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A Histochemical Study on Lectin Binding Patterns of Acquired Cholesteatoma Matrix †

Myung Whun Sung and Tae Hoon Jinn

Department of Otolaryngology,
Seoul National University College of Medicine,
Seoul 110-744, Korea

= Abstract =**Lectins are non-immune origin proteins or glycoproteins binding to specific carbohydrates in the cell, which have been widely used as probes in cytochemical and oncogenic studies. The authors investigated the lectin binding patterns of the human acquired cholesteatoma matrix and compared them to those of normal ear canal skin. Using an enzyme histochemical method with avidin-biotin-peroxidase complex(ABC), which is applicable to formalin-fixed paraffin-embedded tissue sections, the binding patterns of seven lectins were investigated ; concanavalin A(Con A), wheat germ agglutinin(WGA), *Ricinus communis* agglutinin(RCA-1), soybean agglutinin(SBA), peanut agglutinin(PNA), *Ulex europaeus* agglutinin(UEA-1) and *Dolichos biflorus* agglutinin(DBA). The results observed under a light microscope were as follows : 1)The specificity of the lectin binding in cholesteatoma epithelium was confirmed in the control experiment. 2) SBA and UEA-1 lectins did not show staining in the basal layer of the canal skin and tympanic membrane, but revealed positive staining in the basal layer of cholesteatoma. 3) DBA lectin showed negative staining in all 3 layers in both cholesteatoma and canal skin. From these results we can suggest that there is some altered carbohydrate specificity on the keratinocyte membrane of the chlolesteatoma that may lead to characteristic differentiation of cholesteatoma.**

Key Words: *Cholesteatoma, Lectin binding patterns*

INTRODUCTION

Membrane glycoconjugates are believed to play an important role in differentiation and maturation in many normal and malignant cells(Nogami *et al.* 1988; Jin and Lin, 1989; Saku and Okabe, 1989). The human epidermis undergoes constant renewal and stepwise maturation and many authors have found altered lectin-binding specificities in the human epidermal layers(Ookusa *et al.* 1983).

Acquired cholesteatoma is believed to be caused by migration of the squamous epithelium from the tympanic membrane(Abramson *et al.* 1977). Since Virchow has described the histopathology of cholesteatoma in 1855, enormous investigations have been carried out to discover the exact biologic characteristics of this disease. By conventional histologic examination, the cholesteatoma and the epidermis are identical except for the abscence of the pilo-

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서울대학교 의과대학 이비인후과학교실: 성명훈, 진태훈

sebaceous unit and melanocytes in the former. Cholesteatoma can invade and destroy the epitympanum, mesotympanum and mastoid cavity(Sheehy *et al.* 1977). In this way it can bring about serious complications such as facial nerve paralysis, labyrinthine fistula and intracranial complications(Sheehy *et al.* 1977). The answer to the pathogenesis and aggressive behavior of human acquired cholesteatoma is still not clear in spite of many clinical and experimental studies.

The authors studied the lectin binding patterns of acquired choleseatoma epithelium and compared them to those of the ear canal skin and tympanic membrane skin to see if there was any difference in differentiation of the keratinocytes.

MATERIALS AND METHODS

During ear surgery, a piece of cholesteatoma epithelium was taken. As a control, the ear canal skin was also taken during the surgery. Using a histochemical method with avidin-biotin-peroxidase complex(ABC), the binding patterns of seven lectins were investigated.

All specimens were fixed in 10% formalin for 24 hours and 6 μm paraffin sections were cut. Deparaffinized sections were immersed in 0.3% H_2O_2 dissolved in methanol for 30 minutes to block the activity of intrinsic peroxidase and washed 3 times with 0.05M phosphate buffered saline(PBS). The sections were covered with 1% bovine serum albumin(BSA) in 0.05M PBS for 30 minutes. After washing with PBS, the sections were incubated with 7 different biotinylated lectins(Vector lab. U.S.A.) for 60 minutes at room temperature. The biotinylated lectins were diluted to 50 M/ml in PBS just before use. After washing with PBS, the sections were immersed in 0.03% 3,3'-diaminobenzidine tetrahydrochloride(DAB, Sigma Co. U.S.A.) and 0.03% H_2O_2 for 10 minutes at room temperature. The specimens were counterstained with Harris's hematoxylin.

In order to confirm the specificity of lectin staining, the sections were pre-incubated with appropriate hapten sugars and then incubated with biotinylated lectins in the presence of the hapten sugar. The lectins and their hapten sugars used in this experiment are listed in Table 1. Endogenous peroxidase activity was checked by incubating untreated sections with the DAB-H_2O_2 solution.

Table 1. Lectins and corresponding sugar specificity(Vector lab. U.S.A.)

Lectin (abbreviation)	Sugar specificity	Blocking hapten sugar
Concanavalin A (Con A)	-D-mannosyl- -D-glucosyl-	α-methyl-mannoside
Wheat germ agglutinin(WGA)	β-(1-4)-D-GlcNA[1] NeuNAc	NeuNAc[2]
Ricinus communis agglutinin(RCA-1)	β-D-Galactosyl-	Lactose
Soybean agglutinin (SBA)	α-D-GalNAc[3]	α-D-GalNAc
Peanut agglutinin (PNA)	Gal-β-(1-3)-GalNAc	Lactose, D-galactose
Ulex europaeus agglutinin(UEA-1)	α-D-fucose	L-fucose
Dolichos biflorus agglutinin(DBA)	α-D-GalNAc	α-D-GalNAc

* 1) GlcNAc : N-Acetyl-Glucosamine
2) NeuNAc : Acetyl-neuraminic acid (sialic acid)
3) GalNAc : N-Acetyl-Galactosamine

RESULTS

The binding characteristics of each lectin are summarized in Table 2. The staining intensity was scored from (-)negative to (++) strong response.

Ear canal skin and tympanic membrane : Con A strongly stained the keratinocytes in the basal, spinous and granular cell layers(Fig. 1).

WGA, RCA-1, PNA also showed positive staining in the basal, spinous and granular layers. With SBA and UEA-1, the stainings were restricted to the spinous and granular layers (Fig. 2). DBA revealed negative staining in all

3 layers.

Cholesteatoma : Con A, WGA, RCA-1, and PNA showed no significant difference in the staining pattern, compared to the ear canal skin and tympanic membrane. But with SBA and UEA-1, the staining patterns were different from those of the canal skin and tympanic membrane giving positive-stainings in all three layers(Fig. 2, Table 2). Positive staining of SBA and UEA-1 on the basal layer could be found in all 5 cases of cholesteatoma. DBA also showed negative staining in all layers of cholesteatoma.

DISCUSSION

Acquired aural cholesteatoma is characterized by the invasion of keratinizing strati-

Table 2. Lectin binding patterns in cholesteatoma, canal skin and tympanic membrane

Lectins	Canal skin (N=5)			Tympanic menbrane (N=3)			Cholesteatoma (N=5)		
	B	S	G	B	S	G	B	S	G
Con A	++	++	++	++	++	++	++	++	++
WGA	+	+	++	+	+	++	+	++	+
RCA-1	+	+	+	+	+	+	+	+	+
SBA	−	+	++	−	+	+	+	+	++
PNA	+	++	+	+	+	+	+	+	+
UEA-1	−	+	+	−	+	+	+	+	+
DBA	−	−	−	−	−	−	−	−	−

B: basal layer, S: spinous layer, G: granular layer
staining intensity(−: no response, +: weak response, ++: strong response)

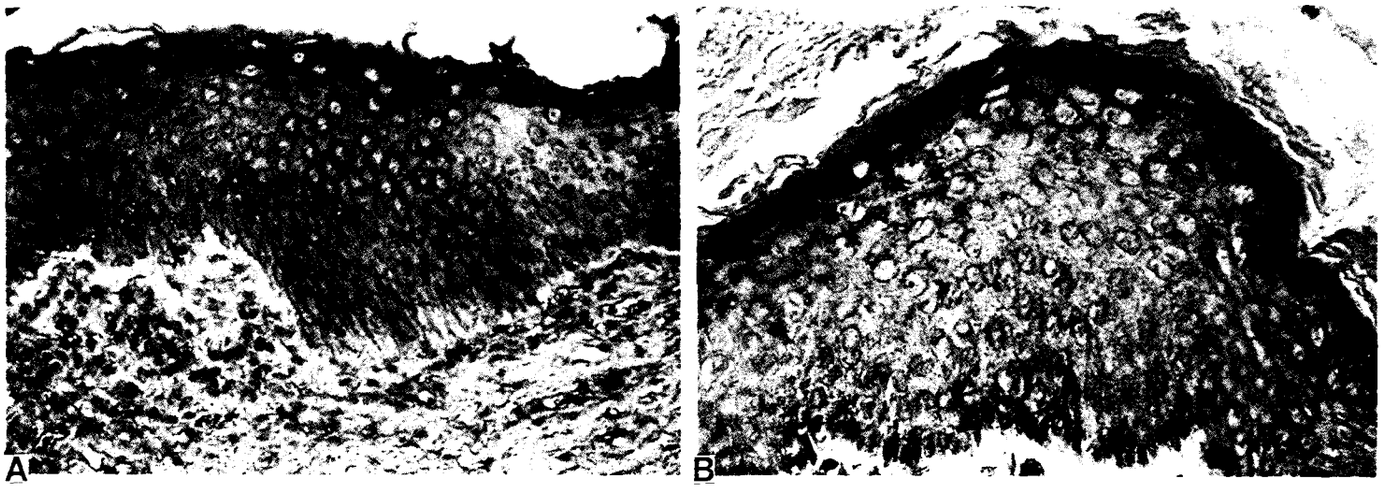

Fig. 1. Con A strongly stained the basal, spinous and granular layers in both the canal skin(A) and cholesteatoma(B)(x250).

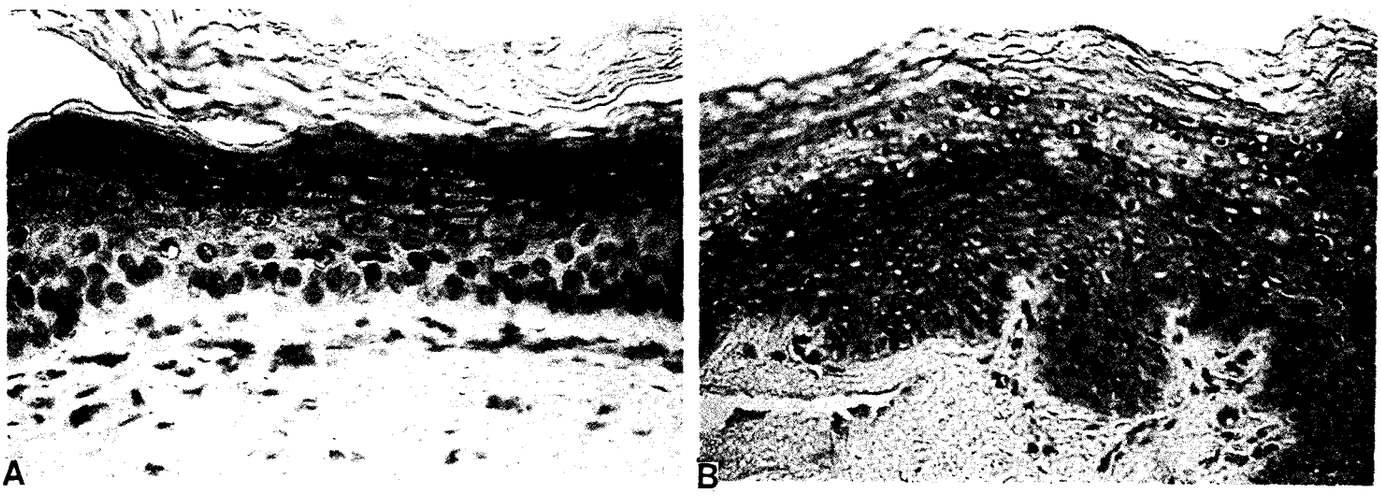

Fig. 2. UEA-1 stained spinous and granular layers in the canal skin(A). In cholesteatoma, the basal layer showed positive staining(arrow head)(B)(x250).

fied squamous epithelium into the normally aerated epitympanum and mesotympanum. When this occurs, a sequence of destructive events ensues which requires surgical intervention to prevent functional impairment and life-threatening complications.

The epidermis of human skin can be considered as a multilayered organ in which undifferentiated basal cells undergo stepwise maturation. During this differention, the expression of glycoconjugates on the keratinocyte membrane alters in each epidermal layer (Ookusa *et al.* 1983; Zieske *et al.* 1982).

In this study, Con A, which is specific for mannose-like sugar, strongly stained all three layers of both the canal skin and cholesteatoma epithelium. Con A is thought to stain both the cell membrane and cytoplasm because mannose-like sugars are commonly distributed in the cytoplasmic reticulum, nuclear envelope and Golgi apparatus. Thus Con A is not supposed to be a good marker for cell surface differentiation(Ookusa *et al.* 1983).

In the case of WGA, RCA-1 and PNA, the canal skin and cholesteatoma epithelium showed similar binding patterns. Generally, the lectin binding increased during ketatinocyte differentiation. These findings coincide with previous reports by many authors(Iwano *et al.* 1989).

In the canal skin and tympanic membrane epithelium the binding of SBA and UEA-1 was restricted to the spinous and granular layers. In cholesteatoma epithelium, however, SBA and UEA-1 stained the basal layer, too. Ookusa *et al.* (1983) showed that the binding of RCA, PNA, and SBA increased during keratinocyte differentiation in human skin. Iwano *et al.* (1989) reported positive staining of PNA and UEA-1 on the basal layer of cholesteatoma epithelium, compared to the lack of binding of PNA and UEA-1 on the basal layer of canal skin. These findings seem to be related to the suggestion of Zieske *et al.* (1982) that -fucosyl residues were added to the glycoproteins on the cell surfaces of differentiated cells as epidermal cells move out of the basal cell layer. Therefore, positive staining of SBA and UEA-1 in the basal layer of cholesteatoma might represent early maturation of this layer compared with normal skin.

DBA does not stain any layer of either canal skin or cholesteatoma epithelium. Ookusa *et al.* (1983) found positive DBA staining in the sweat glands of human skin but not in the epidermis. Hormia *et al.* (1988) used DBA as a tool to detect mast cells in connective cells.

No positive staining of the corneal layer was seen with the lectins tested in the present study. This might be due to the masking or shedding of lectin-reactive glycoconjugates during the keratinization process(Bell and Skerrow 1984; Ookusa *et al.* 1983).

It is thought that many factors can influence the binding patterns of lectins. The source of conjugate, tissue processing method, the effectiveness of inhibitor hapten sugar and individual variation may affect the profile of specific binding charaterisics for each lectin(Bell and Skerrow 1984).

In conclusion, we can suggest that early maturation of the basal layer keratinocyte of the cholesteatoma epithelium could alter the expression of glycoconjugates on the keratinocyte membrane.

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