tracings in Fig. 3, show the result of simulation enough for the present purpose. The three sets of tracings in Fig. 3, B., show the result of simulation with the use of the circuit in Fig. 1, B., with three different values of time constant (4.5, 9, and 18 sec) and each rod potential in Fig. 3, A., used as an input. The tracings show that the difference in time constant does not affect critically the configuration of the simulated slow PHI but that a value of 9 sec gives tracings closest to the actual slow PHI's, supporting the result of Matsuura et al. A slight deviation seen in the decay phase between the actual and simulated slow PHI's suggests a time constant a little longer than 9 sec for a better fit but not necessarily a change in our interpretation of the underlying mechanism.

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From the Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Conn. This work was supported in part by NIH Research Grant EY02528 from The National Eye Institute, salary support for T. Tomita from Foresight, Inc., and Postdoctoral Research Fellowship to M. Fujimoto from Fight for Sight, Inc., New York City. Submitted for publication Jan. 12, 1979. Reprint requests: Dr. Tsuneo Tomita, Department of Ophthalmology and Visual Science, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510. *Present address: Department of Physiology, St. Marianna University School of Medicine, Sugao, Kawasaki, 213 Japan.

Key words: c-wave, KRG, slow PHI, rod potential, aspartate

REFERENCES


A distinct system of immunity in a variety of animals is located subjacent to epithelial surfaces and is typified by the predominance of immunoglobulin A (IgA) and secretory component (SC) in various external secretions, including tears. The present study examined normal rabbit lacrimal gland, conjunctiva, and cornea for the presence of immunoglobulin and SC. IgA-staining plasma cells predominated within lacrimal gland and conjunctival stroma, and SC was found in the epithelial cells of both of these tissues but not within corneal epithelium. These observations are consistent with findings for other secretory sites in both rabbits and humans and establish lacrimal gland and conjunctiva as integral parts of the rabbit secretory immune system.

A unique system of mucosal immunity exists in a number of animal species. Typically this immune system demonstrates a predominance of immunoglobulin A (IgA) in the mucosal secretions, produced by plasma cells within the secretory tissues. The secretory IgA (SIgA) molecule differs from serum IgA by the addition of an epithelial cell-derived peptide, secretory component (SC). Thus the typical components of a secretory immune tissue are an epithelial surface producing SC and subjacent tissue containing IgA plasma cells. SIgA has been demonstrated in rabbit tears and in human tears, where it exists in relatively high proportion compared with other immunoglobulins. It has been shown that the human lacrimal gland constitutes part of the secretory immune system, with its predominance of IgA plasma cells in the gland interstitium and the presence of SC within the epithelial cells. The present study examines whether ocular adnexal tissues constitute part of the rabbit secretory immune system.

Materials and methods

Animals. New Zealand albino rabbits weighing
Fig. 1. Numerous acini surround a ductule in rabbit lacrimal gland. Specific staining for IgA shows individual and clusters of plasma cells (x) and interstitial localization along basement membranes (arrows). (Rhodamine-conjugated anti-IgA serum; ×480.)

Fig. 2. Rabbit lacrimal gland stained for IgG shows interstitial and basement membrane localization. A single plasma cell (p) was found within gland interstitium. (Rhodamine-conjugated anti-IgG serum; ×480.)

Fig. 3. Rabbit lacrimal gland acinar cells and acinar lumens (short, thick arrows) and ductule epithelial cells (thin arrow) stained for SC. (Anti-SC serum, then fluorescein-conjugated antiserum; ×480.)

intravenous sodium pentobarbital. Tissues were removed immediately, fixed in 10% neutral buffered formalin and 30% sucrose, and embedded as previously described. Serial frozen sections were prepared with a cryostat and were stained individually with each fluorescent reagent for the three major rabbit immunoglobulins by the direct method. Tissue examined for SC was immediately frozen onto blocks with embedding medium; cryostat sections were prepared, ethanol-fixed,7 stained by the indirect method, and then observed under a Zeiss fluorescence microscope.

Fluorescent antibody reagents. Fluorescein- and rhodamine-conjugated goat anti-α, anti-γ, and anti-μ rabbit heavy chains were obtained from Dr. John Cebra of the Biology Department, Johns Hopkins University and from a commercial source (Cappel Laboratories, Downington, Pa.). The reagents were tested for specificity by immuneelectrophoresis and by demonstration of accepted proportions of isotype-specific plasma cells in spleen, lymph node, and ileum.2,7 Goat antiserum for rabbit SC was also obtained from Dr. Cebra and used in an indirect technique with a fluorescein-conjugated rabbit anti-sheep immunoglobulin (Cappel). Rabbit anti-sheep immunoglobulin can be seen to cross-react strongly with goat immunoglobulin in gel precipitation and by fluorescence microscopy.

Results. All lacrimal glands demonstrated the typical tubuloalveolar structure, with plasma cells

approximately 3 kg were used for these studies. Hand-light and slit-lamp examination of all 20 animals used in this study failed to show any evidence of pre-existing ocular disease.

Tissue preparation and fluorescent antibody technique. Rabbits were killed by an overdose of
Table I. Localization of immunoglobulins and SC in lacrimal gland

<table>
<thead>
<tr>
<th>Location</th>
<th>Antisera</th>
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<tbody>
<tr>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>Acinar cells</td>
<td>(+)*</td>
</tr>
<tr>
<td>Acinar intercellular spaces</td>
<td>+</td>
</tr>
<tr>
<td>Interstitium</td>
<td>+</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>Trace</td>
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</table>

*Surface only.

Table II. Localization of immunoglobulins and SC in conjunctiva and cornea

<table>
<thead>
<tr>
<th>Location</th>
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<tbody>
<tr>
<td></td>
<td>IgA</td>
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<tr>
<td>Con junctiva:</td>
<td></td>
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<tr>
<td>Epithelium</td>
<td>-</td>
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<tr>
<td>Stroma</td>
<td>+</td>
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<tr>
<td>Plasma cells</td>
<td>Trace</td>
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<tr>
<td>Cornea:</td>
<td></td>
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<tr>
<td>Epithelium</td>
<td>(+)*</td>
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<td>Stroma</td>
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*Surface only.

scattered throughout the gland interstitium. In some areas these cells were found to occur in clusters, often associated with a duct (Fig. 1). IgA staining was localized to the tips of acinar epithelial cells and occasionally could be found in the intercellular spaces between these epithelial cells (Fig. 1). The gland interstitium showed a definite staining, and the overwhelming majority of plasma cells were positive with this reagent. Only an occasional plasma cell stained for immunoglobulin G (IgG), but interstitial IgG staining was intense (Fig. 2). No areas positive for immunoglobulin M (IgM) could be found within the gland. SC was localized to epithelial cells of both acinar structures and ducts (Fig. 3). In addition, the acinar intercellular spaces and lumens were occasionally positive (Fig. 3). These findings are summarized in Table I.

The conjunctival epithelium stained for IgM only along the outer surface. SC could also be localized to the conjunctival epithelium (Fig. 4, A), and the stroma stained faintly for IgA (Fig. 4, B) and IgG and less so for IgM. Plasma cells were somewhat variable in the conjunctival stroma, but those present stained exclusively for IgA (Fig. 4, B, and Table II). The corneal stroma showed moderate staining for IgG but very faint staining for IgA and IgM. No SC could be found within the corneal stroma (Table II).

Discussion. The present experiments describe the distribution of the major classes of immunoglobulins and SC in rabbit cornea, conjunctiva, and lacrimal gland. Plasma cells producing almost exclusively IgA were found in the conjunctival stroma and adjacent to lacrimal gland epithelial cells. These findings are similar to the distribution of IgA plasma cells and SC along other mucosal surfaces of the rabbit which constitute the secretory immune system.²

The secretory immune system represents a general mechanism of surface or mucosal immunologic defense, defined by relatively high proportions of SIgA in the mucosal secretions as well as adjacent epithelial cell production of SC and subepithelial IgA-producing plasma cells.¹ It has been proposed that the IgA gains access to the mucosal surface by complexing with SC and then
passing through the epithelium. Previous studies of human lacrimal gland established that organ as part of the secretory immune system, and the present study shows that the distribution of IgA and SC in the rabbit lacrimal gland is similar to that described in the human.

The conjunctival localization of SC is a curious finding because stratified epithelial surfaces synthesizing SC have not been previously described. Other studies of ocular adnexal tissues failed to examine conjunctiva for SC. An alternative explanation to production of SC could involve passive adsorption, but this seems unlikely with the absence of SC in corneal epithelial cells and its presence in the deeper conjunctival epithelial cells.

The extracellular distribution of immunoglobulins is similar to previous descriptions for the human and rabbit cornea and conjunctiva and the human lacrimal gland. IgG and IgM are most likely derived from serum, whereas IgA appears to originate from both serum and local production. The localization of IgM to the outermost corneal and conjunctival epithelial cell surfaces may represent passive adsorption, since IgM has been found in low concentration in occasional human tear samples although its presence has not been established in rabbit tears. An epithelial transport mechanism for IgM similar to the IgA system seems unlikely because of the absence of subepithelial cell IgM plasma cells.

The predominance of IgA plasma cells in ocular adnexal tissues as well as along other mucosal surfaces and glands suggests that some component of these tissues in some way influences the localization of IgA cells. Other experiments have suggested that mammary gland produces a factor capable of causing the selective lodging of B-lymphocytes already committed to IgA production. Such a mechanism may also account for the lodging of IgA-committed cells in other tissues, including the conjunctiva and lacrimal gland.

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Key words: secretory immunoglobulin A, immune response, plasma cells, lacrimal gland, conjunctiva

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