Lymphocyte Adhesive Interactions With Lacrimal Gland Acinar Epithelial Cells in Primary Culture

Nancy L. O'Sullivan, Rajiv Raja, and Paul C. Montgomery

Purpose. In lacrimal glands, cell–cell interactions control the localization of lymphocyte populations that play a role in immune defense at the ocular surface. This study describes lymphocyte adhesive interactions with cultured lacrimal gland acinar epithelial cells.

Methods. Primary cultures of lacrimal gland epithelial cells were used as targets for in vitro lymphocyte binding assays. The relative adherence of lymphocyte populations was determined. Various physiologically active agents and putative ligand analogs were tested for their effect in the binding assay.

Results. Thoracic duct lymphocytes (TDL) bound to cultured lacrimal acinar epithelial cells in greater numbers than did thymocytes (54 cells/mm² versus 8 cells/mm²). B cells showed preferential adherence compared with T cells (75% slg+, 14% W3/13+). Thoracic duct lymphocyte binding required intact metabolic and membrane–cytoskeletal function and was inhibited by treating the lymphocytes with sodium azide, formaldehyde, or cytochalasin B (23%, 12%, and 10% of control binding, respectively). Further, adherence was dependent on divalent cations. Ethylenediaminetetraacetic acid-mediated inhibition (42% of untreated) was restored by replacing calcium (89%) but not magnesium (41%). Lymphocyte adherence was inhibited in the presence of fucoidin or phosphomannan polysaccharides (36% and 48% of control binding, respectively). Fibronectin peptides, which are involved in certain types of integrin-mediated adherence, had no effect in this system. Lacrimal culture supernatants contained a factor that was inhibitory for TDL adherence (more than 50% inhibition when concentrated 5 or 10 times).

Conclusions. Thoracic duct lymphocyte adherence to cultured lacrimal gland acinar epithelial cells shows good correlation with previously reported adherence to lacrimal gland frozen sections. Further, lacrimal cell culture supernatants contain soluble factors that inhibit TDL adherence to epithelial cells. These findings suggest that the lacrimal molecules involved in lymphocyte localization are shed and that lacrimal epithelial cell cultures will be useful for ligand isolation and characterization. Invest Ophthalmol Vis Sci. 1995;36:2246–2253.

The mucosal immune system protects the ocular surface from microbial and antigenic challenge, primarily through secretory immunoglobulin A (IgA) antibodies. The lacrimal gland is the predominant source of tear IgA antibodies. All the cell populations required to produce a local immune response, including plasma cells, T cells, B cells, dendritic cells, and macrophages, are found in the gland’s interstitium. Lacrimal acinar epithelial cells produce secretory component and actively transport polymeric immunoglobulins into the tears. The presence of IgA antibodies in the tears results from interactions between emigrating IgA-committed B cells and resident lacrimal regulatory immunocytes, parenchymal epithelial cells, or both, leading to the recruitment and retention of relevant populations as well as the delivery of appropriate differentiation signals.

In vivo studies have established that IgA-positive cells from mucosa-associated lymphoid tissues traffic to, and accumulate in, lacrimal glands. In vitro assays have been used to examine adhesive interactions between lymphocytes and lacrimal gland tissues. Lymphocyte adherence to lacrimal gland cryosections is...
primarily to the acinar epithelial cells.\(^5\) Binding requires lymphocyte viability and intact metabolic and cytoskeletal function, is dependent on the presence of calcium ions,\(^3\) and predominantly involves B cells.\(^3\) Although it remains unclear which adhesion molecules mediate lymphocyte–lacrimal gland interactions, attempts to inhibit binding with a panel of monoclonal antibodies initially implicated the involvement of L-selectin and the Peyer’s patch homing receptor.\(^4\)

To analyze further the adhesive interactions between lymphocytes and lacrimal gland epithelial cells, we used short-term primary cultures of rat lacrimal acinar epithelial cells. The current study sought to evaluate the capacity of lymphocyte populations to adhere to cultured lacrimal cells; to characterize and compare the physiologic aspects of the adhesive interactions with the binding previously reported for lacrimal gland cryosections, and to determine whether molecules mediating lymphocyte–lacrimal interactions could be isolated from cultured lacrimal acinar cells.

**MATERIALS AND METHODS**

**Animals and Surgical Procedures**

Male Fischer 344 CDF/Cr1BR rats, each weighing 175 to 275 g, were purchased from Charles River Breeding Laboratories (Kingston, NY) and used as the source of thymocytes or thoracic duct lymphocytes (TDL). Male Fisher 344/NHsd rats, each weighing 55 to 85 g, were obtained from Harlan Sprague–Dawley (Indianapolis, IN) and used at 4 to 5 weeks of age for lacrimal gland tissue cultures. Thoracic duct cannulations were performed under ether anesthesia using previously described techniques.\(^6\) Animal care and treatment was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Cell Isolation From Lacrimal Glands**

Rats were killed by CO\(_2\) asphyxiation, and the exorbital lacrimal glands were removed and processed for acinar cell isolation using published procedures.\(^6\) Capsules were removed, and the glands were minced into 1-mm\(^2\) pieces, placed into 25-ml Erlenmeyer flasks, and then washed with Hank’s balanced salt solution (HBSS without Ca\(^++\) or Mg\(^++\); Gibco, Grand Island, NY). The fragments were suspended in 10 ml HBSS containing 0.76 mg/ml ethylenediaminetetraacetic acid (EDTA; Fisher Scientific, Pittsburgh, PA) at pH 7.6, gassed for 10 to 15 seconds with 95% O\(_2\) and 5% CO\(_2\), and incubated at 37°C for 17 minutes in a shaking water bath (120 rpm). The fragments were washed twice with Dulbecco’s modified Eagle’s medium (DMEM, Gibco) containing 0.1 mg/ml soybean trypsin inhibitor (STI; Sigma Chemical, St. Louis, MO). The fragments were resuspended in 5 ml of enzyme solution consisting of collagenase (200 U/ml), hyaluronidase (700 U/ml), and DNase I (10 U/ml) (all from Sigma) in DMEM–STI and then were gassed and incubated in the shaking water bath for 17 minutes. The fragments were washed with HBSS, and the EDTA–enzyme incubations were repeated, except that the final enzyme treatment was 45 minutes. The resultant loose fragments were rinsed with 20% fetal calf serum (FCS, in DMEM; HyClone, Logan, UT) and were disrupted further by two passages through a 20-gauge needle. The suspensions were filtered sequentially through sterile 500-μm, then 25-μm, Nitex mesh (Tekno, Elmford, NY) and pelleted by centrifugation at 50g. The cells were resuspended in 20% FCS–DMEM, layered over a Ficoll 400 (Pharmacia, Piscataway, NJ) gradient (4%, 3%, and 2% in 20% FCS–DMEM), and spun at 50g for 15 minutes. The resultant cell pellet was composed primarily (>90%) of acinar epithelial cells based on cell size (>15 μm) and the presence of zymogen-like granules.\(^6\) The pellet was resuspended in enriched culture medium, cell number and viability were determined, and the cells were placed in 24-well Falcon Primaria plastic tissue culture plates (Fisher).

**Cell Culture and Supernatant Collection**

Epithelial cells were cultured with or without a coating of Matrigel (Collaborative Biomedical Products, Bedford, MA) at an initial concentration of 20 μl Matrigel/well and 1 × 10⁵ cells/well. The enriched culture medium was DMEM:Ham’s F12 (1:1; Gibco) supplemented with 10% FCS, dexamethasone (10 ng/ml), putrescine (1 mM), epidermal growth factor (50 ng/ml), L-ascorbic acid (25 μg/ml), insulin (5 μg/ml), transferrin (5 ng/ml), and selenous acid (5 ng/ml), all from Sigma, and with 100 ng/ml acidic fibroblast growth factor (Collaborative). The medium was replaced on days 3, 5, and 7, and the cultures were used on day 6 or 7 for the adherence assays. Spent medium (collected on days 5 and 7) from cells grown in the absence of Matrigel was centrifuged at 300g to remove cells, and the supernatant was stored at −20°C.

**Secretory Component Production**

Day 7 cultures were stained for secretory component production using an indirect immunoperoxidase assay. The cultures were fixed with 0.5% glutaraldehyde (GAH, E.M. grade; Polysciences, Warrington, PA) in 0.1 M cacodylate (Sigma) for 10 minutes at room temperature, washed with cacodylate, and blocked with 0.2 M lysine (in cacodylate; Sigma). Endogenous peroxidase was inhibited by treating the cultures with a 1:4 solution of 3% H\(_2\)O\(_2\) (Sigma) in metha-
nol (Sigma). Nonspecific protein binding was blocked by incubating the cultures with 3% bovine serum albumin in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Rabbit anti-rat secretory component, protein-A affinity-purified antibody (prepared in this laboratory at a previously determined appropriate dilution), was added to the wells and incubated overnight at 4°C. The wells were washed by filling with three changes of PBS + 0.05% Tween-20 (Sigma) during a total period of at least 10 minutes. Goat anti-rabbit horseradish peroxidase-conjugated antibodies were added for 30 minutes at room temperature, the cultures were washed three times, and 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma–Fast Tablets; Sigma) was added to the wells for 10 to 20 minutes. Color development was monitored using an inverted microscope, the reaction was stopped by washing with water, and the cells were counterstained with methyl green–thionin (Fisher). Nonspecific staining did not occur when PBS–3% bovine serum albumin was substituted for the primary or primary and secondary antibodies.

Peroxidase Activity

Seven-day cultures of cells grown on Matrigel-coated Lab-Tek 8-well chamber slides (Nunc, Naperville, IL) were fixed with 0.5% GAH in PBS for 10 minutes at room temperature and stained for peroxidase activity using the method of Fahimi. The cultures were rinsed three times with 0.1 M Tris-HCl buffer, pH 8.5, then incubated in the same buffer containing 0.5 mg/ml DAB and 0.02% H2O2 at 37°C for 3.5 hours in the dark. Control wells were incubated in the absence of H2O2. The wells were rinsed with water, counterstained with methyl green–thionin, and examined by light microscopy, and then the percentage of positive cells was determined.

Lymphocyte Adherence Assay

The assay was modified from a previously described binding assay that used frozen sections of lacrimal gland as the target tissue. Culture medium was removed, and the cells were gently rinsed in the wells with 0.1 M cacodylate then fixed for 10 minutes with 3% GAH (in cacodylate). The wells were rinsed three times with cacodylate, then blocked with 0.2 M lysine for 10 minutes. After rinsing three times with RPMI-1640 medium (Gibco; without bicarbonate but with additional NaCl to maintain osmolarity), the cultures were covered with RPMI–FCS (1%) and stored at 7°C until used, generally within 1 hour.

Thymocytes were prepared by mincing thymus tissue and gently pressing the fragments through an 80-mesh stainless steel sieve into RPMI medium. Thoracic duct lymphocytes were isolated by centrifugation from lymph collected, during a 2- to 6-hour period, into PBS–heparin, 1 or 2 days after thoracic duct cannulation of one to four donor rats. Thymocytes and TDL were washed three times with RPMI and resuspended at 2.5 × 10⁶/ml in RPMI–FCS. The adherence assay was performed in quadruplicate by incubating 1.25 × 10⁶ cells (in 0.5 ml) in wells containing fixed, cultured lacrimal gland cells for 30 minutes at 7°C with 80 rpm horizontal rotation on a clinical rotator (Fisher). Nonadherent cells were rinsed with PBS, and the cultures, with the adherent cells, were fixed (3% GAH, 10 minutes, room temperature), rinsed with tap water, and stained with methyl green–thionin for 10 minutes at 37°C. The wells were rinsed with water, air dried, and stored (covered) until counted. For counting, the wells were wet with water and examined at ×200 using an inverted microscope (Olympus, Tokyo, Japan) equipped with a calibrated ocular reticule. Lymphocytes bound to 2.5 mm² (15 grids) of cultured lacrimal cells were counted. Care was taken to enumerate only lymphocytes bound to areas of confluent lacrimal gland epithelial cells, disregarding any matrix-adherent cells. Adherent cell density was calculated by dividing the total number of cells counted by the area examined. To facilitate comparisons in the inhibition studies, the data were expressed as percentage of control binding using the formula: (Mean Number of Treated Cells/mm² − Mean Number of Untreated Cells/mm²) × 100. Significance of differences between groups was determined using the Student’s t-test. Photomicrography was performed after similar adherence assays using acinar cells grown on Matrigel-coated Nunc chamber slides.

Physiological Inhibitors

To test the effects of sodium azide (30 mM, Sigma), EDTA (10 mM) ± cations (12 mM CaCl₂ or MgSO₄, Fisher), fucoidin (10 μg/ml, Sigma), and PPME (polyphosphomannan core from Hansenula hostii phosphomannan prepared by Slodkey ME, US Department of Agriculture [Northern Regional Research Center, Peoria, IL], and provided by Leon MA [Wayne State University, Detroit, MI]) or the fibronectin peptides (200 mM, GRGDS, SDRG, Sigma, and the CS-1 peptide, GPQILNVPST, synthesized at the Macromolecular Core Facility [Wayne State University, Detroit, MI]), the TDL were resuspended in solutions containing these agents and incubated with the lacrimal cells in their continued presence. In studies using cytochalasin B (1.0 μg/ml; Sigma), TDL were pretreated for 20 minutes at 37°C and then assayed in its presence. Formaldehyde fixation was achieved by pretreating the cells with 1.0% formaldehyde for 10 minutes at room temperature and then washing with RPMI–FCS. Thoracic duct lymphocytes were pretreated with 100 U/ml trypsin (Sigma) for 10 minutes at 37°C, washed with RPMI–FCS, and then assayed.
Lymphocyte–Lacrimal Gland Adhesive Interactions

under standard conditions. Control cells were treated in a similar manner except for the absence of test reagents. To test the effect of culture supernatant on adherence, the TDL were assayed in either supernatant or culture medium that had been concentrated by ultrafiltration (Diaflo, 10-kDa cut-off; Amicon, Beverly, MA).

Phenotype of Adherent Cells

The cultures were incubated with TDL using standard adherence assay conditions and then were fixed with GAH (3%, 10 minutes, room temperature). Appropriate dilutions of the unlabeled primary antibodies, namely MRC-W3/13 (CD43, pan T-cell marker; Accurate Chemical and Scientific, Westbury, NY) or rabbit anti-rat IgG F(ab')2 (slg, B-cell marker; Sigma), were added to the wells, and the plates were incubated for 30 minutes at room temperature and washed three times. Species-specific biotin-labeled sheep secondary antibodies (Sigma) were added to the wells and incubated at room temperature for 30 minutes. After washing, a tertiary reagent (Extravidin–HRP; Sigma) was added for an additional 30-minute incubation, the wells were washed, and color was developed using DAB. For control studies PBS–bovine serum albumin was substituted for the first and second antibodies or for the antibodies and Extravidin–HRP.

RESULTS

Culture Characteristics

Lacrimal cells were isolated by EDTA–enzymatic digestion of tissues obtained from 4- to 5-week-old male Fischer 344 rats and grown on a biologic extracellular matrix (Matrigel) in enriched culture medium. By day 6 or 7, the cells had formed a monolayer that was 80% to 90% confluent. The cultures had a heterogeneous appearance when examined by light microscopy, with the majority of cells having an epithelial morphology. These cells were polygonal in shape, had granular vesicles, and appeared to be acinar epithelial cells. Other cells, which had either reaggregated or were never completely dissociated during isolation, attached themselves to the Matrigel and grew as loose clusters with elongated outgrowths organized into discrete networks. These outgrowing cells also had granular vesicles and were considered to be acinar cells. A second cell type was sometimes observed. These had large Weibel–Palade-like vesicles that stained positively for von Willebrand’s factor by immunoperoxidase assay (data not shown) and were presumed to be endothelial cells. Fibroblast-like, spindle-shaped cells that were free of granules and that stained with anti-fibronectin antibody (data not shown) rarely were seen.

A subpopulation of cultured lacrimal cells retained a differentiated phenotype. Data in Table 1 show that peroxidase activity could be detected in approximately 20% of the cells after 7 days of culture. In control wells where H2O2 was omitted, no reactivity was detected. Secretory component, the polymeric IgA receptor and a product of acinar and ductal epithelial cells, could be detected in almost 40% of day 7 cells using an immunoperoxidase assay.

Adherence Assays

Lymphocytes were incubated over fixed lacrimal gland epithelial cell cultures at 7°C and with 80 rpm, conditions similar to those reported previously to evaluate lymphocyte binding to fixed lacrimal gland cryosections. Preliminary experiments determined that the density of adherent TDL under these conditions was comparable to their binding to live lacrimal epithelial cultures at 37°C with no rotation (data not shown). Because fixation prevented loss of lacrimal cells from the wells during the assay and subsequent staining steps and allowed a more direct comparison of data from earlier studies using fixed cryostat sections of lacrimal tissue, fixed cultures were used for the current investigations.

The experiments depicted in Figure 1 and enumerated in Figure 2 were performed to compare the adherence properties of mature, circulating lymphocyte populations, which contain mucosa-seeking precursors of IgA plasmacytes (TDL, Fig. 1A), with immature, noncirculating cells (THY, Fig. 1B). Thoracic duct lymphocytes had a sevenfold greater propensity to adhere to the cultured lacrimal gland epithelial cells than thymocytes. Immunoperoxidase assays, summarized in Figure 3, showed that 75% ± 3% of adherent TDL were surface immunoglobulin-positive B cells, whereas 14% ± 4% stained with the monoclonal antibody W3/13, which recognizes CD43, a T-cell marker. The overlay TDL suspension contained 38% ± 4% B cells and 61% ± 7% T cells.

Table 2 presents data delineating the effects of agents used to probe physiological and molecular aspects of TDL adherence to cultured lacrimal acinar

TABLE 1. Peroxidase Activities and Secretory Component Production by 7-Day Cultured Lacrimal Gland Epithelial Cells

<table>
<thead>
<tr>
<th>Marker*</th>
<th>Percent Positive†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase activity</td>
<td>19.2 ± 1.0</td>
</tr>
<tr>
<td>Secretory component expression</td>
<td>36.4 ± 2.6</td>
</tr>
</tbody>
</table>

* Endogenous peroxidase activity was measured by diaminobenzidine color development, and secretory component expression was determined using an indirect immunoperoxidase assay as detailed in Materials and Methods.
† Mean ± SEM percentage of cells positive determined in four separate wells.
epithelial cells. Interference with TDL cellular metabolism by pretreating the lymphocytes with sodium azide, then performing the binding assay in its continued presence, inhibited binding by 75%. Killing the lymphocytes by formaldehyde fixation obliterated their ability to adhere to cultured cells. Pretreatment of TDL with cytochalasin B and its continued presence during the adherence assay nearly eliminated lymphocyte binding to lacrimal acinar epithelial cells. This agent blocks actin polymerization and interferes with cytoskeletal movements involved in surface membrane modulations that occur during adhesive interactions.\textsuperscript{12,13} Divalent cations were chelated using EDTA, resulting in partial inhibition of TDL binding. Binding was restored by the replacement of calcium but not completely by the replacement of magnesium. Together, these data indicate that TDL adherence to fixed lacrimal acinar epithelial cells was an active, energy-dependent process requiring viable lymphocytes with intact surface membrane function and was at least partially calcium dependent.

A further series of experiments investigated the nature of the lymphocyte receptor(s) involved in TDL adherence to lacrimal acinar epithelial cells. A trypsin-sensitive protein appeared to be involved because trypsin treatment of the TDL significantly decreased binding (treatment E). One type of cell–cell adhesive interaction (selectin mediated) involves lectin-like proteins that recognize carbohydrate ligands.\textsuperscript{14,15} Inclusion of either fucoidin, a sulfated poly-L-fucose carbohydrate, or PPME (polyphosphomannan ester, a polysaccharide consisting entirely of mannose-phosphate), both of which inhibit L-selectin-mediated adhesion,\textsuperscript{14,15} in the incubation medium caused significant reduction of lymphocyte binding. Certain integrin-mediated cell–cell adhesive interactions can be blocked by peptides derived from cell-binding regions of the fibronectin molecule.\textsuperscript{16} Neither the CS-1 peptide nor an arginine, glycine, aspartate (RGD)-containing peptide had any effect on TDL adherence when present in the assay medium, suggesting that integrins such as VLA-4 or VLA-5 are not involved. The control, reverse-sequence peptide, aspartate, glycine, aspartate (DGR), did not cause significant inhibition.

Adhesion molecules are known to be shed from cell surfaces and to retain their functions in the circulation.\textsuperscript{16} Therefore, supernatants from lacrimal gland cell cultures were tested for inhibitory effects in the binding assay. Initial studies determined that medium that had been incubated in Matrigel-coated wells was itself inhibitory, perhaps because of the breakdown or release of extracellular matrix components. The lacrimal gland cells were, therefore, grown in uncoated Primaria tissue culture wells. These cells grew as loose aggregates in suspension, with some cells attaching to the plastic and forming patches of incompletely confluent epithelial cells. Fibroblasts also were seen occasionally in these cultures. The medium was replaced on days 3 and 5. Supernatants were collected on day 5 (cultured from days 3 to 5) and on day 7 (cultured from days 5 to 7) and were concentrated by ultrafiltration. Dilutions of the concentrate were made with culture medium and tested for their effect in the binding assay. The data presented in Figure 4 show that both ×5 and ×10 concentrations of lacrimal gland cell culture supernatants caused more than 50% inhibition of binding compared to similarly concentrated medium. Concentrated culture supernatant diluted to ×1 was not inhibitory. Also, medium (×10 concentrated) did not cause significantly different binding of TDL to the lacrimal acinar epithelial cells than assays performed in the presence of RPMI-1% FCS (standard assay conditions; data not shown).
Lymphocyte–Lacrimal Gland Adhesive Interactions

FIGURE 2. Adherence of thoracic duct lymphocytes (TDL) and thymocytes (THY) to cultured lacrimal gland acinar epithelial cells. Data are the mean ± SEM number of bound lymphocytes per mm² cell monolayer in four wells in three separate experiments.

DISCUSSION

Previous binding studies³,⁴ have demonstrated that viable TDL bind to the acinar epithelial cells of fixed, cryostat, lacrimal gland sections. The assay used was a modification of the Stamper and Woodruff¹⁷ in vitro binding assay, which has been important for the study of adhesive interactions between recirculating lymphocytes and the high endothelial venules of lymph nodes and Peyer’s patches. In high endothelial venule binding, lymphocytes adhere to exposed luminal surfaces of the postcapillary vessels but not to other vasculature or to the parenchyma. One potential limitation in using frozen-tissue section assays for investigating lymphocyte adhesive interactions with normal glandular tissues is that TDL bind not only to acinar cell membranes but also to exposed cytoplasm as well as to the surrounding acinar basement membrane. To confirm the specificity of the interaction and to begin to assess the physiological relevance of the adherence process, we tested the ability of lymphocytes to bind to intact lacrimal acinar epithelial cells grown in tissue culture.

Subpopulations of cells in the lacrimal gland acinar epithelial cultures retained peroxidase activity or continued to produce secretory component after 7 days in culture, implying partial retention of their differentiated state. Because the cultures were not pure acinar cells and the cell layer was not completely confluent, adherent lymphocytes were counted visually using a calibrated ocular reticle for determining area.

Thoracic duct lymphocytes bound to cultured lacrimal gland cells in greater numbers than previously reported for binding to fixed, frozen sections (~100 cells/mm² when incubated at 2 × 10⁹/ml).³,⁴ Incubating similar numbers of TDL over cultured lacrimal cells resulted in heavy binding that was difficult to count visually. Therefore, TDL numbers were reduced to 2.5 × 10⁶ per milliliter to facilitate enumeration. Thoracic duct lymphocyte binding was specific for the

TABLE 2. Physiological Characterization of Thoracic Duct Lymphocyte Adherence to Cultured Lacrimal Gland Cells

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Percent of Control Binding†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium azide</td>
<td>23.4 ± 9.8†</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>11.9 ± 2.6†</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>19.9 ± 1.3†</td>
</tr>
<tr>
<td>EDTA</td>
<td>42.2 ± 10.9†</td>
</tr>
<tr>
<td>EDTA + CaCl₂</td>
<td>89.0 ± 17.2</td>
</tr>
<tr>
<td>EDTA + MgSO₄</td>
<td>41.2 ± 9.3†</td>
</tr>
<tr>
<td>Trypsin</td>
<td>36.5 ± 6.6†</td>
</tr>
<tr>
<td>Fucoidin</td>
<td>36.0 ± 2.6†</td>
</tr>
<tr>
<td>PPME</td>
<td>47.9 ± 2.9†</td>
</tr>
<tr>
<td>CS-1</td>
<td>104.2 ± 6.4</td>
</tr>
<tr>
<td>RGD</td>
<td>100.6 ± 10.5</td>
</tr>
<tr>
<td>DGR</td>
<td>85.8 ± 12.9</td>
</tr>
</tbody>
</table>

* Assays were performed in the presence of NaN₃; EDTA + CaCl₂ or MgSO₄; fucoidin or PPME polysaccharides; or the CS-1, RGD, or DGR peptides. Thoracic duct lymphocytes were pretreated, then washed before assay to determine effects of formaldehyde fixation or trypsinization. Cells were pretreated with cytochalasin B, then assayed in its presence.

† Data are mean ± SEM percentage of control binding in the absence of test agents and represent three to four separate experiments.

* Difference from control binding is significant (P ≤ 0.05 as determined by Student’s t-test).

FIGURE 3. Phenotype of thoracic duct lymphocytes adherent to lacrimal gland acinar epithelial cultures. The percentages of B cells (sIg+) and T cells (W3/13+) were determined using an immunoperoxidase assay. Data represent the mean ± SEM percentage positive for each marker determined in three separate experiments.
Supernatant Concentration

FIGURE 4. Effect of lacrimal gland culture supernatants on thoracic duct lymphocyte adherence to cultured lacrimal acinar epithelial cells. The adherence of thoracic duct lymphocytes suspended in concentrations of culture supernatant were tested for adherence to cultured lacrimal gland acinar epithelial cells. The data represent the mean ± SEM percentage of control binding (using similarly concentrated medium) in three separate experiments.

Acinar epithelial cells in the cultures, with no binding to the other epithelial cell types, fibroblasts, or endothelial cells occasionally present in the cultures. Binding was a property of mature, circulating cells because thymocyte adherence occurred only at low levels. The majority of TDL bound to acinar epithelial cells in both cultures and sections were B lymphocytes (approximately 75% of adherent cells were slg positive in both cases). These data are consistent with binding selectivity because the majority (60%) of TDL in the overlay suspension were T cells. The physiological characteristics of TDL-lacrimal acinar epithelial cell adhesive interactions were similar whether binding was to lacrimal sections or to cultured cells. Both required intact metabolism on the part of the lymphocyte with >70% inhibition of binding in the presence of 10 mM sodium azide. Further, formaldehyde-fixed TDL did not bind well to either sections or cultured cells. Both binding assays were sensitive (>90% inhibition) to the action of cytochalasin B, which disrupts actin microfilaments, implying that the cytoskeleton has an essential role in the formation of stable adherence. Thoracic duct lymphocyte binding to both lacrimal sections and cultured cells was dependent on the presence of calcium but not magnesium ions. Fucoidin and yeast polyphosphomannan ester, polysaccharides that are potent inhibitors of L-selectin-mediated binding to lymph node high endothelial venules, decreased adherence to both sections (93% and 80% inhibition, respectively) and to cultured cells (64% and 52% inhibition, respectively; Table 2). Although integrins are used by a variety of cells for adhesive interaction with the extracellular matrix, several are used by lymphocytes in cell-cell adhesive interactions. VLA-5 binds RGD-containing sites in fibronectin and is present on lymphocytes. Inclusion of synthetic peptides containing the RGD, or the reverse DGR, sequence in the assay did not affect TDL binding to either lacrimal gland sections or cultured cells. Integrin VLA-4 (a4/β1) and the related a4 chain integrin LPAM-1 (a4/β7, Peyer’s patch homing receptor) recognize sequences in the CS-1 fragment of fibronectin. Inclusion of a synthetic decameric CS-1 region peptide in the incubation solution did not affect adherence to either section or to cultured acinar cells. These data suggest that TDL-lacrimal interactions are not mediated by a4 or a5 integrins. The aE/β7 integrin, present on mucosal lymphocytes, has been reported to mediate lymphocyte adherence to cultured epithelial cells. Investigation of this adhesion molecule awaits the availability of rat-reactive antibodies.

Interestingly, lacrimal gland culture supernatants had inhibitory activity for TDL binding to both lacrimal sections (98% at 10X concentration) and cultured cells (Fig. 4). These data suggest that functional molecules mediating lymphocyte adherence are shed from the cultured lacrimal acinar epithelial cells. Characterization of these molecules is in progress.

The current studies show that the previously reported lymphocyte interactions with lacrimal gland cryosections correlate with and extend to cultured lacrimal acinar epithelial cells. Because it appears that lymphocyte homing to and entry into lacrimal gland is random with respect to T- and B-cell populations and subsequent lymphocyte localization and retention is controlled either by interactions with glandular epithelial cells or a secreted product of these cells, the lacrimal gland cultures provide a system to study the effects of a variety of factors (e.g., cytokines, hormones, growth factors) that may regulate the adherence process. In addition, the findings suggest that these cultures will be useful for isolating the acinar epithelial cell molecules that mediate lymphocyte adherence and localization within lacrimal glands.

Key Words

acinar cell culture, cell adhesion, lacrimal gland, lymphocytes, rat

Acknowledgments

The authors thank Dr. David Sullivan for his assistance and advice in establishing the lacrimal gland culture methodol-
Lymphocyte–Lacrimal Gland Adhesive Interactions

ogy and Mr. Ronald Barrett for his help with the photomicrography.

References


