

Lectin Cytochemistry of the Lacrimal Sac Epithelium in Experimental Dacryocystitis

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Purpose: To study the glycoconjugates in the lacrimal sac epithelium of Japanese white rabbits with experimentally induced chronic dacryocystitis.

Methods: Chronic dacryocystitis was induced by a subcutaneous injection of albumin followed by an injection of *Staphylococcus aureus* into the lacrimal sac. The histological appearance of the lacrimal sac was studied using the alcian blue-periodic acid-Schiff sequence. In addition, the specific binding to the lacrimal sac epithelium of *Ulex europaeus* agglutinin 1, *Ricinus communis* agglutinin 1, peanut agglutinin, and soybean agglutinin was also studied.

Results: Staining with alcian blue-periodic acid-Schiff sequence showed hyperplasia of the goblet cells in the inflamed lacrimal sac epithelium. Lectin cytochemistry revealed specific binding of *Ulex europaeus* agglutinin 1, *Ricinus communis* agglutinin 1, peanut agglutinin, and soybean agglutinin to the lacrimal sac epithelium.

Conclusions: These results indicated that the composition of glycoconjugates in the lacrimal sac epithelium is markedly changed in dacryocystitis. There seems to be a fundamental abnormality in glycoconjugate synthesis in the chronically inflamed lacrimal sac epithelium. **Jpn J Ophthalmol 1999;43:69-74** © 1999 Japanese Ophthalmological Society

Key Words: Experimental dacryocystitis, glycoconjugates, lectin cytochemistry, rabbit.

Introduction

Chronic dacryocystitis is characterized by recurrent infection in the lacrimal sac with tearing and regurgitation of a mucopurulent substance. Most knowledge about the morphological changes in dacryocystitis is derived from surgically treated patients (by dacryocystorhinostomy).¹⁻⁴ Even in such cases, the morphological findings are usually variable. Although the pathogenesis of chronic dacryocystitis is still not completely known, few investigators have sought to elucidate it by conducting experimental research.

In an effort to clarify such etiological ambiguities, we induced dacryocystitis in rabbits and examined

the pathomorphological changes. In the present investigation, the main focus was on the cytochemical characteristics of the glycoconjugates in the lacrimal sac epithelium of rabbits with dacryocystitis. We examined the glycoconjugate composition using classic procedures, eg, alcian blue-periodic acid-Schiff sequence and by the application of four different types of plant lectins.

Materials and Methods

Nineteen male, Japanese, white rabbits weighing about 2.0 kg were used in this study. The rabbits were maintained in accordance with the ARVO statement on the care and use of animals in ophthalmic research.

Induction of Experimental Dacryocystitis

Purified egg white albumin was emulsified in complete Freund's adjuvant and injected subcutaneously

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into the backs of the 19 rabbits (1.0 mL/rabbit). An additional adjuvant, inactivated *Bordetella pertussis*, was injected intravenously (10^{10} cells/rabbit). After 4 weeks, the same antigen was injected into the right lacrimal sac through the lacrimal punctum.

The following procedure was conducted on 11 of the 19 rabbits. *Staphylococcus aureus* (ATCC25923) was incubated in growth media for about 5 hours at 37°C to grow to the order of 10^7 cells/mL, and 0.3 mL of this bacterial suspension was injected into the right lacrimal sac of the 11 rabbits via the lacrimal punctum. The remaining 8 rabbits, which had been injected with purified egg white albumin, but not inoculated with the bacterial suspension, served as controls.

The rabbits were monitored for 6 months after the inoculation of *Staphylococcus aureus*, and then sacrificed by an overdose of ketamine injected into a marginal ear vein. At this time, a bacterial culture was started with a sample of the mucopurulent material from the sac.

Histological Procedure

The right lacrimal sac was removed, dissected in chilled saline, and fixed in 4% paraformaldehyde-0.1 mol/L phosphate buffer solution for 24 hours. The specimens were then embedded in paraffin and sections were cut at 5 μ m thickness. The sections were examined for glycoconjugate composition with alcian blue (AB)-periodic acid-Schiff (PAS) sequence (pH 2.5).⁵

The lacrimal sac epithelium of the control rabbits was prepared in the same way.

Lectin Cytochemistry

Reagents. Biotinylated Ulex europaeus 1 (UEA-I), Ricinus communis agglutinin 1 (RCA-I), peanut agglutinin (PNA), and soybean agglutinin (SBA) (Vector Laboratories, Burlingame, CA, USA) were obtained commercially. Haptenic sugars, L-fucose (Fuc), D-galactose (Gal), Gal, and N-acetyl-D-galactosamine (GalNAc) were used against UEA-I, RCA-I, PNA, and SBA, respectively.

Staining with biotinylated lectins. After immersion fixation in 4.0% paraformaldehyde-0.1 mol/L sodium cacodylate solution for 30 minutes, the lacrimal sacs with or without bacterial inoculation were embedded in OCT compound and sectioned at 10 μ m thickness on a cryostat at -20°C. The cryosections were incubated with 25 μ g/mL biotinylated lectins, and then with ABC complex consisting of avidin and biotinylated horseradish peroxidase (Vector Labora-

tories). After several rinsings, the sections were developed with diaminobenzidine solution (Sigma Chemical, St. Louis, MO, USA).

The specificity of lectin binding was checked by competitive inhibition of haptenic sugars at 200 mmol/L.

Results

Acute dacryocystitis was induced in all rabbits within 1 week after the injection of egg white albumin into the lacrimal sacs. The signs of acute inflammation such as swelling and redness in the lacrimal sac region were observed. In the infection experiment, all 11 rabbits exhibited swelling and redness in the lacrimal sac region and produced mucopurulent materials from the lacrimal punctum 1 week after inoculation with *S. aureus*. The inflammation in the lacrimal sac persisted for over 6 months in 6 rabbits, a chronic dacryocystitis induction rate of 55% (6 of 11). Chronic dacryocystitis showed tearing and hair loss in the lacrimal sac region, and mucopurulent substances were discharged when pressure was applied to this region. *S. aureus* and *Pasteurella multocida* were cultured from these mucopurulent discharges. The detection rate was 67% for *S. aureus* and 100% for *P. multocida*.

In contrast, the rabbits without bacterial inoculation showed similar acute inflammatory signs within 1 week after injection of egg white albumin into the lacrimal sac, but the inflammation did not persist for more than 1 week.

Appearance of Lacrimal Sac

In the control animals, the lining of the lacrimal sac epithelium had a two-layered columnar appearance (Figure 1A). The superficial layer comprised light cells with pale cytoplasm intermixed with dark cells. The basal cells rested on the basal lamina. We could not find any goblet cells or apparent PAS- or AB-positive substances in the epithelium. The substantia propria comprised well-developed connective tissue. There were no differences between the histological features of normal lacrimal sacs (naive) and the albumin-injected (control) sacs.

In the animals with chronic dacryocystitis, the epithelium of the lacrimal sac had a thickness of three to four layers of cells, and occasionally showed focal ulcerations (Figure 1B). Goblet cells were distributed in the superficial layer. The mucus was stained purplish-red, indicating that the mucus contained neutral mucopolysaccharides (MPs). The epithelial cells in the middle layers had an AB-PAS-positive

Figure 1. Light micrographs of PAS and AB stained sections of lacrimal sac epithelium from control (A) and dacryocystitis rabbit (B). A. Epithelium comprised superficial dark and light cells (arrowheads) and basal cells (arrows). B. Mucus granule of goblet cells stained red-purple (arrowheads). Supranuclear regions of middle layer epithelial cells also positive for PAS and AB. Original magnification, $\times 125$. Bar = 100 μm .

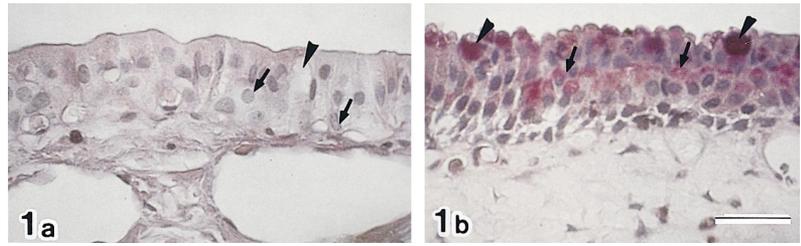


Figure 2. UEA stained frozen sections of lacrimal sac epithelium of control (A) and treated rabbit (B). A. Epithelium was negative for UEA. B. Epithelium showed strong reaction for UEA. Original magnification, $\times 100$.

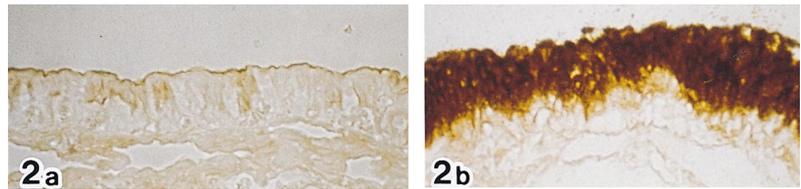


Figure 3. RCA stained frozen sections of control (A) and treated rabbit (B). A. RCA strongly positive for substantia propria (sp). B. RCA specifically bound to supranuclear regions of middle layer epithelial cells (arrows). Original magnification, $\times 100$.

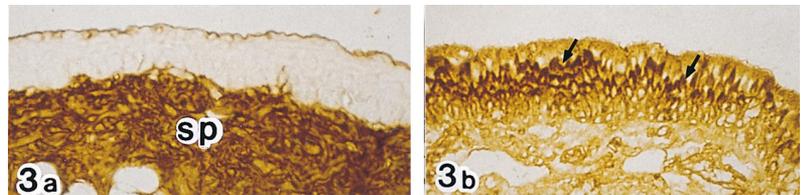


Figure 4. PNA stained frozen sections of control (A) and treated rabbit (B). A. PNA strongly positive for substantia propria (sp). B. Basal cells in epithelium specifically stained with PNA (arrows). Original magnification, $\times 100$.

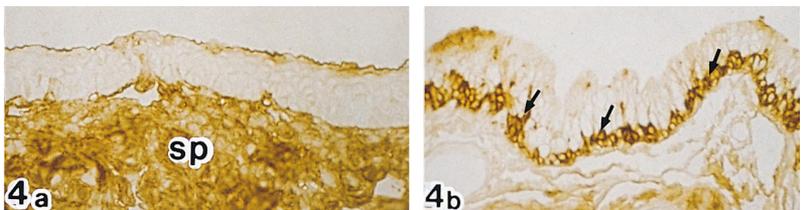
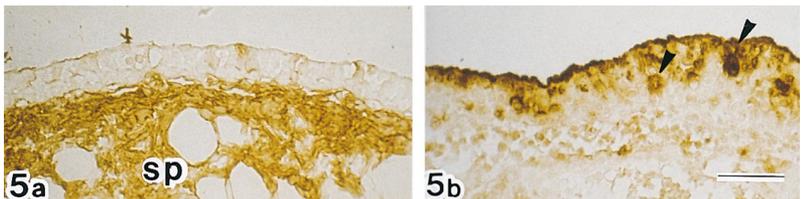


Figure 5. SBA stained frozen sections of control (A) and treated rabbit (B). A. SBA strongly positive for substantia propria (sp). B. Secretory granules seem specifically stained with SBA (arrows). Original magnification, $\times 100$. Bar (for Figures 2–5) = 100 μm .



substance in the supranuclear region of the cell body. There seemed to be no positive reaction in the basal cells. The substantia propria comprised connective tissue and was infiltrated by a number of mononuclear cells. There were few residual epithelial

cells or inflammatory leukocytes in the lumen of the lacrimal sac.

There appeared to be no differences in lectin binding reactions between the conjunctival and the nasal sites of the lacrimal sac.

Lectin Staining

UEA-I. There was a vestigial reaction to UEA-I in the lacrimal sac epithelium of the control rabbits (Figure 2A). By contrast, UEA-I was strongly bound to all the epithelial cell layers of the rabbit with dacryocystitis (Figure 2B). There seemed to be no positive reaction in the substantia propria of both sets of rabbits.

RCA-I. RCA-I was strongly bound to the substantia propria of the control rabbits, and there were no positive reactions to RCA-I in the epithelium (Figure 3A). In the rabbits with chronic dacryocystitis, RCA-I was specifically bound to the supranuclear region of the epithelial cells in the middle layers, and weakly to the luminal surface of the epithelium (Figure 3B).

PNA. PNA was moderately bound to the substantia propria of the control rabbits, and there were no positive reactions to PNA in the epithelium (Figure 4A). In the rabbits with dacryocystitis, the basal cells in the stratified epithelium were specifically stained with PNA. PNA was vestigially bound to the substantia propria (Figure 4B).

SBA. SBA was moderately bound to the substantia propria of the rabbit in the control experiment, whereas there were no positive reactions to SBA in the epithelium (Figure 5A). In the rabbits with dacryocystitis, the luminal surface and the secretory granules containing goblet cell mucus seemed to be specifically stained with SBA. SBA was vestigially bound to the substantia propria (Figure 5B).

In the specimens incubated with a mixture of biotinylated lectin and a specific haptenic sugar, reactions to lectin was significantly reduced over all regions. The observations of lectin binding in the lacrimal sac are summarized for each type of epithelial cell in Table 1. We could not find any difference in lectin binding reaction between the specimens with and without epithelial ulceration.

Discussion

It is widely held that infections in the lacrimal sac are usually secondary to a blockage of the nasolacrimal duct.⁶ In the present study of experimental dacryocystitis, the nasolacrimal duct appeared to be closed by edema of the epithelium, which might have been induced by an allergic reaction to purified egg white albumin.^{7,8} However, the rabbits sensitized with albumin did not develop chronic inflammation without bacterial infection. Therefore, bacterial in-

oculation seems to be a prerequisite for inducing chronic dacryocystitis.

S. aureus used in the present study is not often found in human dacryocystitis. The most common organisms of infection are pneumococci.⁶ It is possible that the allergic reaction might have enhanced the sensitivity of the lacrimal epithelium to the bacteria employed. It is also interesting that the detection rate of *S. aureus* in the mucopurulent substance of chronic dacryocystitis is lower than that of *P. multocida*, which is a component of the normal bacterial flora of the rabbit. In the experimental rabbit model of pneumococcal sinusitis, the pneumococcal infection is gradually replaced by both aerobic and anaerobic types of bacteria.⁸ This phenomenon is considered to be related to the reduction in antral oxygen pressure, which encourages the growth of anaerobic bacteria.⁹ If this is the case, these results might suggest that the initial staphylococcal infection is gradually replaced by anaerobic *P. multocida* because of the chronic inflammation.

To date, few investigations using experimental dacryocystitis have been published. By contrast, a number of cytochemical studies of the epithelium with chronic inflammation have been reported concerning experimental rabbit sinusitis¹⁰⁻¹² or *Nippostrongylus brasiliensis* infected rat intestine.¹³ In experimental sinusitis, there seem to be both quantitative and qualitative changes in the mucus secreted by the goblet cells. Furthermore, the conversion of neutral MPs into acidic MPs, mainly sulfated MPs, was shown.¹ These findings seem to be fairly consistent

Table 1. Lectin-binding to Lacrimal Sac Epithelium of Rabbit With and Without Chronic Inflammation

	LS	Epithelial layer			SP
		Superficial	Middle	Basal	
UEA-I c	-	-	-	-	-
I	+++	+++	+++	+++	-
RCA-I c	-	-	-	-	+++
I	++	+	+++	-	-
PNA c	-	-	-	-	++
I	-	-	-	+++	-
SBA c	-	-	-	-	++
I	++	++	++	-	-

LS: luminal surface of lacrimal sac epithelium; SP: substantia propria of lacrimal sac; c: lacrimal sac epithelium of control rabbit; I: inflamed lacrimal sac epithelium of rabbit with dacryocystitis; UEA-I: Ulex europaeus agglutinin 1; RCA-I: Ricinus communis agglutinin 1; PNA: peanut agglutinin; SBA: soybean agglutinin.

Intensity of lectin labeling was qualitatively evaluated visually as negative (-), weak (+), moderate (++), or intense (+++).

with the present experimental dacryocystitis. AB-PAS staining revealed neutral MPs in the hyperplasia of the goblet cells in chronic dacryocystitis. MPs are long polysaccharide chains comprising repeated disaccharide units, and they adopt the so-called random-coil conformation that could contribute to the stability of the mucus.¹⁴ Miller and colleagues¹⁵ reported that the microorganisms were trapped in a thick mucus layer. Mucus newly generated by goblet cells in dacryocystitis seems to act as a mechanical barrier against microbial invasion.

It has been suggested that the real site of infection is the epithelium itself, and mucopurulent substances are the yield of bacterial inflammation in chronic sinusitis.⁹ However, a series of cytochemical studies of experimental sinusitis^{10–12} focused on the quantitative and qualitative changes in the mucosal barrier secreted by goblet cells. Nevertheless, there are few reports concerning the constitutional changes of glycoconjugates in the inflamed epithelium.

Using lectin cytochemistry, it has been discovered recently that the glycoconjugate composition in the epithelium is remarkably changed in the various tissues with chronic inflammation. An increase in the amount of α -L-Fuc and GalNAc has been reported in chronically inflamed gingiva.¹⁶ The expression of α -L-Fuc has also been observed related to inflammation in the pelvic ileo-anal reservoir.¹⁷ In the present study, the expression of specific receptors for SBA and UEA-I, which have affinities for GalNAc and α -L-Fuc, respectively, was revealed in the lacrimal sac epithelium during chronic dacryocystitis. These findings may reflect a fundamental abnormality in glycoconjugate synthesis in the epithelium during chronic inflammation.

RCA-I, which has an affinity for β -D-Gal, is known as a sensitive marker for microglia responding to mechanical injury or focal inflammation in HIV encephalitis.¹⁸ In the present study, RCA-I bound specifically to the middle layers of cells in the inflamed lacrimal sac epithelium. By contrast, the basal cells of the epithelium were specifically bound by PNA. PNA, which has an affinity for Gal β 1,3 GalNAc, is also a marker of ulcerative colitis, in which PNA-positive substances accumulate in the supranuclear region of colonic surface epithelial cells.¹⁹ In the normal lacrimal sac epithelium, such substances responding to inflammation are not found. It has been discovered that the glycoconjugates in cutaneous basal cells are altered in their differentiation to more apical layers.²⁰ The specific bindings of RCA-I and PNA might indicate that the glycoconjugates associated with chronic inflammation are first

expressed in the basal layer, and modified during differentiation to a more apical layer. However, in the present study, the precise mechanism of sugar modification is still unknown.

The precise role of newly expressed glycoconjugates in the lacrimal sac epithelium during chronic inflammation remains unclear. It has been proposed that the immune system may regulate glycosyl transferase activity in the Golgi apparatus of the *Nippostrongylus brasiliensis*-infected cells.¹³ In fact, attachment of microbial organisms to the epithelium is prevented by glycoconjugates having altered sugar residue.²¹ However, there is a possibility that the newly generated glycoconjugates contribute to the onset and maintenance of chronic inflammation.²² If this is the case, the constitutional change of the glycoconjugates may reflect an adaptive response of the epithelial cells to the new environment associated with chronic dacryocystitis.

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