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).

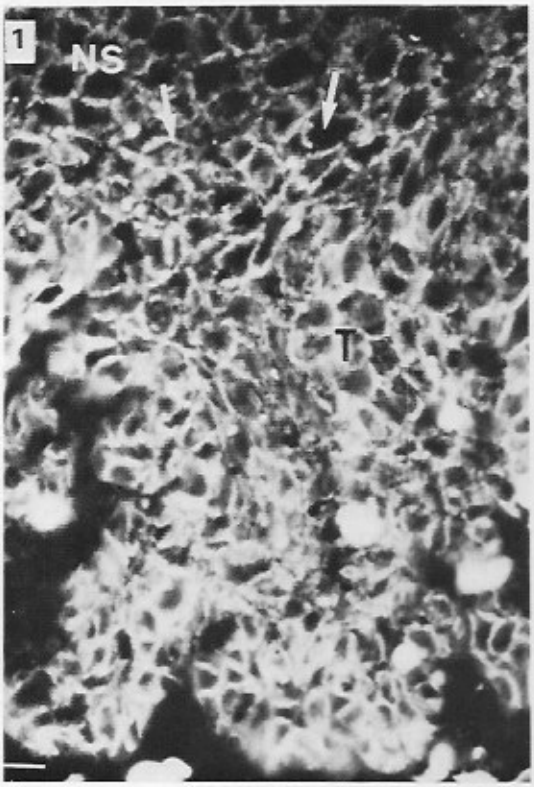
RESULTS

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.



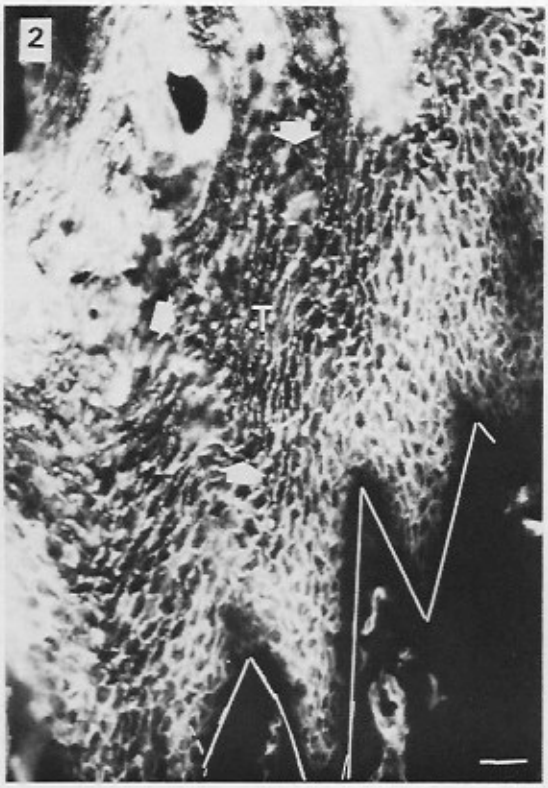


Fig. 1. Squamous cell carcinoma grade I reacted with Con-A. *NS*, Normal skin; *T*, tumour. *Arrows*, Margins of tumour.

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.

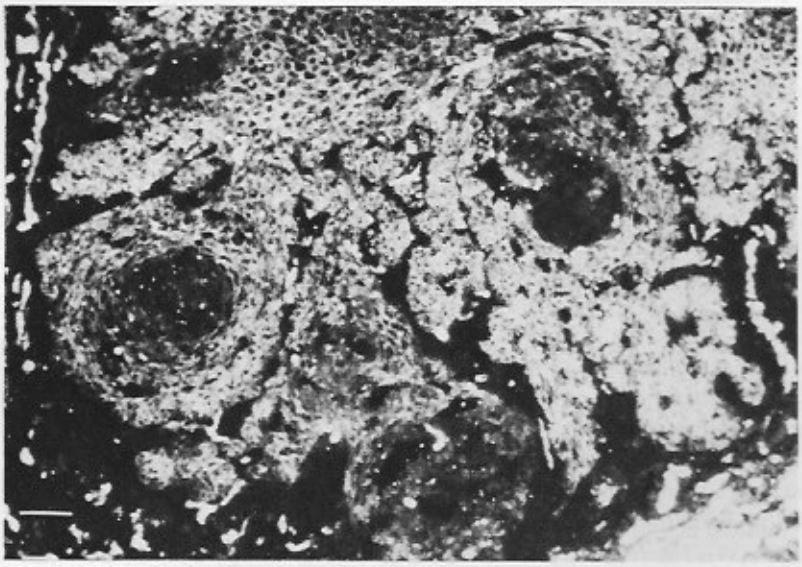


Fig. 3. Squamous cell carcinoma grade II reacted with PHA-L. Intercellular pattern in tumour, does not observe peritumoral pattern.

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.

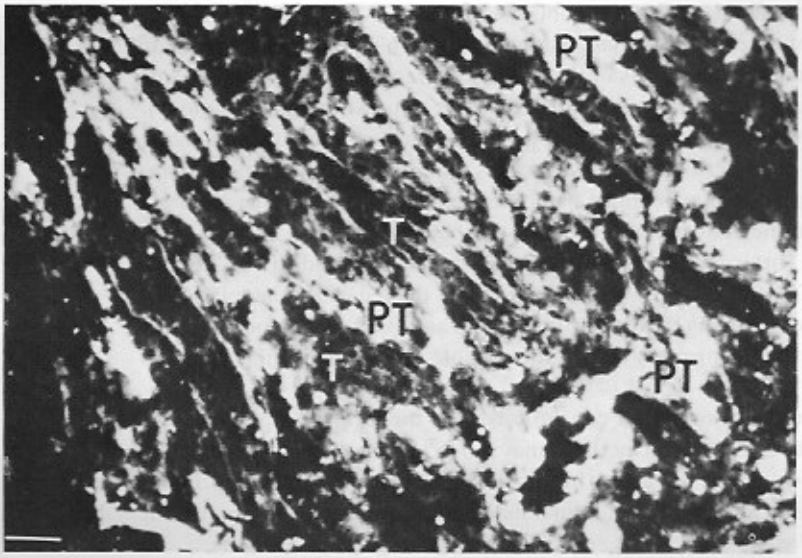


Fig. 4. Squamous cell carcinoma grade III reacted with RCA-120. *T*, tumour cells; *PT*, peritumoral pattern.