Inductive Pathways Leading to Rat Tear IgA Antibody Responses

Deanne M. Ridley Lathers, Randall F. Gill, and Paul C. Montgomery

PURPOSE. To define the inductive pathways leading to rat tear IgA antibody responses.

METHODS. Fluoresceinated dinitrophenylated bovine serum albumin was encapsulated in poly(lactide-co-glycolide) microparticles and was administered by intranasal, ocular topical, or gastrointestinal routes. Histologic methods were used to determine the microparticles' ability to access tissues associated with mucosal inductive pathways. Rats were immunized with microencapsulated antigen by intranasal or ocular topical routes. Tear IgA and serum IgG antibody concentrations were assessed by radioimmunoassay. The frequency of antibody-secreting cells in tissues, postulated to function in tear IgA induction, was measured by enzyme-linked immunospot assay.

RESULTS. Although uptake of microencapsulated antigen was greatest at the site of delivery, ocular topical administration resulted in antigen uptake in the conjunctiva and in nasal-associated lymphoid tissue. Intranasal immunization resulted in earlier and significantly higher tear IgA and serum IgG antibody responses and in higher frequencies of antibody-secreting cells in corresponding draining cervical lymph nodes and lacrimal glands than did ocular topical immunization.

CONCLUSIONS. Nasal-associated lymphoid tissue functions as a primary inductive site for tear IgA antibody responses by contributing triggered IgA-committed B cells to the lacrimal gland. (Invest Ophthalmol Vis Sci. 1998;39:1005-1011)

Mammalian lacrimal glands (LGs) serve as the predominant source of IgA in tears and function as major effector sites for ocular mucosal IgA antibody responses. Cell trafficking data and antibody expression data have provided documentation that LGs are linked to the mucosal immune network and suggest that the mucosal immune system contributes to acquired immune defenses at the ocular surface. In addition to traditional mucosal immunization by oral or gastrointestinal routes, topical administration of particulate antigen or microencapsulated soluble antigen to the conjunctiva (ocular topical) elicits tear IgA antibody responses. More recent evidence now confirms that nasal-associated lymphoid tissue (NALT) also serves as a major inductive site for the mucosal immune network and suggests that NALT may also serve as an inductive site for tear IgA antibody responses.

The mechanism by which ocular topical immunization elicits tear IgA antibody responses is not well defined. Two inductive pathways have been proposed. In the first pathway, antigen would be taken up by the conjunctiva. After conjunctival uptake, it is unknown whether the antigen triggers B cells within the conjunctiva or whether it accesses the draining superior cervical lymph node (sCLN) and triggers B cells within the sCLN. In the second pathway, antigen would pass through the nasolacrimal canal and be taken up by microfold cells in the NALT. Antigen would appear in NALT, possibly access the draining posterior cervical lymph node (pCLN), and, ultimately, would trigger a B-cell response. Although the site of B-cell triggering is unknown for antigens taken up by conjunctiva or NALT, antigen-reactive IgA-committed B cells from either of these inductive pathways would gain access to the circulation, traffic to the LG, and differentiate into IgA-secreting cells.

Previous studies in our laboratory using dinitrophenylated Streptococcus pneumoniae demonstrated that a particulate antigen could elicit a tear IgA response after intranasal administration and suggested that NALT could serve as an inductive site. Subsequent studies have shown that carbohydrate microspheres were taken up by conjunctiva and NALT after ocular topical administration and indicated that intranasal immunization using a microencapsulated, soluble antigen elicited tear IgA antibody responses. The present study was designed to extend these earlier observations and to define the primary mucosal inductive pathway for ocular mucosal IgA antibody responses. The rat model was used to determine the capacity of poly(lactide-co-glycolide) (PLG) microparticles containing fluoresceinated antigen to access tissues with a postulated role in tear IgA antibody induction and to compare the ability of antigen-containing microparticles administered by the intranasal or ocular topical routes to elicit tear IgA responses. The appearance of antibody-secreting cells in tissues relevant to tear IgA induction was also studied.

MATERIALS AND METHODS

Animals

Ten- to 12-week-old female Fischer 344 rats (126–140 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN),
TABLE 1. Tissue Distribution Scores of Poly(lactide-co-glycolide) Microparticles Containing Fluorescein Isothiocyanate-Dinitrophenylated Bovine Serum Albumin at Selected Times After Intranasal, Ocular Topical, or Oral Administration

<table>
<thead>
<tr>
<th>Tissue Distribution</th>
<th>10 Minutes</th>
<th>30 Minutes</th>
<th>3 Hours</th>
<th>6 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intranasal Administration</td>
<td>Conjunctiva</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nasal-associated lymphoid tissue</td>
<td>1-2</td>
<td>1-2</td>
<td>1-2</td>
<td>1-2</td>
</tr>
<tr>
<td>Posterior cervical lymph node</td>
<td>0</td>
<td>0-1</td>
<td>1</td>
<td>0-1</td>
</tr>
<tr>
<td>Superior cervical lymph node</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ocular Administration</td>
<td>Conjunctiva</td>
<td>2-3</td>
<td>3</td>
<td>3-4</td>
</tr>
<tr>
<td>Nasal-associated lymphoid tissue</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1-2</td>
</tr>
<tr>
<td>Posterior cervical lymph node</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Superior cervical lymph node</td>
<td>0</td>
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<td>1-2</td>
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<tr>
<td>Peyer’s patch</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oral Administration</td>
<td>Conjunctiva</td>
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<tr>
<td>Nasal-associated lymphoid tissue</td>
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<tr>
<td>Posterior cervical lymph node</td>
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<tr>
<td>Superior cervical lymph node</td>
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</tr>
<tr>
<td>Peyer’s patch</td>
<td>0</td>
<td>0-1</td>
<td>1-2</td>
<td>0-1</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Tissue sections were scored as follows: 0 = 0-5; 1 = 6-20; 2 = 21-50; 3 = 51-100; 4 = more than 100 microparticles per square millimeter. Scores represent data obtained from a minimum of eight sections.

Antigen and Microparticle Preparation

Dinitrophenylated bovine serum albumin (DNP-BSA) was prepared and conjugated to fluorescein isothiocyanate (FITC; Sigma, St. Louis, MO) as detailed previously.20 Protein concentration was determined by a bicinchoninic acid assay (Sigma). DNP-BSA or FITC-DNP-BSA were prepared using an oil-in-water solvent evaporation method.23 Loaded PLG microparticles (mean diameter, 1.7 μm) were washed and lyophilized, and protein entrapment was determined as described previously.13

Microparticle Uptake

The PLG microparticles containing FITC-DNP-BSA (PLG-FITC-DNP-BSA microparticles) were diluted in phosphate-buffered saline (PBS; pH 7.2) and were administered by intranasal, ocular topical, or gastrointestinal routes to anesthetized rats. For intranasal and ocular topical administration, the total dosage of PLG-FITC-DNP-BSA microparticles was 500 μg protein, administered in 20-μl aliquots to each nostril (intranasal route), or 10-μl aliquots administered in 5-μl increments to each conjunctival surface (ocular topical route) after blotting of tears. Oral intubation (gastrointestinal route) was carried out with 2500 μg PLG-FITC-DNP-BSA microparticles in 500 μl PBS. Rats were killed at 10 minutes, 30 minutes, 3 hours, or 6 hours after the administration of the microparticles. Conjunctiva, NALT, sCLN, pCLN, Peyer’s patches (PPs) and LGs were surgically removed as described previously. All tissues were embedded in ornithine carbamoyltransferase (OCT Compound; Miles Scientific, Naperville, IL) and were frozen in liquid nitrogen. Cryostat sections were cut at 8 μm, placed on microscope slides, and mounted using Fluoromount G (Southern Biotechnology, Birmingham, AL). Sections were examined for the presence of PLG-FITC-DNP-BSA microparticles using a fluorescence microscope (Nikon Optiphot; Columbus, OH) and a minimum of eight sections were scored by the following criteria: 0 = 0 to 5; 1 = 6 to 20; 2 = 21 to 50; 3 = 51 to 100; 4 = more than 100 PLG-FITC-DNP-BSA microparticles per square millimeter.

Immunization and Sample Processing

Immunization was carried out with PLG microparticles containing DNP-BSA. Poly(lactide-co-glycolide) DNP-BSA microparticles were reconstituted in PBS (pH 7.2) and administered by intranasal (20 μl per nostril) or ocular topical (10 μl per conjunctival surface) routes in three cycles on days 0, 1, and 2 (first cycle); 25, 36, and 37 (second cycle); and 77, 78, and 79 (third cycle). Each animal received a total dosage of 500 μg protein during each 3-day immunization cycle. Tear and serum samples were obtained from individual animals in each group on day 11 after primary immunization and on days 4, 11, and 25 or 4, 11, and 18 after respective secondary or tertiary immunization. Unstimulated tears (5 μl from each animal) were collected by gentle aspiration with a 5-μl calibrated pipette and housed under conventional conditions, and given water and laboratory chow (Ralston Purina, St. Louis, MO) ad libitum. Animal care and treatment were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
FIGURE 1. DNP-BSA antibody responses at selected times (days after primary, secondary, and tertiary immunization [arrows]) with PLG microparticles containing 500 μg DNP-BSA. Rats were immunized by the intranasal (IN) or ocular topical (OT) routes. Data represent the mean ± SD of tear IgA or serum IgG antibody concentrations. * Intranasal group values that differ significantly from the ocular topical group for that time point at \( P ^{0.05} \). DNA-BSA, dinitrophenylated bovine serum albumin; PLG, poly(lactide-co-glycolide).

Radioimmunoassay

Tear and serum samples were assayed respectively for IgA or IgG anti-DNP-BSA antibodies by using a solid-phase radioimmunoassay described elsewhere.\(^9\) The concentrations of antibody isotypes were calculated from values obtained with calibrated reference standards using a data analysis program (Excel 5; Microsoft, Redmond, WA). The limit of sensitivity for this assay system was 1.6 ng/ml for tear IgA and 3.3 ng/ml for serum IgG. Samples were run in triplicate, and the data are presented as mean ± SD.

Enzyme-Linked Immunospot Assay

ELISPOT assays were performed using an adaptation of previously described methods.\(^24\) In brief, LG and lymph node (sCLN and pCLN) cells were counted by trypan blue exclusion (Life Technologies) and were plated on pretreated 96-well nitrocellulose-coated plates (Multiscreen Filtration System; Millipore, Bedford, MA). Twenty-four hours before assay, wells were coated with 100 μg sterile DNP-BSA (50 μg/ml) in PBS (pH 7.4), and the plates were stored in a humidified chamber at 4°C. On the day of the assay, wells were washed three times with 200 μl/well sterile PBS and were blocked for 1 hour with 200 μl/well IMDM-5 at 37°C. Ten minutes before adding the cell suspensions, wells were further blocked with IMDM containing 10% fetal bovine serum at room temperature. Triplicate dilutions of cells (10^4, 10^5, and 10^6 cells/well) in 100 μl IMDM-5 were added to the wells. The plates were incubated overnight at 37°C in 5% CO₂ and were washed 10 times with PBS. After a final wash with dH₂O, 50 μl alkaline phosphatase-conjugated anti-IgA or anti-IgG antibodies (The Binding Site, San Diego, CA), diluted 1:1000 in PBS and 1% BSA, were added to the appropriate wells. Plates were incubated 2 hours at room temperature and washed six times with PBS and Tween 20. After another wash with PBS, 50 μl of BCIP-NBT (Sigma-Fast Blue Tabs; Sigma) was added to each well. Plates were incubated for 45 minutes at room temperature in the dark, washed three times with dH₂O, and allowed to air dry at least 24 hours before spot-forming cells were counted by using a dissecting microscope (Bausch & Lomb, Rochester, NY). All experiments were run in duplicate, and data were expressed as the mean number of antibody-secreting cells per 10^6 cells ± SD.

Statistical Analysis

Radioimmunoassay and ELISPOT assay data were analyzed using an unpaired \( t \)-test. \( P \) values ≤ 0.05 were considered significant.
The uptake and distribution of the PLG-FITC-DNP-BSA micro-

elements were seen in the conjunctiva at all time points studied (Table

TABLE 2. Frequency of IgA Antibody-Secreting Cells at Selected Times After Primary, Secondary, or Tertiary

Intranasal or Ocular Topical Immunization with Poly(lactide-co-glycolide) Microparticles Containing 500 μg

Dinitrophenylated Bovine Serum Albumin

<table>
<thead>
<tr>
<th>Cycle/Day</th>
<th>Lacrimal Gland</th>
<th>Posterior</th>
<th>Superior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IN</td>
<td>OT</td>
<td>IN</td>
</tr>
<tr>
<td>1/14</td>
<td>30 ± 0°†</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>2/7</td>
<td>137 ± 2.5†</td>
<td>20 ± 0.7</td>
<td>30 ± 0.7†</td>
</tr>
<tr>
<td>2/14</td>
<td>137 ± 2.9†</td>
<td>83 ± 0.4</td>
<td>7.7 ± 1.1†</td>
</tr>
<tr>
<td>2/28</td>
<td>27 ± 0.4†</td>
<td>7 ± 0.4</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>3/7</td>
<td>305 ± 1.1†</td>
<td>57 ± 1.6</td>
<td>47 ± 0.9†</td>
</tr>
<tr>
<td>3/14</td>
<td>140 ± 1.3†</td>
<td>83 ± 1.1</td>
<td>13 ± 0.4†</td>
</tr>
<tr>
<td>3/21</td>
<td>143 ± 0.4†</td>
<td>90 ± 0.7</td>
<td>17 ± 0.4†</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean frequency of DNP-BSA specific IgA-secreting cells per 10^6 total cells ± SD.
† Denotes statistically significant values of corresponding groups for each tissue at P ≤ 0.05.

IN, intranasal; OT, ocular topical.

RESULTS

The uptake and distribution of the PLG-FITC-DNP-BSA microparticles were studied at various time points after intranasal, ocular topical, or gastrointestinal administration. At all time points (10 minutes, 30 minutes, 3 hours, and 6 hours), intranasally administered microparticles were found in the NALT (Table 1). Microparticles were seen in the pCLN at 30 minutes, 3 hours, and 6 hours after administration but not in the sCLN, conjunctiva, PP, or LG at any of the time points studied (Table 1). Microparticles administered by the ocular topical route were seen in the conjunctiva at all time points studied (Table 1), in the NALT at 30 minutes, 3 hours, and 6 hours and in the sCLN 30 minutes and 3 hours after administration. They were not seen in the pCLN, PP, or LG at any time point (Table 1). Gastrointestinallly administered microparticles were not seen in the conjunctiva, NALT, pCLN, sCLN, or LG at any time point studied but were seen in the PP 30 minutes, 3 hours, and 6 hours after administration (Table 1). In all cases, the number of microparticles was highest in tissues at the delivery site. As seen in Table 1, after intranasal and ocular topical administration, microparticle uptake occurred at the respective delivery sites (NALT and conjunctiva) at the earliest time point (10 minutes); and after gastrointestinal administration, the uptake by PP was evident at 30 minutes.

Antibody expression in tears after primary, secondary, and tertiary intranasal or ocular topical immunization is shown in Figure 1. Intranasal immunization elicited significantly higher serum IgG antibody responses after each immunization; the antibody levels were low and were only detected at two time points. The control (nonimmunized) group displayed no tear IgA antibody responses (data not shown).

Antibody expression in serum after primary, secondary, and tertiary intranasal or ocular topical immunization is shown in Figure 1. Intranasal immunization elicited significantly higher serum IgG antibody responses after each immunization;
the most vigorous response was seen after tertiary immunization. Although ocular topical immunization elicited serum IgG antibody responses and a booster effect was noted after tertiary immunization, the antibody levels were lower and less consistent than the responses noted in the intranasally immunized group. The control (nonimmunized) group displayed no serum IgG antibody responses (data not shown).

The frequency of DNP-BSA-specific IgA-secreting cells was determined in the LG, pCLN, and sCLN after primary, secondary, and tertiary intranasal or ocular topical immunization (Table 2). The highest frequency of DNP-BSA-specific IgA-secreting cells was seen in LG tissue. Intranasal immunization resulted in significantly higher numbers of IgA-secreting cells than did ocular topical immunization. The highest frequency of DNP-BSA-specific IgA-secreting cells occurred after tertiary immunization. The frequency of these cells in LG duplicated the kinetics of tear IgA antibody responses observed in the intranasal group (Fig. 1). In pCLN, DNP-BSA-specific IgA-secreting cells were seen only in animals receiving intranasal immunization. In sCLN, no IgA antibody-producing cells were seen until after tertiary immunization; and although at two time points (days 7 and 14) the frequencies were higher in animals immunized by the ocular topical route, the values were low, and a statistically significant difference was recorded only at day 7. Control (nonimmunized) animals displayed no DNP-BSA-specific IgA-secreting cells at any time point in any tissues studied (data not shown).

The frequency of DNP-BSA-specific IgG secreting cells was determined in LG, pCLN, and sCLN tissues after primary, secondary, and tertiary intranasal or ocular topical immunization (Table 3). The highest frequencies of DNP-BSA-specific IgG-secreting cells were seen in LG and pCLN. In both tissues, intranasal immunization resulted in significantly higher numbers of IgG antibody-secreting cells than did ocular topical immunization. The highest frequency of DNP-BSA-specific IgG-secreting cells occurred after tertiary immunization. In sCLN, the frequencies of IgG antibody-secreting cells were significantly lower than in LG and pCLN and were significantly higher in animals immunized intranasally. The frequency of DNP-BSA-specific IgG secreting cells was generally lower than that of IgA-secreting cells in LG of intranasally immunized animals but was significantly greater than that seen in pCLN and sCLN of comparable groups immunized by the intranasal and ocular topical route. The frequency of IgG antibody-secreting cells in all three tissues generally followed the kinetics of the serum IgG antibody responses observed in the intranasal and ocular topical groups (Fig. 1). Control (nonimmunized) animals displayed no DNP-BSA-specific IgG-secreting cells at any time point in the tissues studied (data not shown).

**DISCUSSION**

In many species, including rodents and humans, NALT serves as an important mucosal inductive site. Although the present investigations examined IgA antibody induction, it is important to note that antigen presentation to NALT may also downregulate responses or induce tolerance to autoantigens and allergens. Although the mechanisms for tolerance induction are not well defined, peptide antigens appear to exert their effect on the T cell. In IgA antibody induction, antigenic stimulation at mucosal inductive sites results in the triggering of IgA antigen-specific B-cell populations. These B cells emigrate from the inductive sites, enter the lymphatics and traffic to mucosal effector sites where IgA-committed B cells differentiate into antibody-secreting cells, and IgA transport by epithelial cells occurs. Intranasal vaccination has been documented to elicit protective antibodies in tears, parotid saliva, nasal secretions, and serum. The present data define the inductive pathways leading to tear IgA antibody production in the rat model and clearly document the central role of NALT in ocular mucosal IgA antibody responses after either intranasal or ocular topical antigen administration.

The microparticle trafficking data show that the most rapid and pronounced uptake occurred at targeted delivery sites. Appearance of microparticles administered by intranasal or ocular topical routes at 3 hours in the cervical lymph nodes appeared to reflect drainage of microparticles from NALT and conjunctiva to the pCLN and sCLN, respectively. Although it is not surprising that microparticles taken up by microfold cells in the NALT can ultimately access the pCLN, it is of particular interest that the microparticles were seen in the sCLN, which drains the conjunctiva. These data do not address the location of the initial triggering event (conjunctiva versus sCLN), but the appearance of microparticles in the sCLN provided further confirmation that they are taken up by the conjunctiva. Microparticles administered by the ocular topical route also access NALT by 30 minutes, confirming previous data obtained with fluorescent carboxylate microspheres. These appears to result from nasolacrimal duct passage. Although previous observations have demonstrated that radiolabeled soluble antigen can reach the stomach of rats 1 to 2 hours after topical application to the ocular surface, we did not observe microparticles in the PP after intranasal or ocular topical administration. However, the current data show that gastrointestinal administration of microparticles resulted in uptake by PP in gut-associated lymphoid tissue. This latter observation is in keeping with previous findings that orally administered PLG microparticles containing rhodamine are detected in the PP of rats. These data demonstrate that delivery-site-specific uptake could occur in the conjunctiva as well as the NALT and gut-associated lymphoid tissue. Although it is possible that multiple factors may influence the ability of an antigen to access the various tissues with documented (NALT or gut-associated lymphoid tissue) or postulated (conjunctiva) mucosal inductive properties, at no time were microparticles seen in the LG. This latter observation suggests that, although LG contains all of the cellular components to function as an inductive site, under the immunization conditions used in these studies, LG functioned exclusively as an effector site serving as a repository for B cells triggered elsewhere.

The increased frequency of IgA antibody-secreting cells in LG after intranasal immunization compared with ocular topical immunization correlates with the IgA antibody response data and is consistent with previous observations using particulate and microencapsulated soluble antigens. These data and the tear IgA antibody data show that intranasal immunization is more effective than ocular topical immunization in eliciting ocular mucosal IgA antibody responses. The correlation between the numbers and appearance of IgA antibody-secreting cells in LG and the kinetics of tear IgA antibody responses also further confirms previous observations that the LG is the source of IgA in tears. In addition, the appearance of significantly higher numbers of IgA-secreting cells in LG after intranasal and ocular topical immunization compared with
those in pCLN, which drains NALT, and sCLN, which drains conjunctiva, indicates that the LG is the major site for the differentiation of IgA-committed B cells.

Regarding the frequency of IgG antibody-secreting cells, it is interesting to note that substantial numbers of these cells were detected in the three tissues studied (LG, pCLN, sCLN) and that the numbers were significantly higher in tissues from intranasal rather than ocular topical groups. Although there is some general correlation between these data and the serum IgG antibody response data, it is less precise than that noted in the tear IgA antibody responses. This is not surprising, because the relative contributions of antibody produced in these tissues (LG, pCLN, sCLN) to the serum IgG pool is unknown, and not all tissues that could contribute to the serum IgG antibody pool were studied. Based on the microparticle trafficking data, it appears that antigen could access the pCLN, which drains NALT, and the sCLN, which drains conjunctiva, indicating that IgG antibody-secreting cells could be triggered in these tissues. The appearance of IgG antibody-secreting cells in pCLN of ocular topical immunized animals may result from trafficking of these cells from the sCLN. It is more difficult to account for the appearance if the IgG antibody-secreting cells in sCLN of intranasal animals as well as in LG of animals in the intranasal and ocular topical groups. In each group, microparticles could not be detected in these tissues, suggesting that limited triggering of resident B cells occurs at these sites and more likely results from the random recirculation of IgG antibody-secreting cells that gain access to the circulation after activation in other tissues. Regarding LG, this latter alternative is consistent with results in previous studies suggesting that lymphocyte entry into LG may be random and that the selective accumulation of IgA-secreting cells appears to occur by interactions that take place within the LG tissue. The higher numbers of IgA antibody-secreting cells in LG of the intranasal and ocular topical groups lends support to this latter possibility.

In summary, these data indicate that PLG microparticle-encapsulated soluble antigen can be used to elicit LG-mediated tear IgA antibody responses and serum IgG antibody responses. Intranasal immunization is a more effective route than ocular topical immunization in eliciting these antibody responses, and NALT functions as a primary inductive site for tear IgA antibody responses by contributing triggered IgA-committed B cells to lacrimal glands. These studies define the pathways leading to ocular mucosal IgA antibody production and provide new strategies for the induction of protective antibody-mediated immune responses at ocular surfaces.

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References


