malignant tumours, benign tumours retain their ability to express most of the glycoconjugates of the normal epidermis. Previous data point to the absence of intercellular glycoproteic antigens in SCC, in contrast to benign tumours, where they are unaffected (16). Such glycoconjugates probably represent target components in the rejection mechanisms of epidermal tumour cells. It is therefore tempting to speculate that malignant cells may escape immune control by non-expression of the surface oligosaccharide receptors involved in the control mechanism of cell behaviour.

In this study we noted that surface receptors for lectins CON-A, HPA, and RCA-120 were not present in malignant SCC; this absence was related to the degree of the neoplasia. In addition, we found peritumoral staining patterns with CON-A and RCA-120 associated with malignancy. Thus, it appears that lectins may constitute valuable probes in distinguishing between KA and SCC.

However, this investigation does not permit conclusions as to the mechanisms giving rise to the present data. Although histochemical methods may fail to indicate the presence of lectin-binding residues, these would be detected by biochemical methods following tissue homogenenization. Biochemical analyses of epidermal tissue glycoproteins are in progress in our laboratory.

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