Class II Antigen Expression by Lacrimal Epithelial Cells

An Updated Working Hypothesis for Antigen Presentation by Epithelial Cells

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It has been suggested that aberrant expression of Class II histocompatibility antigens (HLA) is involved in T cell activation and leads to autoimmunity. Although Class II antigen expression was found in various nonlymphoid tissues, including salivary glands, its expression on lacrimal epithelial cells has not been reported. In this study, 12 cadaver lacrimal glands were analyzed for HLA-DR and for the numbers and distributions of T suppressor cells (Ts), T helper cells (Th), B cells, and macrophages. None of these cases exhibited the high numbers of inflammatory cells, tissue damage, and fibrosis characteristic of Sjogren's syndrome. The HLA-DR-positive epithelial cells were detected in ten cases; they represented from less than 1% to more than 70% of the epithelial cells. In these ten positive cases, there were greater numbers of T cells per millimeter squared (229 ± 94 [mean ± the standard error of the mean]) than in the two HLA-DR-negative cases (37 ± 1 [mean ± range]). Three lacrimal gland specimens tested were negative for immunoglobulin (Ig) G-bearing B cells, and two of the three specimens tested had IgA-bearing cells. Acinar cells were isolated from rat and rabbit lacrimal glands and cultured overnight in serum-free media supplemented with several potential mediators of Class II antigen expression: interferon-γ, carbachol, or isoproterenol. Freshly isolated cells did not express Class II antigens at detectable levels, but in most experiments, they began to express the antigen even in the absence of putative mediators. In light of results from recent studies of antigen presentation and epithelial cell membrane dynamics, these findings suggest a hypothesis in which Class II antigen-expressing epithelial cells present antigenic peptides that are generated in the intracellular compartments in communication with the basal–lateral membrane assembly and recycling pathway. Invest Ophthalmol Vis Sci 32:2302–2310, 1991

The lacrimal glands contribute a substantial portion of the volume of the aqueous layer of the precorneal tear film, and lacrimal dysfunction is a major cause of tear film disorders and resulting ocular morbidity. Lacrimal insufficiency often is attributed to aging and to an autoimmune disease, Sjogren's syndrome. It is generally presumed that during progression of the disease in Sjogren's syndrome, inflammatory infiltrates inhibit the secretory function of glandular epithelial cells; the end stage of the disease is characterized by complete destruction of the secretory epithelium and fibrosis of the gland.1–3 Because aging is associated with increasing lymphocytic infiltration and fibrosis of the lacrimal gland, it has been suggested that the factors impairing lacrimal secretion may be similar in aging and autoimmune disease.4

The lacrimal glands are important components of the mucosal-associated lymphoid system,5–7 and their participation in this system depends, in part, on their ability to recruit T and B lymphocytes.7 There is currently little information concerning the normal mechanisms of lymphocytic recruitment or the ways these mechanisms might be perturbed to cause excessive lymphoproliferation. One theory for the etiology of autoimmune disease places special emphasis on the inappropriate or aberrant expression of Class II histocompatibility antigens by nonlymphoid cells. The Class II antigens, HLA-DR in humans and major histocompatibility complex (MHC) Ia in rodents, are transmembrane cell surface structures composed of two polypeptides of molecular weights 33 and 28 kilodaltons.8 These antigenic determinants are found on resting mature B cells, macrophages, and dendritic cells throughout the body.9–12 Class II antigens func-
tion in antigen presentation to T cells in conjunction with the T cell receptor and foreign antigens. According to one theory, the inappropriate expression of Class II antigens by a cell population allows that population to present its own surface constituents, rendering it a target for an immune response.

There are now numerous instances in which autoimmune disease has been correlated with inappropriate Class II antigen expression. These include thyroid epithelial cells in autoimmune thyroiditis and salivary duct cells in Sjögren's syndrome. However, Class II antigen expression by epithelial cells of the lacrimal glands has not been discussed to our knowledge, either for humans or for relevant animal models.

Before attempting to consider the role inappropriate Class II antigen expression might play in either age- or autoimmune-related lymphoproliferation in the lacrimal glands, it seemed essential to confirm that lacrimal gland epithelial cells can, in fact, express Class II antigens. We report here the presence of Class II antigens on epithelial cells and the correlation between this antigen expression and the numbers of T lymphocytes and macrophages in human lacrimal glands. Furthermore, in an attempt to evaluate possible mechanisms for Class II antigen expression, we used isolated rat and rabbit lacrimal acinar cells maintained in short-term culture. In most experiments, the acinar cells began expression of Class II antigens even in the absence of interferon-γ or other potential mediators of Class II antigen expression. On the basis of these observations, we present a hypothesis for antigen presentation by Class II antigen-expressing epithelial cells that incorporates concepts derived from recent studies of antigen presentation by macrophages and of plasma membrane dynamics in secretory epithelial cells.

Materials and Methods

Materials

Cadaver lacrimal glands were obtained as excess tissue from the Department of Anatomy and Cell Biology within 24 hr post mortem (IRB number 06077). Male Sprague-Dawley rats, 5–7 weeks old, were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Male New Zealand white rabbits (2 kg) were obtained from Irish Farms, Norco, CA. All animals were used in accordance with the ARVO Resolution on the Use of Animals in Research.

The following substances were used: OCT (Miles, Elkhart, IN); Ham’s F-12 medium, Dulbecco’s Modified Eagle’s Medium (DME), and fetal calf serum (Irvine Scientific, Irvine, CA); purified collagenase (Gibco, Gaithersburg, MD); hyaluronidase (Worthington, Freehold, NJ); Hank’s medium and DNase type I (Sigma, St. Louis, MO); and human recombinant interferon (Collaborative Research, Bedford, MA).

The following antibodies were obtained: Ox-6 (immunoglobulin [Ig] G1, mouse monoclonal against rat Ia; Sera-lab, Sussex, UK) and Leu 2 (anti-CD8, T suppressor cells), Leu 3 (anti-CD4, T helper cells), Leu 14 (anti-CD22, B cells), Leu M5 (anti-CD11c, macrophages), and anti-HLA-DR (all from Becton-Dickinson, San Jose, CA). Samples of the supernatant of monoclonal culture 2C4, which contains an antibody directed against the rabbit MHC Class II antigen Ia, were provided by Drs. Katherine L. Knight (Stritch School of Medicine, Loyola University) and Robert A. Prendergast (The Johns Hopkins University School of Medicine). Biotinylated horse anti-mouse antibody, biotinylated goat anti-rabbit antibody, and avidin-peroxidase complex were obtained from Vector (Burlingame, CA). Polyclonal rabbit antibodies to human IgA and IgG were from Dako (Carpentaria, CA).

Other reagents were from standard suppliers.

Tissue Preparation

Human lacrimal glands were placed in physiologic saline at 4°C and transported to the laboratory. Fat and readily accessible connective tissue were removed. Glandular tissue was blotted dry, snap frozen, and stored at −70°C.

The rats were killed by CO2 narcosis. The rabbits were sedated with an intramuscular injection of ketamine and xylazine and then given lethal injections of sodium pentobarbital. Lacrimal glands to be used for cryosections were rinsed in physiologic saline, then placed in OCT, snap frozen, and stored at −70°C. The glands to be used for isolation of acinar cells were placed in sterilized Ham’s F-12 medium supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), L-glutamine (2 mM), linoleic acid (0.084 μg/ml), HEPES (10 mM), soy bean trypsin inhibitor (0.05 mg/ml), and bovine serum albumin (5 mg/ml). All solutions were sterilized by filtration through 0.2-μm pore-size filters (Costar, Cambridge, MA). Acinar cells were isolated with the modification of Oliver’s procedure described by Hann et al. They were maintained in 50% Ham’s F-12 medium with 50% low-glucose DME. The medium did not contain serum, but it was supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), glutamine (4 mM), hydrocortisone (5 nM), transferrin (5 μg/ml), insulin (5 μg/ml), epiderma growth factor (10 ng/ml), butyrate (2 mM), and linoleic acid (0.084 μg/ml). Agents tested for their ability to induce Class II antigen ex-
pression were: interferon-γ (1000 U/ml); carbachol (10 μM and 1 mM); and isoproterenol (10 μM and 1 mM). Overnight culture was done in a 37°C water-jacketed incubator under an atmosphere of 95% air and 5% CO₂. After culturing, acinar cells were transferred to Falcon centrifuge tubes (Becton Dickinson, Lincoln Park, NJ), diluted to 15 ml with medium, and sedimented at 100 X g in a preparative centrifuge. They were washed once by resuspension and centrifugation, then resuspended in Hank’s medium, pH 7.4, containing 5% bovine serum albumin. Aliquots of 200 μl were sedimented onto polylysine-coated slides at 800 rpm for 5 min in a cytocentrifuge (Shandon, Pittsburgh, PA). Cell preparations were allowed to dry overnight and then used for immunocytochemistry.

**Immunocytochemistry**

Immunoperoxidase staining of cryostat sections and cytocentrifuge preparations was done as described previously. Briefly, the sections were fixed in acetone for 5 min, then incubated in phosphate-buffered saline (PBS), pH 7.4, for 5 min. Human, rat, and rabbit specimens were pretreated sequentially with avidin and biotin according to the vendor’s instructions (Vector) to reduce nonspecific background staining. Tissue samples were incubated with primary antibodies in a humidified chamber for 30 min at 25°C, then washed for 10 min with 1% bovine serum albumin in PBS. Depending on the source of the primary antibody, the tissues were then incubated either with biotinylated horse anti-mouse antibody or biotinylated goat anti-rabbit antibody for 30 min, rinsed, and washed. They then were incubated with avidin-biotin-peroxidase complex for 20 min and again rinsed and washed. Amino-ethyl carbazole, which produces a red precipitate in the presence of peroxidase, was added to all the slides and incubated for 10 min, followed by Mayer’s hematoxylin for 3 min. The slides were rinsed in tap water for 10 min and mounted in glycerol and PBS. Controls for the staining procedure included both the use of an irrelevant antibody and the use of PBS in place of primary antibody.

The slides were examined using a 1-mm² grid. Macrophages, T lymphocytes, and B cells could be resolved in almost all cases. They were counted in three separate areas per tissue; the results are expressed as mean numbers of cells per millimeter squared ± the standard deviation. It was not possible to resolve individual Class II-positive epithelial cells, and they were scored as: – (negative); +/− (< 1% of epithelial cells positive); ++ (1–30% of epithelial cells positive); and +++ (> 70% of the epithelial cells positive).

**Results**

Lacrimal glands from five women (age range, 71–91 yr; mean, 83 yr) and two men (age range, 87–88 yr) were examined. The HLA-DR was detected on lymphoid cells, as expected, and on 1–100% of the epithelial cells in 10 of the 12 samples tested (Fig. 1). Both the cytoplasm and the surface of the acinar cells appeared to stain for HLA-DR. The staining in an acinar cluster was not uniform but appeared in a mosaic pattern, suggesting differences in the levels of HLA-DR expression by individual epithelial cells. Two of the 12 lacrimal tissues showed no HLA-DR-positive epithelial cells; the lymphocytes in these tissues stained positively for HLA-DR (Fig. 2).

As illustrated in Figures 3–5, T helper and suppressor lymphocytes and macrophages were distributed among the acinar clusters, with 3- to 11-fold more lymphoid cells than macrophages. The data in Table 1 indicate that there were wide ranges in the numbers of helper T lymphocytes, suppressor T lymphocytes, and macrophages. The ratio of T helper to T suppressor cells for the positive specimens did not suggest any particular pattern. Although the number of samples with no epithelial HLA-DR expression was too small to permit generalization, it may be noteworthy that the two negative cases examined had fewer lymphoid cells than nine of the ten samples with positive epithelial cells.

Three of the human lacrimal gland samples also were surveyed for IgA- and IgG-producing lymphocytes. All three samples were negative for IgA. One sample (from a 71-year-old woman) was also negative for IgG. One sample (from a 91-year-old woman), both of which had HLA-DR-positive epithelial cells. The two other samples examined (from an 87-year-old man and a 91-year-old woman), both of which had HLA-DR-positive epithelial cells, were also positive for IgA cells.

In a series of experiments designed to test the abilities of various agents to induce Class II antigen expression, lacrimal acinar cells were isolated from male Sprague-Dawley rats and cultured overnight in control medium and in media supplemented with interferon-γ, isoproterenol, or carbachol. Preliminary experiments also were done with a combination of 1 ng/ml of TPA and 1 μg/ml of A23187 as potential mediators of Class II antigen expression; however this combination was cytotoxic. In one experiment, control cells and cells maintained in the presence of 10 μM carbachol were negative for Class II antigen (Fig. 6), but Class II antigen-positive cells could be detected after overnight culture in the presence of either isoproterenol or interferon-γ (Fig. 7). In two additional
Fig. 1. Human lacrimal gland tissue from an 88-yr-old man is stained with anti-HLA-DR and shows intensely positive acinar cells. Magnification ×100.

Fig. 2. Human lacrimal gland tissue from an 88-yr-old woman is stained with anti-HLA-DR and shows no DR-positive epithelial cells but occasional DR-positive leukocytes (arrows, magnification ×250).

Fig. 3. Human lacrimal gland tissue from an 88-yr-old man is stained with anti-CD4 and shows low numbers of positive T helper cells among the acini. Magnification ×100.

Fig. 4. Human lacrimal gland tissue from an 88-yr-old man is stained with anti-CD8 and shows T suppressor cells distributed among the acini. Magnification ×100.

Fig. 5. Human lacrimal gland tissue from an 88-yr-old man is stained with anti-CD11 and shows a modest number of macrophages. Magnification ×100.

Fig. 6. Isolated rat lacrimal acinar cells from overnight culture show no Class II antigen expression. Magnification ×400.
Table 1. Summary of expression of HLA-DR on epithelial cells and quantitation of leukocyte subtypes present in human lacrimal gland samples

<table>
<thead>
<tr>
<th>Case</th>
<th>Epithelial cells (T&lt;sub&gt;suppressor&lt;/sub&gt;)</th>
<th>Leu5 (macrophages)</th>
<th>Leu14 (B-cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>88-yr-old F</td>
<td>18 ± 5</td>
<td>20 ± 5</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>71-yr-old F</td>
<td>5 ± 1</td>
<td>34 ± 5</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>94-yr-old F</td>
<td>25 ± 13</td>
<td>57 ± 15</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>97-yr-old M</td>
<td>75 ± 18</td>
<td>13 ± 6</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>86-yr-old F</td>
<td>97 ± 20</td>
<td>90 ± 11</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>88-yr-old M</td>
<td>76 ± 13</td>
<td>89 ± 11</td>
<td>27 ± 14</td>
</tr>
<tr>
<td>87-yr-old M</td>
<td>2 ± 1</td>
<td>30 ± 7</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>91-yr-old F</td>
<td>144 ± 33</td>
<td>27 ± 13</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>94-yr-old M</td>
<td>70 ± 19</td>
<td>29 ± 10</td>
<td>85 ± 21</td>
</tr>
<tr>
<td>66-yr-old F</td>
<td>108 ± 17</td>
<td>9 ± 5</td>
<td>34 ± 15</td>
</tr>
<tr>
<td>38-yr-old M</td>
<td>119 ± 13</td>
<td>930 ± 113</td>
<td>225 ± 27</td>
</tr>
<tr>
<td>75-yr-old M</td>
<td>152 ± 31</td>
<td>195 ± 6</td>
<td>69 ± 9</td>
</tr>
</tbody>
</table>

Values presented are mean numbers of positive cells per mm<sup>2</sup>; nd, not done; F, female; M, male.

* Positively staining cells in this sample appeared in clusters and were difficult to identify and count; values given for this sample are estimated percentages of the total tissue area occupied by the clusters.

Discussion

These observations show that epithelial cells of human, rat, and rabbit lacrimal glands, like those of the thyroid gland, salivary glands, hepatic duct, mammary gland, small intestine, and retinal pigment epithelium, are capable of expressing Class II HLA-DR.

In general, the degree of epithelial HLA-DR expression tends to correlate with the extent of lymphocytic infiltration in various autoimmune diseases. This relationship is consistent with the hypothesis that aberrant Class II antigen expression may be capable of triggering an autoimmune response. However, as will be discussed, the cause-and-effect relationship has not been proved.

The observation (Table 1) that the total numbers of lymphocytes were smaller in the two samples with HLA-DR-negative epithelial cells than in nine of the ten samples with positive epithelial cells suggests a general, but by no means absolute, correlation between epithelial Class II antigen expression and numbers of lymphocytes in the lacrimal gland. A similar relationship was suggested by preliminary experiments with lacrimal glands from NZB/W mice; these animals develop lacrimal lymphocytic infiltrates similar to those observed in Sjogren's syndrome. Although these results were difficult to quantify because of an excessive level of background staining, Ia was not apparent on epithelial cells at 7 wk and 12 wk, but it could be detected on small numbers of the epithelial cells at 20 wk, a time interval over which the number of Ia-positive leukocytes roughly doubles (18 per mm<sup>2</sup> at 7 wk, 15 per mm<sup>2</sup> at 12 wk, and 30 per mm<sup>2</sup> at
Fig. 7. Isolated rat lacrimal acinar cells from the same preparation show occasional Class II antigen-positive cells (arrows) after overnight culture in the presence of 1000 U/ml interferon γ. Magnification ×400.

Fig. 8. Normal rabbit lacrimal gland tissue shows no Class II antigen-positive acinar cells, whereas results for leucocytes are positive. Magnification ×100.

Fig. 9. Freshly isolated rabbit lacrimal acinar cells show no Class II antigen expression. Magnification ×400.

Fig. 10. Rabbit lacrimal acinar cells after 3 hr in control culture medium showed positive results for Class II antigen expression. Magnification ×400.

Fig. 11. Rabbit lacrimal acinar cells after 18 hr in control culture medium showed positive results for Class II antigen expression and marked clustering. Magnification ×400.

Thus, it appears that in lacrimal glands, as in other epithelial tissues, the degree of epithelial Class II antigen expression tends to correlate with the degree of lymphocytic infiltration.

Despite the apparent relationship between epithelial Class II antigen expression and lymphocytic infiltration, the observations summarized in Table 1 suggest that epithelial expression of Class II antigens does not lead inexorably to the vigorous autoimmune response characteristic of Sjogren's syndrome. With the exception of the 38-year-old man, all of the cases described in Table 1 showed total numbers of lymphocytes in the range of non-Sjogren's lacrimal glands as described in a younger population of donors by Wiecezek et al.3 Furthermore, none of the cases in our study exhibited the tissue destruction and fibrosis typical of Sjogren's syndrome.2-3 The IgG-bearing B cells, a hallmark of Sjogren's infiltrates,3 were not detectable in the three samples we analyzed.

Attempts to understand the cause-and-effect relationship between epithelial Class II antigen expres-
sion and lymphocytic activation are complicated by the fact that there may be several different mechanisms for induction of epithelial cell Class II antigen expression. One mechanism, now believed to operate in several different epithelial tissues, including the thyroid gland, the salivary gland, and the retinal pigment epithelium, involves interferon-γ released by activated T cells and macrophages in inflammatory infiltrates.

The observation of a lacrimal gland sample with unusually low numbers of lymphocytes and an unusually large number of HLA-DR-positive epithelial cells (from an 87-year-old man, Table 1) raises the possibility that factors other than locally released cytokines might also be able to induce epithelial HLA-DR expression. Because thyroid-stimulating hormone, the hormone which controls thyroid hormone release, induces Class II antigen expression by thyroid epithelial cells in culture and prolactin induces Class II antigen expression by mammary epithelial cells, we attempted to determine whether lacrimal epithelial cell Class II antigen expression might be influenced by interferon-γ, carbachol, or isoproterenol, the latter two neurotransmitter receptor agonists which trigger lacrimal secretion by different intracellular signal transduction pathways. Our results were variable because some rat acinar cell preparations and all rabbit acinar cell preparations contained cells that expressed Class II antigen in culture even in the absence of added potential mediators. Although these experiments yielded little information about the control of Class II antigen expression by lacrimal acinar cells, they confirmed that these cells are capable of expressing the Class II antigen. In most experiments, the cells spontaneously began expression Class II antigen when placed into culture media, and therefore we can conclude that either (1) Class II antigen expression is inhibited by the normal milieu of the lacrimal gland or (2) intracellular or autocrine mediators released as the cells adapt to the conditions of cell culture have the effect of inducing Class II antigen expression. In either case, it appears that the normal lacrimal acinar cell may be ready to begin expressing Class II antigens in response to various potential stimuli, including, for example, interferon-γ, other cytokines, or excessive or sustained (compared with pulsatile) secretomotor stimulation.

If lacrimal epithelial cells begin expressing Class II antigens, then it is likely that there are several other events that would have to occur if they are to activate lymphocytes and instigate an autoimmune response. Recent work with macrophages and other antigen-presenting cells indicates that Class II antigens normally do not present native plasma membrane constituents of the antigen-presenting cell. When macrophages and dendritic cells activate T lymphocytes, they do so by presenting antigenic fragments that have been generated during the proteolytic processing of larger molecules. The processing events occur in an endocytic compartment, and the antigenic fragments return to the cell surface bound to a specific domain of the Class II molecule.

Recent work on the membrane dynamics of secretory epithelial cells suggests the possibility that there might be circumstances in which Class II antigen-expressing lacrimal epithelial cells are able to present intracellularly generated antigenic fragments in a manner analogous to the presentation of foreign antigens by macrophages. There are now several indications that the lacrimal acinar cell basal–lateral plasma membrane, ie, the membrane domain in immediate contact with the glandular interstitium, is highly dynamic. Cholinergic stimulation triggers a rapid redistribution process in which Na+/K+ adenosine triphosphatase and muscarinic receptors are mobilized from cytoplasmic pools, presumably associated with the Golgi complex, and inserted into the basal–lateral membrane. In addition β-adrenergic stimulation has been found to accelerate the internalization of basal–lateral membrane constituents. It now appears that the stimulation-associated insertion and internalization phenomena both are superimposed on a high steady-state rate of basal–lateral membrane recycling; isolated lacrimal acinar cells take up and release the fluid phase marker, Lucifer yellow, with half-times of less than 5 min.

This background information suggests that there may be several different mechanisms by which potentially antigenic fragments are generated inside the acinar cell and enter membrane compartments that communicate with the membrane assembly and recycling pathway traversed by Class II antigens and other basal–lateral membrane constituents. These include a back flux of partially degraded proteins from lysosomes to recycling compartments, a mistargeting of lysosomal proteases to the recycling compartments, and a premature activation of newly synthesized lysosomal proteases during their transit through the Golgi complex.

This working hypothesis is still speculative. It incorporates the observations that lacrimal epithelial cells are capable of expressing Class II antigens and that they maintain an extensive traffic of membrane constituents between the Golgi complex, rapidly recycling intracellular compartments, and the basal–lateral plasma membrane. Although there is no direct evidence for the subcellular targeting errors we postulate to account for the generation of potentially antigenic peptides by lysosomal proteases, we are aware of one precedent for the inappropriate accumulation of
lyosomal proteases in a compartment containing peptides destined for other cellular loci. This occurs in rats treated with supramaximal doses of cerulein, an agonist for the cholecystokinin receptor. In this animal model, lysosomal proteases and secretory zymogens, including trypsinogen, accumulate in aqueous vacuoles in the apical cytoplasm of pancreatic acinar cells. It is believed that limited proteolysis by the lysosomal proteases results in activation of secretory zymogens, releasing activated proteases into the acinar cytoplasm and triggering an acute pancreatitis.42

We anticipate that the mistargeting events that would generate potentially antigenic peptides in lacrimal epithelial cells normally are rare and that the low rate at which they occur could be one of several factors accounting for the observation that epithelial Class II antigen expression alone is insufficient to trigger autoimmune reactions.

Key words: Sjogren's syndrome, dacryoadenitis, membrane recycling, antigen presentation

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