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# Lectin Histochemistry of the Glycoconjugates in Partridge and Quail Conjunctival Epithelia

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**ABSTRACT** The aim of this study was to examine the distribution of the glycoconjugates in conjunctival epithelium of partridge (*Alectoris chukar*) and quail (*Coturnix coturnix*). Eyelid samples harvested from both species were subjected to routine tissue processing. Lectin histochemistry was applied to sections in order to demonstrate the expression of glycoconjugates. Six different HRP-conjugated lectins (Con A, UEA-I, PNA, HPA, MAA and BSA I-B4) were used for this purpose. The conjunctival epithelium in both partridge and quail consisted of goblet cells and nongoblet cells. Both PNA and HPA bound, to varying degrees, to the goblet cells and nongoblet cells in conjunctival epithelium of partridge and quail. Con A reacted with nongoblet cells in both species. While partridge conjunctival goblet cells showed no reaction to Con A, quail conjunctival goblet cells had a very weak reaction to Con A. UEA-I did not bind to any cells in partridge, however, quail conjunctival goblet cells and nongoblet cells could react to UEA-I. In both species, conjunctival epithelial surfaces reacted with Con A, UEA-I, PNA and HPA. However, MAA and BSA I-B4 did not bind any cell and/or part in both partridge and quail conjunctiva. The present data suggest that composition of glycoconjugates could be different between goblet cells and nongoblet cells of these species, but it could be very similar at conjunctival epithelial surfaces on which the content of the goblet cells and nongoblet cells is released.

***Keywords:*** *Conjunctiva, Glycoconjugates, Goblet cell, Lectin histochemistry, Partridge, Quail*

**ÖZ**

## Keklik ve Bıldırcın Konjunktiva Epitelindeki Glikokonjugatların Lektin Histokimyası

Bu çalışmada keklik (*Alectoris chukar*) ve bıldırcın (*Coturnix coturnix*) konjonktiva epitelindeki glikokonjugatların dağılımının belirlenmesi amaçlandı. Her iki türden alınan gözkapağı örnekleri rutin doku takibi işlemlerinden geçildi. Glikokonjugat ekspresyonunu göstermek için alınan kesitlere lektin histokimyası uygulandı. Bu amaçla altı farklı HRP-bağlı lektin (Con A, UEA-I, PNA, HPA, MAA ve BSA I-B4) kullanıldı. Hem keklikte hem de bıldırcında konjunktiva epiteli, goblet hücreleri ve goblet olmayan hücrelerden oluşmaktadır. Hem PNA hem de HPA değişen derecelerde bıldırcın ve keklik konjunktival goblet hücreleri ve goblet olmayan hücrelere bağlanmıştır. Con A her iki türde goblet olmayan hücreler ile reaksiyon vermiştir. Keklik konjunktival goblet hücreleri Con A'ya karşı herhangi bir reaksiyon göstermezken; bıldırcın konjunktival goblet hücreleri Con A'ya karşı çok zayıf reaksiyon göstermiştir. UEA-I, keklikte herhangi bir hücre tipine karşı reaksiyon göstermezken; bıldırcın konjunktival goblet hücreleri ve goblet olmayan hücreler UEA-I ile reaksiyon vermiştir. Her iki türde konjunktival epitelyal yüzeyler Con A, UEA-I, PNA ve HPA ile reaksiyon vermiştir. Bununla birlikte, MAA ve BSA I-B4 her iki türde herhangi bir hücre ve/veya kısma bağlanmamıştır. Bu sonuçlar glikokonjugat komposizyonunun bu türlerin goblet ve goblet olmayan hücrelerinde farklı olabildiğini, ancak goblet ve goblet olmayan hücrelerin içeriklerinin salındığı konjunktival epitelyal yüzeylerde çok benzer olabildiğini göstermiştir.

***Anahtar Kelimeler:*** *Bıldırcın, Glikokonjugat, Goblet hücresi, Keklik, Konjunktiva, Lektin histokimyası*

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## INTRODUCTION

Conjunctiva is a biological shield of the eye thin (Gipson et al. 2004). It, together with the eyelids, acts as a physical barrier and protects the cornea from environmental allergens. It also properly distributes the tear film, serves as a source of immunological mediators that combat infection, and supplies epithelial cells and blood vessels for corneal healing (Lawton 1998; Stahl et al. 2002).

It consists of a surface epithelium that is unique among the non-keratinized, both stratified squamous type and stratified columnar type, with interspersed goblet cells (Gipson et al. 2004; Yanoff and Cameron 2011), and that is supported by delicate fibrovascular tissue that contains lymphatic channels (Yanoff and Cameron 2011). Within the epithelium are the mucin-secreting goblet cells, which discharge their contents onto the ocular surface (Stahl et al. 2002). Mucins give the corneal surface smoothness by intercalating the microvillus within the epithelium of the cornea (Reece 2005) and function to hold water on the surfaces of the epithelia synthesizing them. Mucins also serve to trap and remove particulates and pathogens from the ocular surface exposed mostly to the outside environment (Gipson and Argueso 2003).

The surface of the eye is covered by a tear film held by a wet-surfaced, stratified, corneal and conjunctival epithelia. The major origin of the tear film mucus glycoproteins has been thought to be goblet cells of the conjunctiva whose morphological and histochemical features were described in great detail (Gipson and Argueso 2003). Maintainance of tear film on the ocular surface, lubrication and provision of a pathogen barrier on this wet surface is mediated by the mucins (Gipson and Argueso 2003). The possible hypothesis regarding mucin function and tear film structure is that the secreted mucins create a hydrophilic blanket to clear debris and pathogens. Mucins impede cell-cell and cell-pathogen adherence. The expression and glycosylation of mucins are affected by drying, keratinizing ocular surface diseases (Komatsu et al. 1997; Argueso et al. 2003; Gipson and Argueso 2003).

Lectins possess a specific binding affinity for the sugar residues of glycoconjugates, so they are versatile probes for examining the incidence and distribution of glycoconjugates in various tissues (Spicer and Schulte 1992). Lectin histochemistry has been successfully employed to evaluate the composition of the glycoconjugates in the eye of some mammals such as human (Hietanen et al. 1995; Iwakiri et al. 1997; Shatos et al. 2001; Mochizuki et al. 2010; Doughty 2012), rat (Ríos et al. 2000), rabbit (Maeda et al. 1998), guinea pig (Latkovic 1991). However, very little information is available on the histochemistry and lectin-based histochemistry of glycoconjugates present in the avian eye.

Therefore, we here aimed to analyze the glycoconjugate distribution in conjunctival goblet cells and non-goblet cells of partridge (*Alectoris chukar*) and quail (Coturnix *coturnix*) using the lectins Con A, UEA-I, PNA, HPA, MAA and BSA I-B4.

## MATERIALS and METHODS

### Study Protocol

The study was approved by the Animal Care and Usage Committee of Süleyman Demirel University (approval date: 29.11.2011, Decision No: B.30.2.SDÜ.0.05.06.00-131). It was in accordance with the guidelines of the International Association for the Study of Pain.

### Histological Specimens

A total of 8 adult specimens, 4 for *Alectoris chukar* and 4 for *Coturnix coturnix*, were used in the lectin histochemical study. Adult birds were anesthetized intramuscularly with ketamine hydrochloride (50 mg/kg). Decapitation was performed under deep anesthesia. Eyelids were excised. The specimens were fixed in Bouin's fluid for 18h at room temperature, as described previously (Öztop et al. 2018). Then, they were washed three times in 50% of alcohol, followed by dehydration in a series of ascending ethanol, and cleared in xylene and embedded in paraffin. Sections at 5-6 µm thickness were cut and gathered on albumin-coated slides.

### Lectin Histochemistry

After deparaffinizing in xylene and rehydrating in ethanol, the sections were rinsed in distilled water. Lectin histochemistry (LHC) was performed, as described previously (Çinar et al. 2016). The sections were incubated with 0.3% H2O2 (v/v) in absolute methanol for 10 min at room temperature so as to quench endogenous peroxidase activity. After washing in distilled water and washed in 0.01 M PBS (Phosphate Buffered Saline) (pH 7.2) containing 1% BSA (Bovine Serum Albumin), the sections were then incubated with a panel of Horseradish Peroxidase (HRP)-conjugated lectins for 30 min at room temperature and washed in PBS.

The HRP-conjugated lectins, their binding specificities and optimal concentrations used in this study were listed in Table 1. The lectin binding sites were then visualized by DAB (3,3-diaminobenzidine tetrahydrochloride) for 10 min at room temperature and appeared as brown or dark-brown colors. Slides developed with DAB were washed in distilled water, dehydrated in ascending grades of alcohol and cleared in xylol, permanently mounted with entellan and examined under a light microscope (Olympus, CX 41) and photographed using a digital camera mounted on the microscope.

**Table 1.** Sources, abbreviations, carbohydrate binding specificities and concentrations of lectins used in the study.

| Lectins | Carbohydrate binding specificity | Concentration (µg/ml) |
|---|---|---|
| Con A | α-D-mannosyl, α-D-glucosyl | 50 µg/ml |
| UEA-1 | α-L-Fucose | 25 µg/ml |
| PNA | β-Galactose-N-acetylgalactosamine | 20 µg/ml |
| HPA | N-acetyl-D-galactosamine | 10 µg/ml |
| MAA | sialic acid-α(2,3)-galactose | 50 µg/ml |
| BSA I-$B_4$ | α-D-galactocyl, N-acetyl-α-D-galactosaminyl | 25 µg/ml |

## RESULTS

With the exception of the stratified non-keratinized epithelium at the initial part of the conjunctiva in partridge and quail, the goblet cells of conjunctiva were clustered in other epithelial parts and they reacted with the lectins in various degrees. The conjunctival epithelial surfaces were more positive than goblet cells. The positivity was also more prominent in non-goblet cells than in goblet cells.

Reaction intensity was generally stronger at conjunctival epithelial surfaces than in goblet and non-goblet cells. Micrographs depict the reactions of the partridge and quail conjunctival epithelia with the lectins Con A, UEA-I, PNA, HPA, MAA and BSA I-B4 (Figures 1-7). The labeling pattern in the conjunctival epithelia varied significantly with the lectins used. Table 2 summarizes the results.

**Table 2.** Lectin-binding pattern in partridge and quail conjunctival epithelia.

| Lectins | Partridge | | | Quail | | |
|---|---|---|---|---|---|---|
| | GC | NGC | ES | GC | NGC | ES |
| Con A | 0 | 3 | 4-5 | 0-1 | 3 | 4-5 |
| UEA-I | 0 | 0 | 2 | 2-4 | 1 | 2 |
| PNA | 1-4 | 1 | 4 | 3-5 | 1 | 2 |
| HPA | 1-5* | 3-4 | 4-5 | 2-5 | 3 | 3-4 |
| MAA | 0 | 0 | 0 | 0 | 0 | 0 |
| BSA I-$B_4$ | 0 | 0 | 0 | 0 | 0 | 0 |

*1-5 indicates that reaction intensity ranged from very weak to very strong. Negative, 0; Very weak, 1; Weak, 2; Mediate, 3; Strong, 4; Very Strong, 5. GC: Goblet cell; NGC: Non-goblet cells; ES: Epithelial surfaces.

Con A was bound strongly to the conjunctival surfaces (Figure 1 and 2) of both avian species. While there seemed to be no positive reactions in partridge conjunctival goblet cells (Figure 1); very weak reactions were found in some goblet cells, without being positive reactions in most of quail conjunctival goblet cells (Figure 2).

UEA-I was bound moderately to the conjunctival epithelial surfaces in *Alectoris chukar* whereas conjunctival goblet cells, non-goblet cells did not show any reactions against this lectin. In *Coturnix coturnix*, weak, moderate and strong positive reactions were found in the conjunctival epithelial goblet cells (Figure 3). Positive reactions were also discernible at the conjunctival epithelial surfaces.

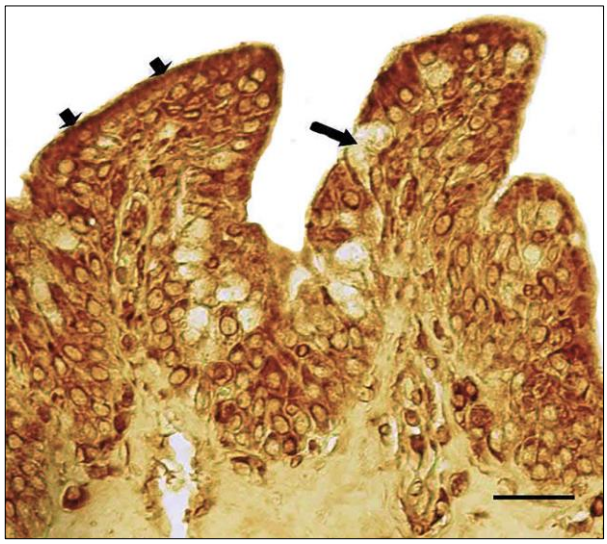

**Figure 1.** Con A staining in partridge conjunctiva. Conjunctival surfaces (short arrows), goblet cells (long arrow). LHC, 40x.

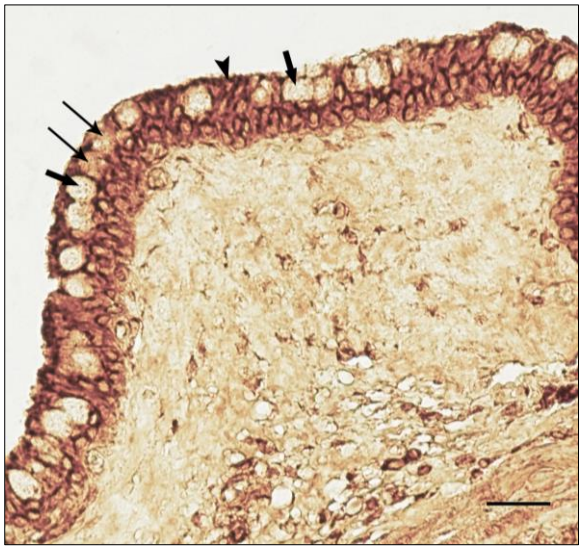

**Figure 2.** Con A staining in quail conjunctiva. Conjunctival surfaces (arrowhead), nonreactive goblet cells (thick arrows), reactive goblet cells (thin arrows). LHC, 40x.

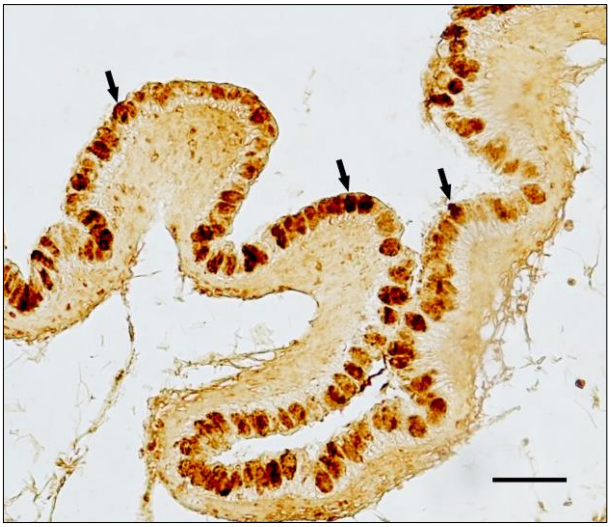

**Figure 3.** UEA- I staining in quail conjunctiva. Reactive goblet cells (arrows). LHC, 40x.

PNA was bound strongly to the partridge conjunctival epithelial surfaces while very weak positive reactions were found in cells on quail conjunctival epithelial surfaces. Partridge conjunctival goblet cells showed different positive reactions from very weak to strong, with being mostly strong reactions (Figure 4). On the other hand, quail conjunctival goblet cells displayed moderate, strong and/or stronger positive reactions (Figure 5).

HPA was bound strongly to the conjunctival epithelial surfaces and epithelial cells in quail and partridge. Both quail (Figure 6) and partridge (Figure 7) conjunctival goblet cells exhibited positive reactions in different intensities from weak to stronger. Weaker positive reactions were also found in partridge conjunctival goblet cells than in quail conjunctival goblet cells. Any cells or surfaces present in conjunctival epithelia studied in quail and partridge did not react to both MAA and BSA I-B4.

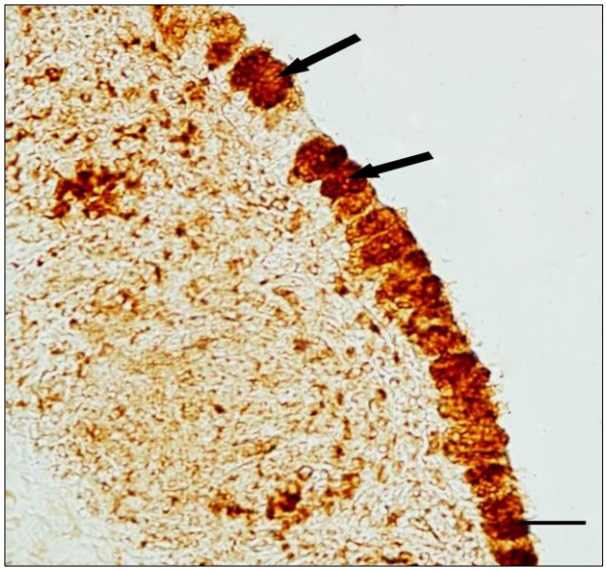

**Figure 4.** PNA staining in partridge conjunctiva. Reactive goblet cells (arrows). LHC, 40x.

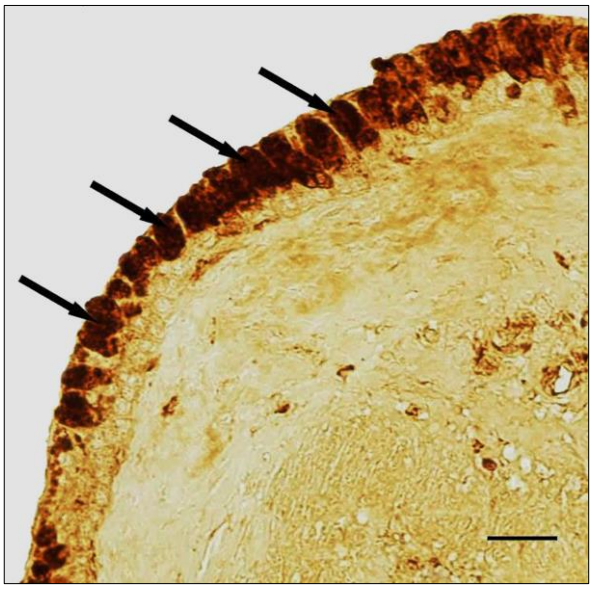

**Figure 5.** PNA staining in quail conjunctiva. Reactive goblet cells (arrows). LHC, 40x.

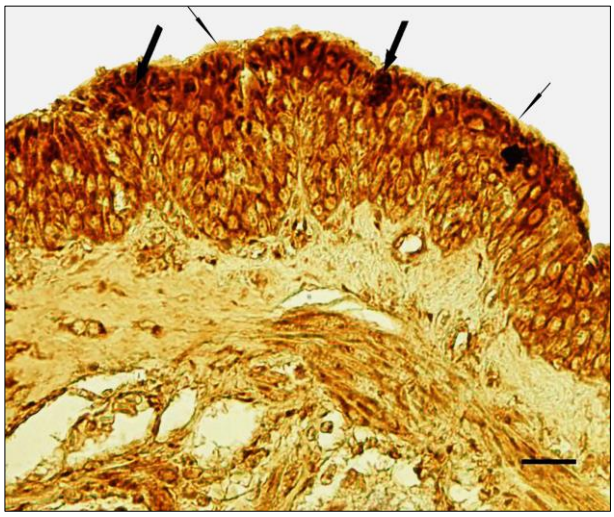

**Figure 6**. HPA staining in quail conjunctiva. Reactive goblet cells (thick arrows), conjunctival surfaces (thin arrows). LHC, 40x.

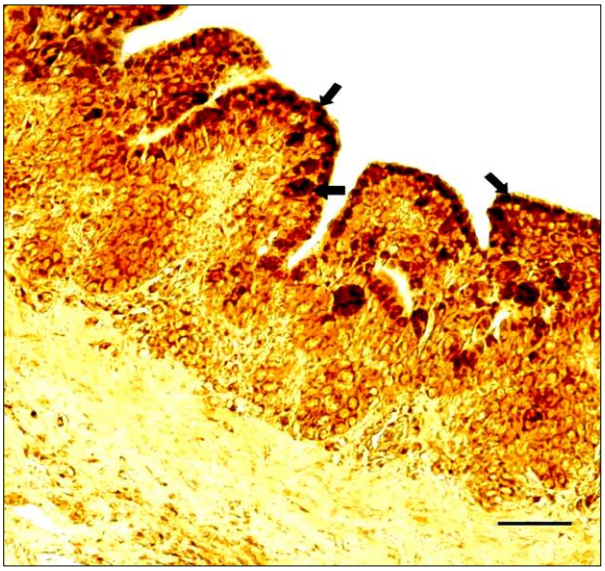

**Figure 7.** HPA staining in partridge conjunctiva. Reactive goblet cells (arrows). LHC, 40x.

## DISCUSSION and CONCLUSION

This study is the first report that investigates the existence of glycoconjugates in the partridge and quail conjunctival epithelia by lectin-based histochemistry. In both species, HRP-conjugated lectins bound more avidly to conjunctival epithelial surfaces than to both goblet cells and non-goblet cells, indicating that the content of the goblet cells and non-goblet cells is indeed released on the conjunctival surface. This is concordant with findings in human conjunctiva (Reid and Clamp 1978). Besides, in both partridge and quail goblet cells, the intensity of lectin binding varied from cell to cell within the same cluster, suggesting that the cells were in different stages of cell differentiation and/or in different stages of the mucin secretion cycle.

With a few exceptions, the prominent reaction of the partridge and quail conjunctival tissues with Con A, UEA-I, PNA, and PNA is agreeable with what would be expected for glycoconjugate types with different histochemical properties and on the basis of the sugar-binding specificity of the lectins (Table 1), they are likely to produce α-D-mannosyl, α-D-glucosyl, β-Galactose-N-acetylgalactosamine, α-L-Fucose, and N-acetyl-D-galactosamine. Thus, conjunctival glycoconjugates have a species-specific glycan expression pattern. As a high percentage of sialylated structures are found in human conjunctival glycoconjugates, fucosylated structures predominate in rabbit and dog conjunctival glycoconjugates (Royle et al. 2008). On the basis of at least the lectins used in this study, we noted that the glycoconjugates with galactosylated and N-acetylated moieties occur in both partridge and quail conjunctiva, and those with fucosylated moieties are more dominant in quail conjunctiva than in partridge conjunctiva. We did, however, not discerned any glycoconjugates with sialylated moieties in the conjunctival epithelium of both species.

Maeda et al. (1998) indicated that Con-A is specifically bound to the non-goblet epithelial cells, but do not react with the goblet cells. Our present findings concerning Concanavalin A, which has an affinity for mannose (Lis et al. 1970), are almost completely consistent with the results of Maeda et al. (1998), except for little differences observed in quail conjunctival goblet cells that reacted

weakly with Con-A. Goblet cells and epithelial surfaces of human conjunctiva did, however, not show any definite reaction to Con A (Kawano et al. 1984). These results indicate that the conjunctival glycocalyx bears a striking resemblance between at least rabbit, partridge and quail, but not human. Hietanen et al. (1995), however, observed that the superficial conjunctival epithelium reacted consistently with concanavalin A in the human conjunctiva of patients with and without exfoliation syndrome.

Maeda et al. (1998) showed that UEA-I recognizes the L-fucose moiety of glycoconjugates at the external surface of the rabbit conjunctiva, and any positive reaction to this lectin is not found in other parts of the conjunctiva and in the vasculature in the substantia propria of the conjunctiva. While our results in partridge are in general agreement with Maeda et al. (1998), our findings in quail conjunctiva are almost completely contrary to those in human conjunctiva (Kawano et al. 1984) and those in rabbit conjunctiva (Maeda et al. 1998). It can be inferred from these results that the glycocalyx of the human, rabbit and partridge conjunctiva shows a similar labeling pattern with UEA-I.

It has been found that human conjunctival goblet cells in vitro react avidly to HPA (Şeftalioğlu et al. 1996) and both goblet cells in vitro and in rat conjunctival tissue show positive reaction to UEA-1 and HPA and no reaction to BSA I-B4 (Shatos et al. 2001). These results are coherent with our HPA positive and BSA I-B4 negative findings in partridge and quail conjunctival goblet cells. Besides, in the present study, whereas positive reaction in quail conjunctival goblet cells is observed with UEA-I, any reaction in partridge conjunctival goblet cells do not discern with UEA-I.

Dartt et al. (1996) and Ríos et al. (1999) indicated that the lectins UEA-I and HPA selectively recognize carbohydrates in secretory products of the adult rat conjunctival goblet cells. Since then, Ríos et al. (2000) observed that goblet cells at different developmental stages react with UEA-I and HPA in various degrees. Their results suggest that the immature goblet cells differentiating during eyelid opening conjunctiva do not express the same carbohydrates in their secretory products as do mature goblet cells. Our present results, which show the reaction to UEA-I and HPA at different intensities from weak to strong in quail conjunctival goblet cells with the exception of no reaction to UEA-I in partridge conjunctival goblet cells, are concordant with literature reports that UEA-I and HPA positive reactions are present in rat conjunctival goblet cells (Dartt et al. 1996; Ríos et al. 1999, 2000).

Kawano et al. (1984) and Hietanen et al. (1995) observed that human conjunctival goblet cells and epithelial surfaces are labeled with PNA intensely. Wells et al. (1988) noticed that goblet cells and epithelial surfaces in normal and pathologic human conjunctival tissues react positively to PNA and non-goblet cells to PNA after treatment with neuraminidase. Our observations conform to those reported in human conjunctiva (Kawano et al. 1984; Wells et al. 1988).

Iwakiri et al. (1997) indicated that MAA binds avidly to human conjunctival goblet cells. This is inconsistent with present findings in partridge and quail conjunctival goblet cells. These observations suggested that the terminal galactosyl residue of the glycoconjugates is alpha 2,3-sialylated in human conjunctival goblet cells, and any sialylated residues do not find in both partridge and quail conjunctival goblet cells. In this regard, the sialylation can be an important process that may modulate immune interactions in conjunctival tissue, perhaps in conjunctiva-associated lymphoid tissue (CALT) or the follicle-associated epithelium of the conjunctiva. Less is known about the glycocalyx above the follicle-associated epithelium of the conjunctiva. It is important to note that M cell in the guinea pig conjunctiva is the only epithelial cell type that expresses α(2-3)-linked sialic acid on the apical surface (Liu et al. 2005). Therefore, it is most likely to find the M cell in the human conjunctiva because the lectin MAA recognizes sialic acid-α(2,3)-galactose in human conjunctival goblet cells (Iwakiri et al. 1997) and M-cell in the guinea pig conjunctiva is the only epithelial cell type that expresses sialic acid. Therefore, it remains to be seen whether the M-cell is present in partridge and quail conjunctiva, in particular, based on using model organism of *Coturnix coturnix*.

In conclusion, the positive reaction to these lectins in both partridge and quail varies from weak to strong and appears to be related to the levels of mucins associated with the cell at the time it is processed for lectin histochemistry. These mucins cover the surface cells of the conjunctiva and cornea. Mucins present in the tear film may play crucial roles in keeping corneal and conjunctival surfaces wet and in the defense of the ocular surface against bactericidal, virucidal agents and/or other infectious agents. There can be an alternation either in glycoconjugate distribution or glycosylation, fucosylation, acetylation or sialylation patterns of glycoconjugates on the conjunctival epithelial surfaces, in conjunctival goblet cells and non-goblet cells in various eye diseases, including conjunctivitis and dry eye. It is considered that present findings may be useful in the search for the importance of glycoconjugates in order to elucidate the pathophysiology and pathogenesis of eye diseases.

## CONFLICTS of INTEREST

The authors report no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Idea / Concept: KÇ, ED
Design: KÇ, ED
Supervision / Consultancy: KÇ
Data Collection and / or Processing: MÖ, ED
Analysis and / or Interpretation: MÖ, KÇ
Literature Review: MÖ
Writing the Article: MÖ
Critical Review: MÖ, KÇ, ED

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