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Carbohydrate Residues in Non-Malignant Prostatic Epithelium as Revealed by Lectins

P. D. Abel[1], A. Leathem[2], A. Aylott[2], C. Marsh[3], D. Henderson[3] and G. Williams[1]

1 Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hospital, London
2 Bland-Sutton Institute of Histopathology, The Middlesex Hospital, London
3 Department of Histopathology, Freeman Hospital, Newcastle-Upon-Tyne

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Summary. Non-neoplastic prostatic epithelium from 39 patients obtained at transurethral resection for outflow tract obstruction and 5 normal prostates from men under 35 years of age obtained at postmortem were formalin-fixed and paraffin-embedded. The distribution of 8 lectin receptors were studied using a peroxidase anti-peroxidase method and an avidin-biotin method. Con A, WGA, and PNA bound to most epithelial cells. Con A and WGA also showed major stromal binding. Approximately 5% to 10% of cells bound UEA1, GS1, DBA, SBA and BPA. No major differences in lectin receptor expression were observed between normal and hyperplastic epithelium with either of the immunohistochemical techniques except that hyperplastic cells stained more strongly than normal epithelium.

Key words: Lectins, Carbohydrates, Non-malignant prostate.

Introduction

Carbohydrates are major components of the glycoproteins and glycolipids on the surface of cells and alter during differentiation and dedifferentiation [5]. Changes in expression of the major ABO(H) blood-group related carbohydrate antigens (BGA), for example, have been reported in urothelial cancer. It has been suggested that reduction or loss of expected BGA on superficial transitional cell carcinomas is associated with or even preceeds subsequent muscle invasion and, therefore, a worse prognosis [2, 11].

Changes in other complex carbohydrates may occur in malignancy. In order to establish a baseline for prospective studies in prostatic cancer, we have studied carbohydrate expression in normal and benign hypertrophied prostates using 8 lectins. Lectins are proteins or glycoproteins of non-immune origin that recognise complex carbohydrates [8, 9].

Table 1. Lectins used to identify carbohydrate structures

Lectin	Abbreviation	Major carbohydrate binding[a]
Jack bean (*Concanavalin enisformis*)	Con A	Man > Glc
Wheatgerm (*Triticum vulgaris*)	WGA	GlcNAc
Peanut (*Arachis hypogaea*)	PNA	Gal-GalNAc
Gorse (*Ulex europeus 1*)	UEA1	Fuc
Bandeiraea (*Griffonia simplificolia*)	GS1	Gal > GlcNAc
Soyabean (*Glycine max*)	SBA	GalNAc > Gal
Horse gram (*Dolichos biflorus*)	DBA	GalNAc
Bauhinia (*Bauhinia purpurea*)	BPA	GalNAc

[a] Man = Mannose; Glc = Glucose; Fuc = Fucose; GlcNAc = N-acetyl glucosamine; GalNAc = N-acetyl galactosamine

Patients and Methods

Prostatic tissue was obtained from 39 patients aged between 64–89 years (mean 71 years) undergoing transurethral resection to relieve outflow tract obstruction and from 5 men between 20 and 35 years within 18 h of death from road traffic accidents. Tissues were formalin-fixed for 24 h and then embedded in paraffin (FFPE).

The lectins used are summarised in Table 1. They were obtained both native and conjugated to biotin from Sigma Ltd and from E–Y Labs Ltd as was avidin-peroxidase. Antisera were raised to the native lectins in rabbits by ourselves [8].

All lectins were diluted to 10 μg/ml in tris buffered saline (TBS) containing 1 mmol each of calcium chloride, magnesium chloride and manganese chloride. TBS was also used for washing.

Five μ thick sections were cut, dewaxed in xylene and brought to water. Sections were trypsinised (400 mg of trypsin, Sigma grade 2 with 400 mg calcium chloride in 400 ml TBS) at 37 °C for 15 min and sites of endogenous peroxidase activity blocked by methanol (360 mls) with hydrogen peroxide (H_2O_2) (40 mls of 30 vol) for 20 min. Sections were then washed in tap water.

In the peroxidase anti-peroxidase (PAP) method, lectins were incubated on sections for sixty minutes at room temperature and then washed in TBS. Rabbit anti-sera to the lectins (Table 1) were diluted to 1:100 in TBS and incubated on sections for 60 min at room temperature. Sections were then washed in TBS. Swine anti-

Table 2. Lectin binding to prostatic epithelium and stroma

	Epithelial cells[a]	Stroma[b]
Con A	+++	+++
WGA	+++	++
PNA	+++	+/–
UEA1	+	–
GS1	+	–
SBA	+	–
DBA	+	–
BPA	+	–

[a] +++ = > 90% binding; + = < 10% binding
[b] +++ = strong diffuse binding; ++ = patchy intermediate binding; + = patchy, weak binding, in some sections only; – = no significant binding

rabbit IgG (Dako) was diluted 1:100 in TBS and incubated on sections for 30 min. After a further washing in TBS, rabbit PAP complexes (Dako), diluted 1:200 with TBS, were added for 30 min.

Peroxidase was demonstrated by addition of DAB, (diaminobenzidine tetrahydrochloride, at a concentration of 1 mg/ml of TBS with 10 µl of 30 vol H_2O_2 added to each ml) for 10 min. Sections were counterstained in Harris' haematoxylin, dehydrated, cleared and mounted.

An avidin-biotin method was also used. Biotinylated lectins at a concentration of 10 µg/ml of TBS were incubated on sections for 60 min, followed by washing with TBS, then avidin peroxidase at 10 µg/ml for 60 min, followed by further washing and then DAB solution, counterstaining and mounting as above.

For negative controls, the native or biotinylated step was omitted. Paraffin sections of normal human kidney were used as positive controls for all the lectins. Kidney epithelium provides an excellent positive control [8]. All patients had H & E sections of their prostates examined to confirm the diagnosis of normal or hyperplastic epithelium.

Results

Results of the lectin staining were identical whether the PAP or avidin-biotin method was used. Three major patterns of lectin binding were found (Table 2). In the first pattern, (Con A and WGA), over 90% of epithelial cells expressed the lectin receptor and there was also major stromal binding. With the second pattern (PNA), greater than 90% of epithelial cells stained positively but only occasional weak stromal binding was observed. PNA also bound to myoepithelial cells. In the third pattern (UEA1, GS1, SBA, DBA and BPA), less than 10% binding to independent groups of epithelial cells and no stromal binding was observed. Lectin receptors were localised in a diffuse, finely granular pattern throughout the cytoplasm of positive cells. Examples of the patterns of staining are given in Figs. 1–6. No differences in lectin binding were observed in patients of different ages.

The binding pattern of each individual lectin was consistent in all 44 prostates studied although hyperplastic cells stained with more intensity than normal cells.

It was not possible to localise lectin receptor expression to specific sites within the prostate because of the random selection of resected tissue.

Discussion

Carbohydrate chains are present on the surface of cells, attached to both proteins and lipids and all are potential antigens. It has been shown that alterations in carbohydrate expression may distinguish tumour cells from their normal counterparts [4]. One group of carbohydrate structures widely studied are the major ABO(H) blood-group antigens (BGA). There appears to be an association between progressive loss of expected BGA in urothelial cancer and increasing malignant potential [2, 11]. Not all reports agree however [6, 7] and at present, changes in BGA expression have no proven clinical role. Therefore it is important to investigate changes in other complex carbohydrates.

Lectins are proteins or glycoproteins of non-immune origin which recognise complex carbohydrates [8, 9]. Vast numbers occur throughout nature in both animals and plants. They are very specific and highly sensitive and the specificity of the commonest lectins are now well characterised. They are cheap and easy to extract, use and label [9]. No systematic study of lectin receptors in prostatic tissue has been undertaken and such a study is a necessary prelude to undertaking work on malignant tumours to see what changes, if any, have occurred.

The present work shows that most non-malignant prostatic epithelial cells express glycoprotein receptors for Con A, WGA and PNA. Smaller sub-groups of cells, less than 10% in each case, independently express glycoprotein receptors for one or other of the five remaining lectins. This suggests that morphologically identical but functionally distinct sub-groups of epithelial cells could be identified by the different carbohydrates on their cell surface. Although staining was slightly more intense in hyperplastic rather than normal cells, no significant differences in staining patterns were noted.

Stroma probably has a role in cellular recognition and stroma-epithelial interactions may be important in normal prostatic development as well as in the development of benign prostatic hypertrophy [3]. It is therefore of interest to study the carbohydrate receptors which are present in the stroma. Only Con A and WGA showed significant stromal binding in this study.

Negative results (that is absent lectin binding) may reflect a true absence of the lectin receptor. An alternative hypothesis for negative results includes the possibility that some receptors are partially or totally lipid based and therefore could be variably extracted by lipid solvents during paraffin processing as has been described with BGA in the urothelium [10, 12]. Our study would only identify glycoprotein based receptors because of this. Alternatively, sialic acid or other carbohydrates may mask and prevent identification of some of the lectin receptors [9].

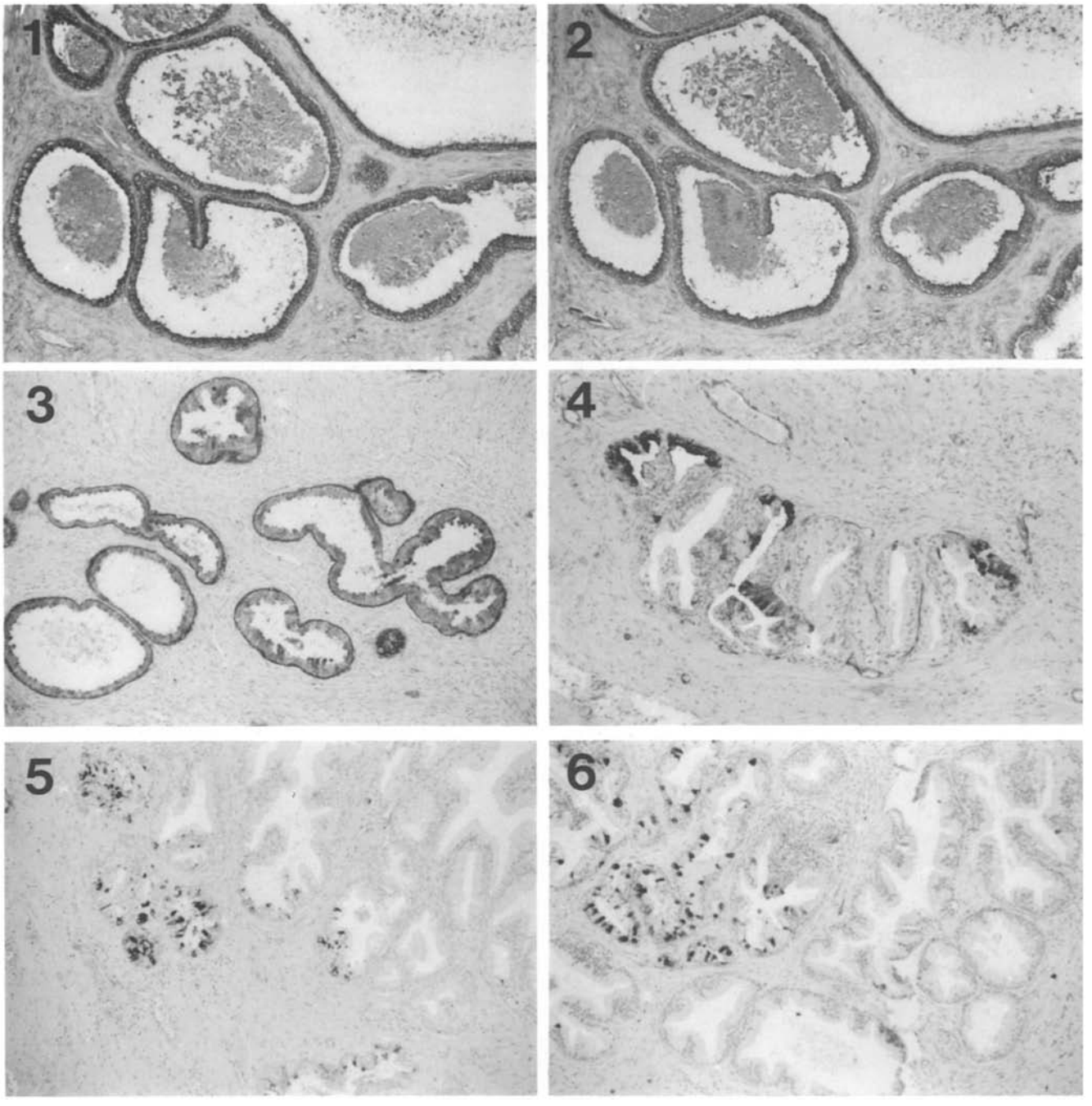

Fig. 1 and 2. Con A and WGA respectively (mag x100) – cells and stroma positive (adjacent sections) (BPH)

Fig. 3. PNA (mag x100) – cells positive, stroma negative (BPH)

Figs. 4–6. UEA1, DBA and GS1 respectively (mag x 100) – < 10% cells positive (BPH)

Another explanation for staining of small populations of epithelial cells is that lectin receptors may alter during development from childhood through to old age and some credance is given to this by the studies of Bischof and Anmuller [1]. Although they could not demonstrate receptors for the ricin lectin at any age, PNA receptor activity on prostatic epithelium varied at different age groups.

Increasing activity was seen in secretory cells as puberty progressed and reached a maximum during adulthood and up to about 60 years of age. Before puberty and after 60 years of age PNA binding activity was low. The authors concluded that PNA binding was possibly an androgen dependent process with a potential role as a marker for the prostatic action of testosterone. Their unpublished work in

normal adult dogs revealed a prostatic PNA binding pattern similar to previously noted patterns in man. In contrast, dramatically lower PNA activity was found in castrated dogs, supporting their theory of testosterone dependence with PNA activity in man. They used FFPE material however and did not consider the possibility that changes from glycolipid based to glycoprotein based PNA receptors could occur as the prostate developed and then atrophied. In our patients, the same patterns of PNA expression were found between early adulthood and old age with all the lectins studies, including PNA.

We have established a pattern of lecting binding with 8 lectins in non-neoplastic tissues. The prostatic epithelium is heterogenous in its expression of carbohydrates. Other lectins need to be studied to increase the number of well characterised receptors available to study the changes that may occur in malignant disease and which may therefore be useful as potential tumour markers.

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P. D. Abel, M.B., F.R.C.S.
Department of Surgery
Royal Postgradulate Medical School
Hammersmith Hospital
Ducane Road
W12 OHS
London
UK