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Accumulation of a Blood Group Antigen Precursor in Oral Premalignant Lesions[1]

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ABSTRACT

Epithelial cell membrane-bound blood group antigens A and B are lost in premalignant and malignant oral lesions. We now show that this loss in premalignant lesions is accompanied by accumulation of a blood group antigen precursor. The precursor structures were: type 2 chain H-antigen, Fucα1 → 2 Galβ1 → 4GlcNAc-R (A and B precursor); and *N*-acetyllactosamine, Galβ1-4GlcNAc-R (H-precursor), in which Fuc is L-fucose, Gal is galactose, and GlcNAc is *N*-acetyl-D-glucosamine. They were demonstrated in tissue sections by immunohistochemical staining techniques with monoclonal antibodies to H-antigen and *N*-acetyllactosamine. Precursors were found only on basal and suprabasal cells of normal mucosa. In all nine of ten premalignant lesions, the H-antigen was found on all cell membranes in the epithelium, in higher titers than in normal adjacent epithelium. Ten carcinomas were studied, and all showed an irregular distribution of H-antigen. *N*-Acetyllactosamine was not found in premalignant or malignant lesions. The accumulation of type 2 chain H-antigen in oral premalignant lesions may prove helpful in early diagnosis of epithelial cancer.

INTRODUCTION

Cell surface glycoproteins and glycolipids are instrumental in defining intercellular recognition and antigenic specificity. They also have a role in regulating cell cycle and replication (2, 6, 8). The roles played by these carbohydrate structures are indicated by changes in their composition, structure, and metabolism during development; differentiation; and oncogenic transformation (11–13).

Blood group A-, B-, and H-antigens are bound to either glycolipids or glycoproteins and carry their specific antigenic determinants at the ends of their carbohydrate chains. The A- or B-antigens and their immediate precursor, H-antigen, are carried by branched or unbranched type 1 ([Galβ1 → 3GlcNAc]$_n\beta$1 → 3Gal → R) or type 2 ([Galβ1 → 4GlcNAc]$_n\beta$1 → 3Gal → R) chains, in which Gal is D-galactose and GlcNAc is *N*-acetyl-D-glucosamine, depending on the type of tissues; *e.g.*, in gastrointestinal epithelia and their secretions, antigens are carried predominantly by type 1 chains, while those in erythrocytes are carried by type 2 chains (12, 22). Recently, monoclonal antibodies directed against 2 precursors of blood group antigens have been prepared, one against type 2 chain H and the other against type 2 chain *N*-acetyllactosamine, the precursor of H-antigen (24). These antibodies do not cross-react with type 1 chain structures (24). In earlier immunofluorescent studies, we observed that the expression of antigens A, B, H, and *N*-acetyllactosamine are markers of differentiation in human stratified oral epithelium (5, 21). The basal cells, which include the proliferating and least mature cells in the epithelium, express precursors to antigens A and B on their cell membranes, but complete blood group antigens are found only on the more mature spinous cells (5). We also reported the deletion of blood group antigen A from premalignant oral mucosal lesions which is regarded as an example of impaired synthesis of cell surface carbohydrates associated with malignant changes (4). We now find immunohistochemical evidence of accumulation of precursor carbohydrates, namely, H-antigen in premalignant lesions. Observation of this antigen may be a useful marker in diagnosis of epithelial cancer.

MATERIALS AND METHODS

Tissue. We studied 10 human oral mucosal biopsy specimens from 5 Blood Group A and 5 Blood Group O persons (Table 1). The lesions are regarded as premalignant based on their histopathological diagnosis of carcinoma *in situ* or severe epithelial dysplasia (23). We also studied 10 specimens from oral squamous cell carcinomas (5 Group A and 5 Group O) and normal oral mucosa from 12 fetuses (7 Group A and 5 Group O) 10 to 20 weeks after fertilization. All tissues were fixed in neutral buffered formalin and embedded in paraffin. Sections were cut at 5 μm and mounted on gelatin-coated slides.

Antigens and Antibodies. The chemical compositions of blood group antigens A and H and the precursor carbohydrate *N*-acetyllactosamine are given in Chart 1.

Monoclonal antibodies against blood group H-antigen (type 2 chain) and *N*-acetyllactosamine were prepared by a hybridoma technique (24). The antibody against blood group antigen A was a human serum described previously (3).

Staining. After the sections were rehydrated, antibodies in serial dilutions (1:15 to 1:1920) were applied to sections. We incubated the tissue with the monoclonal antibodies for 24 hr at 4°. The slides were washed in phosphate-buffered saline, pH 7.2, and the antigen-antibody complex was observed by reaction for 40 min with fluorescein- or peroxidase-conjugated rabbit antiserum to mouse immunoglobulin (Dako). Blood group antigen A was demonstrated by applying human anti-A sera to sections for 40 min, washing the sections as above, and incubating for another 40 min with fluorescein- or peroxidase-conjugated rabbit antiserum to human IgG (Dako). Sections stained with peroxidase-conjugated antibodies were incubated for 5 min in 0.05% 3,3-diaminobenzidine hydrochloride and 0.01% H_2O_2 in Tris buffer, pH 7.4. Details of the staining, conjugates, and method of examination have been described previously (3).

For control, the primary antiserum was replaced with (*a*) normal serum, (*b*) monoclonal antibodies to an irrelevant antigen (trinitrophenol), (*c*) anti-A serum absorbed with erythrocytes, or (*d*) phosphate-buffered saline.

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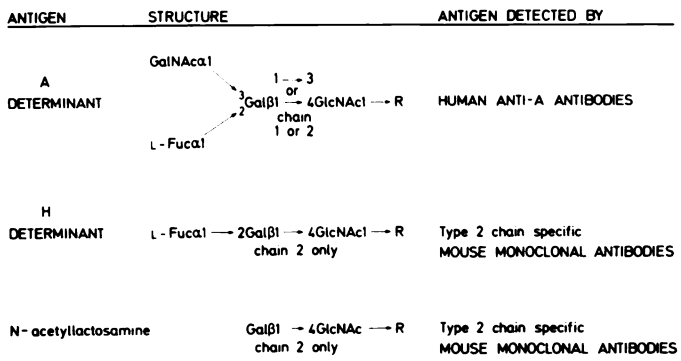

Chart 1. Carbohydrates investigated in this study. *Gal*, galactose; *GlcNAc*, *N*-acetyl-D-glucosamine; *Fuc*, L-fucose.

RESULTS

Staining with antibodies to *N*-acetyllactosamine was positive in normal epithelium in basal and suprabasal cells. This staining was, however, completely lost in premalignant lesions (Table 1).

In all premalignant specimens, except one (Case 5), an intense staining of cell surface membranes with anti-H (type 2) was observed throughout the entire epithelium (Figs. 1 and 2). For normal mucosa adjacent to these lesions, we found that H-antigen is present only on suprabasal cells as described previously (5) (Fig. 1). Titration of the anti-H-antibody indicated that the H-antigen is revealed at greater dilutions in premalignant lesions than in normal tissue (Fig. 2; Table 1). The stainings showed the same distribution of H-antigen in sections from Blood Group O and Blood Group A persons.

Premalignant lesions from Blood Group A persons were stained with both anti-A and anti-H in order to compare the distribution of the 2 antigens. We found a total loss of blood group antigen A in 4 (Cases 3, 6, 8, and 9) of the 5 lesions. In all 4 lesions, H-antigen was observed throughout the epithelium on cells which in normal epithelium express A-antigen but no H-antigen (Fig. 3). This inverse relationship in expressing A- and H-antigen was also seen in the specimen with retained A-antigen (Case 5). In this case, H-antigen was limited to suprabasal cells as in normal epithelium.

In oral squamous cell carcinomas, the distribution of H-antigen was irregular; some tumor cells did not express H-antigen, whereas others did but at low titers (Fig. 4). The staining with anti-*N*-acetyllactosamine was completely negative.

The distribution of antigens A and H in fetal oral epithelium was the same as in the normal adult epithelium; *i.e.*, H-antigen was expressed on the deeper cells and A-antigen on the more superficial cells. A detailed report of these observations is in preparation.[3]

DISCUSSION

The results of this study confirm previous studies which show loss of blood group antigen A in oral premalignant lesions from Group A persons (4, 9, 16, 19). The study also shows an accumulation of type 2 chain H-antigen, a precursor to A-antigen, in premalignant lesions from both Group A and Group O persons. The immunohistochemical methods used in this study do not allow quantitative analysis of H-antigen in the tissue; however, the findings are generally compatible with the results of biochemical analysis which indicate that glycolipid changes in malignant cells can be directed towards simplification due to blocked synthesis and the associated accumulation of precursor carbohydrates (11, 20).

Our findings in Blood Group A persons, where loss of the antigen A was accompanied by accumulation of H-antigen, suggest that the activity of enzymes which convert H-antigen to A-antigen is decreased in premalignant lesions. However, results on the distribution of H-antigen in normal and premalignant epithelium from Blood Group O persons suggest that the accumulation of H-antigen in premalignant cells may not be solely due to blocked synthesis of A-antigen. This is based on the observation of an identical distribution of Blood Group H in the epithelium from Blood Group O and A individuals. Since the normal oral epithelia from Blood Group O individuals do not have the enzymes required to convert the H-antigen to A-antigens, it was expected that blood group antigen H in Group O persons had the same distribution as does the A-antigen in Blood Group A persons. However, Blood Group H was present only in the suprabasal cells of the normal epithelium independent of the patients' blood group. In premalignant tissue, H-antigen is expressed in all cell layers. This change in expression in cell surface antigen in premalignant lesions may be due to an arrest of cells in a stage of differentiation which is comparable to that of the basal layers of normal epithelium. The antibody titrations indicate, however, that there is more H-antigen in premalignant epithelium than in the adjacent normal tissue, suggesting that the premalignant cells cannot be regarded simply as less differentiated normal cells.

The apparent increase of fucosylation in the premalignant lesions could be occasioned by increased levels of fucosyltransferase, which has been demonstrated in some transformed cells and in the serum of patients with tumors (1, 15, 18). In normal epithelium, H-sites could be masked by sialation; whereas in premalignant lesions, fucosyltransferase competes with sialyltransferase for a common substrate, such as the blood group antigen carbohydrate chain. Studies with lectins, however, have not been able to demonstrate sialic acid on normal and malignant epithelial cells (9). Studies with neuraminidase, which can unmask cell surface receptors in normal and malignant cells, would help clarify this problem. Synthesis of new fucolipids can be seen in premalignant tissue. A recent paper describes the occurrence of a new fucoganglioside in premalignant lesions of the rat liver and suggests that the appearance of this ganglioside could be one of the earliest changes associated with a process of oncogenesis (14).

The possibility that the loss of A-antigen and the appearance of H-antigen in oral premalignant lesions could be due to degradation by glycosidases cannot be ruled out. This problem has not been investigated in our study, but recent studies of human skin suggest that glycosidases are involved in degradation of cell surface carbohydrates in normal epidermis (17).

A deficiency of α-2fucosyltransferase that converts *N*-acetyllactosamine to H-antigen has been demonstrated in extracts from human gastrointestinal tumors (15). Our findings in oral carcinomas seem to be in keeping with these studies, but the

[3] P. Vedtofte, E. Dabelsteen, S. Hakomori, and W. W. Young. Blood group antigen in fetal oral mucosa, manuscript in preparation.

Table 1

Maximum dilutions for positive staining of normal and premalignant oral epithelial cells with monoclonal antibodies to N-acetyllactosamine and blood group antigen H (type 2 chain) and with polyclonal antibodies to blood group antigen A

		Normal epithelium			Premalignant epithelium with epithelial dysplasia or carcinoma *in situ*		
Case	Blood Group	Anti-*N*-acetyllactosamine	Anti-H	Anti-A	Anti-*N*-acetyllactosamine	Anti-H	Anti-A
1	O	1:60	1:120	[a]	[a]	1:960	[a]
2	O	1:30	1:480	[a]	[a]	1:1920[b]	[a]
3	A	1:60	1:240	1:120	[a]	1:1920[b]	[a]
4	O	1:30	1:120	[a]	[a]	1:960	[a]
5	A	1:60	1:120	1:30	[a]	1:120[c]	1:30
6	A	1:15	1:120	1:240	[a]	1:960	[a]
7	O	1:60	1:240	[a]	[a]	1:1920[b]	[a]
8	A	1:30	1:240	1:120	[a]	1:1920[b]	[a]
9	A	1:15	1:240	1:240	[a]	1:1920[b]	[a]
10	O	1:60	1:120	[a]	[a]	1:960	[a]

[a] No staining.
[b] Sections have not been stained with dilutions of antiserum greater than 1:1920.
[c] Limited to suprabasal cells as in normal epithelium.

failure to demonstrate *N*-acetyllactosamine in oral malignant tissue also suggests other defects in the carbohydrate synthesis.

To explore the possibility that H-antigen activity in the premalignant lesion could be a retrogenetic expression (10), we studied the distribution of H- and A-antigens in oral epithelium from Blood Group A-positive human fetuses. Since the distribution of the antigen was not different from normal adult mucosa, this possibility is not supported, although we have not studied fetal epithelium at all stages of development.

We now regard the change from an even distribution of blood group antigen H in premalignant tissue to an irregular distribution in carcinomas as a chemical sign of malignant progression. This work may therefore supplement previous studies in showing that tumors consist of heterogeneous cell populations (7).

The present study has shown that antigen H accumulates in premalignant lesions with epithelial dysplasia or carcinoma *in situ* and is irregularly distributed in oral squamous cell carcinomas. The difference in distribution of antigen H in oral epithelial premalignant lesions and normal epithelium suggests that this antigen may be useful in the early diagnosis of epithelial cancer.

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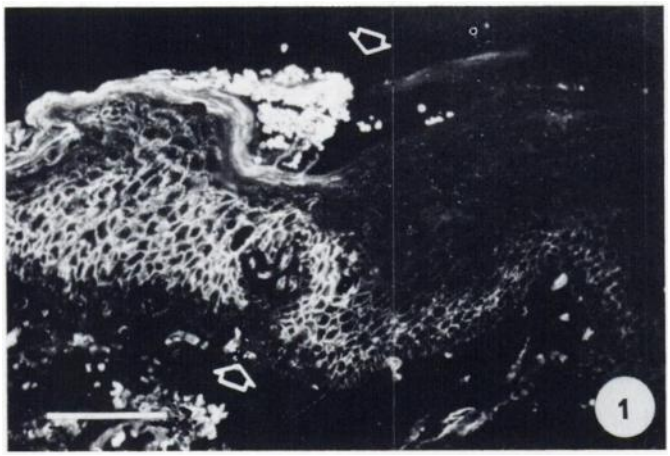

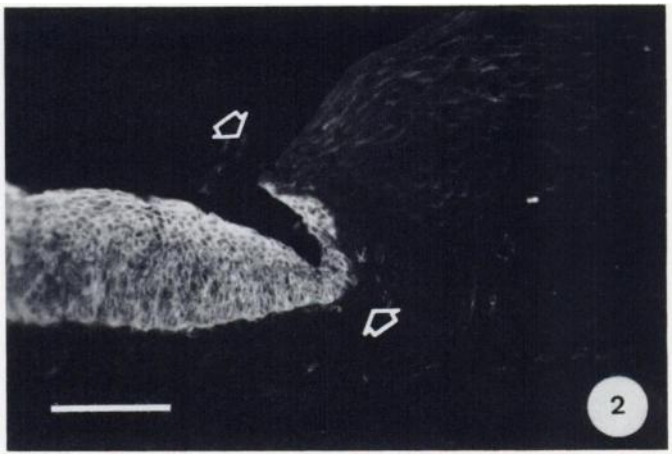

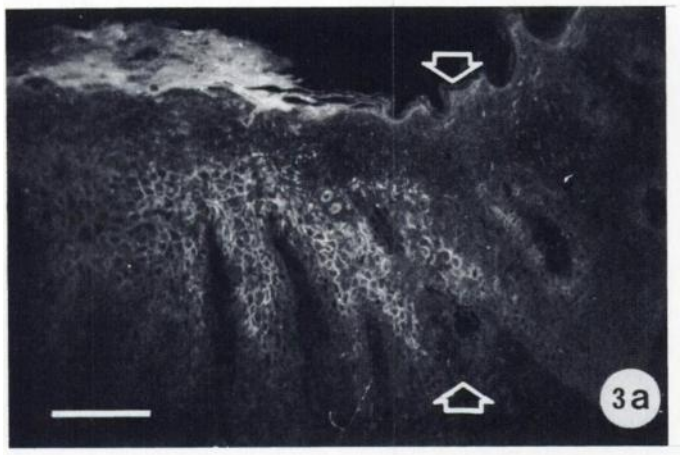

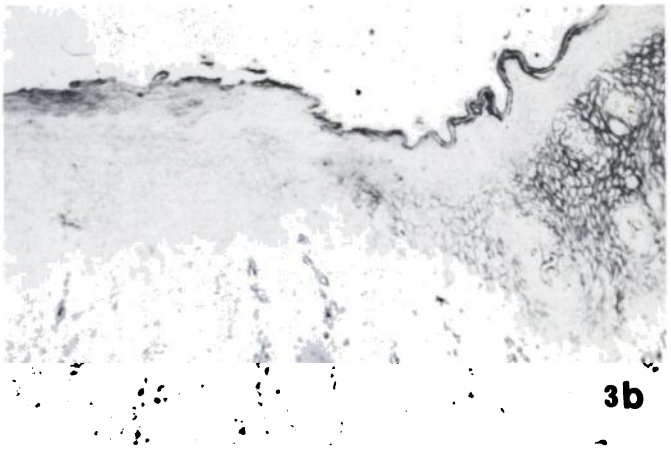

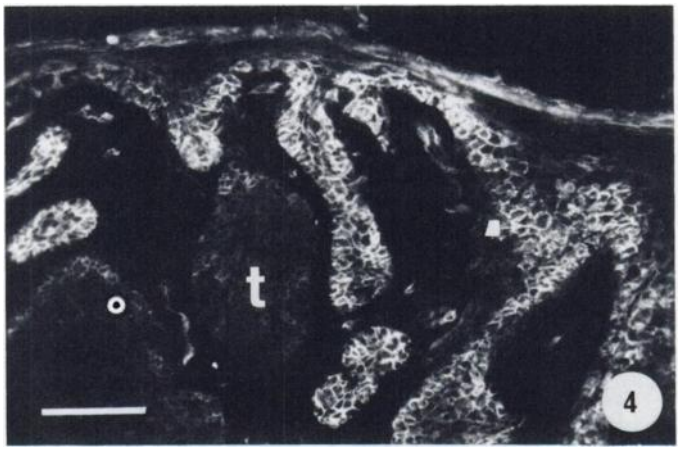

Fig. 1. Section of a biopsy from the border line between normal epithelium (*right*) and dysplastic epithelium (*left*). *Arrows*, border line. Immunofluorescence staining of blood group antigen H (type 2 chain). In the normal epithelium, there is bright staining of the suprabasal cell layers, whereas the dysplastic epithelium demonstrates staining almost to the surface of the epithelium. *Bar*, 100 μm. Case 4.

Fig. 2. Section of a biopsy from border line between normal epithelium (*right*) and carcinoma *in situ* (*left*). *Arrows*, border line. Immunofluorescence staining of blood group antigen H (type 2 chain). The anti-H antibody is diluted 1:960, at which dilution-positive staining is seen in carcinoma *in situ* only. *Bar*, 100 μm. Case 7.

Fig. 3. Section of a biopsy from border line between normal and dysplastic epithelium in a Blood Group A person. *Arrows*, border line. *a*, immunofluorescence staining of Blood Group H (type 2 chain). Positive staining is seen in the dysplastic epithelium (*left*), whereas there is only weak staining in normal epithelium. *b*, the same section as in *a*, with immunoperoxidase staining of blood group antigen A. Positive staining in normal epithelium and no staining in dysplastic epithelium. *Bar*, 100 μm. Case 6.

Fig. 4. Biopsy from invasive carcinoma stained for Blood Group H (type 2 chain). The superficially located cells stain positively, whereas some of the invasive tumor cell masses (*t*) are almost negative. *Bar*, 100 μm.