Does Androgen Insufficiency Cause Lacrimal Gland Inflammation and Aqueous Tear Deficiency?

David A. Sullivan,1 Kathleen L. Krenzer,1,3 Benjamin D. Sullivan,1 Dorothy Bazzinotti Tolls,3 Ikuko Toda,1 and M. Reza Dana1,2

PURPOSE. The current investigators have shown that androgen treatment suppresses inflammation and stimulates the function of lacrimal glands in mouse models of Sjögren's syndrome. Recently, others have hypothesized that androgen insufficiency induces an autoimmune process in lacrimal tissue, leading to inflammation, a Sjögren's syndrome-like pathology, and aqueous tear deficiency. The purpose of the present study was to test this hypothesis.

METHODS. Lacrimal glands were obtained from adult testicular feminized (Tfm) and control mice; castrated rats, guinea pigs, and rabbits; and castrated rats without anterior or whole pituitary glands and were processed for histology and image analysis. Tear volumes were measured in mice, in patients taking antiandrogen medications, and in age-matched human control subjects.

RESULTS. Tfm mice, which are completely resistant to classical androgen action, did not have increased lymphocyte infiltration in their lacrimal glands or decreased tear volumes. No inflammation was evident in lacrimal tissues of male or female rats, guinea pigs, or rabbits 12 to 31 days after castration, no inflammation existed in rat lacrimal glands 15 to 31 days after orchietomy and pituitary removal, and no aqueous tear deficiency was apparent in patients receiving antiandrogen therapy.

CONCLUSIONS. Androgen deficiency may promote the progression of Sjögren’s syndrome and its associated lacrimal gland inflammation, meibomian gland dysfunction, and severe dry eye. However, androgen insufficiency alone does not cause lacrimal gland inflammation, a Sjögren's syndrome-like pathology in lacrimal tissue, or aqueous tear deficiency in nonautoimmune animals and humans. (Invest Ophthalmol Vis Sci. 1999;40:1261-1265)

From the 1 Schepens Eye Research Institute, Departments of Ophthalmology, Harvard Medical School; 2 Brigham and Women’s Hospital; and 3 New England College of Optometry, Boston, Massachusetts.

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Reprint requests: David A. Sullivan, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114.

During the past several decades, researchers have discovered that androgens exert a significant influence on the structural characteristics, functional attributes, and pathologic features of the lacrimal gland. This tissue contains a single class of saturable, high-affinity, and steroid-specific androgen receptors, which are located primarily within the nuclei of epithelial cells and have a dissociation constant and stereochemical selectivity similar to those found in numerous cells and tissues throughout the body. In addition, androgens modulate the morphology, biochemistry, physiology, immunology, molecular biology, pathophysiology, or protein secretion of lacrimal glands in a variety of species and seem to be responsible for many of the gender-related differences identified in this tissue. These combined findings indicate that androgens are among the most potent hormones regulating the lacrimal gland.

One of the most striking effects of androgens is their impact on lacrimal tissue disease in Sjögren’s syndrome. This syndrome is a complex autoimmune disorder that occurs almost exclusively in women and has a devastating influence on the lacrimal gland. Autoimmune sequelae include profound lymphocyte accumulation in lacrimal tissue; marked alterations in the expression of cytokines, adhesion molecules, and apoptotic factors; immune-mediated dysfunction or destruction of acinar and ductal epithelial cells; a precipitous decrease in aqueous tear secretion; and severe dry eye.

Androgen treatment dramatically suppresses the inflammation in, and significantly enhances the functional activity of, lacrimal tissues in mouse models of Sjögren’s syndrome. This hormonal effect seems to be unique, tissue-specific, and mediated through an androgen interaction with receptors in epithelial cell nuclei. It involves altered expression of cytokines and apoptotic factors in the lacrimal gland.

Given these androgen effects, it is of interest that researchers have recently hypothesized that androgen insufficiency induces an autoimmune process in lacrimal tissue, leading to inflammation, a Sjögren’s syndrome-like pathology, and aqueous tear deficiency. These investigators have reported that androgen withdrawal, such as that occurring after ovariec- tomy, triggers lacrimal gland atrophy and appears to be associated with decreased acinar size, acinar cell necrosis, and massive regions of acinar cell degeneration. These changes, in turn, are hypothesized to elicit the generation of autoantigens, the development of lacrimal gland autoimmune disease, and the induction of a Sjögren’s syndrome-like aqueous tear insufficiency. In contrast, androgen treatment apparently prevents lacrimal gland regression after ovariec- tomy. Overall, these hypotheses and the related results suggest that androgens are essential for maintaining fluid secretion by the lacrimal gland.

We predicted that, if these hypotheses were correct, lacrimal glands of testicular feminized (Tfm) mice would contain extensive areas of inflammation. These mice possess dysfunctional androgen receptors, are completely resistant to androgen influence, and are considered to be the most appropriate animal model for evaluating multiple androgen-dependent phenomena. We further predicted that androgen deficiency, such as that occurring after orchietomy, ovariec- tomy, or interruption of the hypothalamic-pituitary axis, would lead to a significant lymphocyte accumulation in lacrimal tissue and that androgen receptor dysfunction (such as that in Tfm mice) and androgen insufficiency (such as in patients taking antiand-
drogen medications) would induce a profound aqueous tear deficiency. The purpose of the present study was to test these predictions.

**Materials and Methods**

**Human Studies**

These studies were approved by the Human Studies Committee of the Schepens Eye Research Institute (Boston, MA) and were conducted in accordance with guidelines established by the Declaration of Helsinki.

Male subjects who had antiandrogen therapy prescribed for prostatic indications were recruited from the Departments of Urology at Brigham and Women’s Hospital and Boston University Medical Center, Massachusetts. These patients, whose average age was 70.9 ± 1.9 years (range, 57–83 years), had had antiandrogen medications prescribed for periods ranging from 3 to 96 months (median, 36 months). These medications included flutamide (an inhibitor of type 2 5α-reductase), leuprolide or goserelin (analogues of luteinizing hormone-releasing hormone), and bicalutamide or flutamide (nonsteroidal antiandrogens). Age-matched control subjects (mean age, 64.8 ± 1.0 years) were recruited from the Boston environs. After providing appropriate consent, subjects underwent a complete ocular surface and anterior segment examination, including 5-minute Schirmer I testing (without anesthesia) on both eyes.

**Animals and Surgical Procedures**

All studies with experimental animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult testicular feminized (Tfm; C57BL/6j-A^tm Td^d/+ A^tm/+ A^tm/+ male control animals (Tabby 6j+; Tabby), and adult female mice (MRL/Mp-lpr/lpr) were purchased from Jackson Laboratory (Bar Harbor, ME). Adult male and female Sprague-Dawley rats (6-8 weeks old), Hartley guinea pigs (8 weeks old), and New Zealand White rabbits (7-8 months old) were obtained from Zivic-Miller Laboratories (Allison Park, PA), Charles River Breeding Laboratories (Wilmington, MA), or Pine Acre Rabbitry (Norton, MA). Animals were maintained in constant temperature rooms with light-dark periods of 12 hours' length. Surgical procedures, including orchietomy, ovariectomy, hypophysectomy, anterior pituitary removal, and appropriate sham operations, were performed on 5- to 7-week-old rats by surgeons at Zivic-Miller Laboratories. Castration of guinea pigs and rabbits was performed by veterinary personnel in the Charles River Breeding Laboratories and in Schepens Institute’s animal facilities, respectively.

To compensate for electrolyte imbalance in rats with whole or partial pituitary gland ablation, animals received a solution containing sodium chloride (2.03 g/l), potassium chloride (0.083 g/l), magnesium chloride (0.017 g/l), and calcium chloride (0.035 g/l), as previously described. To confirm the success of pituitary surgery, serum thyroxine levels were measured with a radioimmunoassay kit (Cambridge Medical Technology, Billerica, MA). Thyroxine was undetectable in sera of rats that had undergone hypophysectomy or anterior pituitary ablation.

**General Procedures**

Tears were obtained from both eyes of mice after ether anesthesia, according to a reported protocol. In brief, the tip of a graded micropipette was placed at the inner canthus and gently moved along the palpebral conjunctiva. This procedure, which was repeated twice on each eye, collected the entire available tear content. Tear volumes were measured accurately to within 0.1 μl. At experimental termination, lacrimal (exorbital) glands and, when indicated, submandibular glands, were removed from animals; cleared of adherent fascia; and processed for histology and image analysis. Statistical analyses of data were performed with the unpaired, two-tailed Student’s t-test.

**Histologic Procedures**

For light microscopic examination, tissue sections were obtained from glands that had been embedded in O.C.T. compound (Miles Laboratories, Elkhart, IN), paraffin, or plastic, depending on the specific experiment. For the preparation of frozen sections, tissues were placed in O.C.T. compound, stored in liquid nitrogen until experimental use, and sectioned (6 μm) at −20°C. Sections were transferred to coated glass slides (Vectorbond; Vector Laboratories, Burlingame, CA) and fixed in acetone. For the processing of paraffin sections, glands were fixed in St. Marie’s solution (19 parts 100% ethanol:1 part glacial acetic acid) at 4°C, dehydrated in increasing ethanol concentrations and xylene, embedded in paraffin, and sectioned (5 μm). Sections were placed on gelatin-coated glass slides and deparaffinized. For the production of plastic sections, tissues were fixed in 10% buffered formalin overnight, dehydrated, exposed to resin (Historesin; LKB, Bromma, Sweden), and sectioned (5 μm). All tissue sections were stained with hematoxylin and eosin.

To determine the percentage of lacrimal gland inflammation, tissue sections were evaluated with an image analysis system, as described elsewhere (Toda et al., unpublished data, 1998). This system accurately measured the areas of the total infiltrate and the entire section. Photographs were obtained by microscope (Carl Zeiss, Oberkochen, Germany), imported into image analysis software (Adobe Photoshop 4.01; Adobe, San Jose, CA) on a computer with 96 megabytes RAM (Power Macintosh 8600/200; Apple Computer, Cupertino, CA), and printed (model XLS 8600; Eastman Kodak, Rochester, NY).

**Results**

**Does Androgen Receptor Dysfunction Cause Inflammation in Lacrimal Glands of Mice?**

To determine whether androgen receptor dysfunction causes inflammation in lacrimal glands of mice, tissues were obtained from young (3 months of age; n = 5-10/group) and old (7-8 months of age; n = 9-10/group) Tabby control and Tfm mice and processed for histology and image analysis. For comparative purposes, submandibular glands, which are also androgen target organs, were collected from the older mice and analyzed for the extent of lymphocyte infiltration.

Androgen receptor dysfunction did not lead to lacrimal gland inflammation (Fig. 1; Table 1). No lymphocytic foci were evident in lacrimal tissues of young Tabby control or Tfm mice. A small amount of lymphocyte accumulation was apparent in some lacrimal glands from older Tabby control and Tfm mice, but the magnitude of the inflammation was not significantly different between these groups and appeared to be age-related.
Analogous findings were obtained in submandibular tissues, in which there was no difference in the percentage of glandular inflammation between Tabby control (0.15% ± 0.07%) and Tfm (0.09% ± 0.04%) mice.

Does Androgen Deficiency Cause Inflammation in Lacrimal Glands of Rats, Guinea Pigs, and Rabbits?

To assess whether androgen deficiency induces inflammation in lacrimal glands of rats, guinea pigs, or rabbits, tissues were collected from animals (n = 8–22/group) that had undergone orchiectomy, ovariectomy, hypophysectomy, and/or anterior pituitary ablation, and glands were then processed for histology.

Androgen deficiency for periods ranging from 12 to 31 days did not result in the appearance of any inflammation in lacrimal glands of male or female rats, guinea pigs, or rabbits (Table 1). Of interest, in eight other experiments involving sham surgery or castration of age-matched young adult rats, no evidence of lacrimal gland regression, indicated by a consistent decrease in weight, was found in the ovariec- tomy- or orchietomized animals (Table 2). Indeed in three of the studies, the absolute lacrimal gland weight increased after ovariec- tomy, compared with that of glands in the control group.

Does Androgen Receptor Dysfunction or Androgen Deficiency Cause Aqueous Tear Insufficiency?

To examine whether androgen receptor dysfunction or androgen deficiency causes aqueous tear deficiency, two experiments were performed. In the first study, tear volumes were measured in 7- to 8-month-old Tabby control (n = 9) and Tfm (n = 10) mice. In the second experiment, tear secretion was quantitated in both eyes of human male subjects (n = 15) who had been taking antiandrogen therapy for 3 to 96 months and in age-matched control subjects (n = 6).

Androgen receptor dysfunction had no influence on the tear volume in mice (Fig. 2). Similarly, androgen deficiency had no impact on the level of tear secretion in humans (Fig. 3).

DISCUSSION

Recently, researchers have hypothesized that androgen insufficiency may trigger an autoimmune process in the lacrimal gland, resulting in lymphocyte infiltration, pathologic characteristics similar to those encountered in Sjögren's syndrome, and aqueous tear deficiency. The results of our present studies do not support this hypothesis.

Analysis of lacrimal glands from young and old Tfm mice showed no evidence of any inflammation due to androgen insufficiency. These mice contain a single-base deletion in the amino terminal domain of androgen receptor mRNA, which leads to a frameshift in translation, a premature termination of androgen receptor synthesis, and the loss of DNA- and steroid-binding domains. Moreover, androgen production in Tfm mice is severely reduced. This deficiency, when coupled with the androgen receptor defect, serves to prevent the classic and nonclassic effects of androgens. Thus, if androgen insufficiency induces lacrimal gland inflammation, this consequence of endocrine imbalance should have been very apparent in Tfm mice. Such inflammation, however, did not exist.

Similarly, androgen deficiency caused by castration and interruption of the hypothalamic-pituitary axis in male and female rats, guinea pigs, or rabbits did not elicit any lymphocyte accumulation in the lacrimal gland. These findings, when combined with the Tfm results, show that androgen insufficiency did not induce lacrimal gland inflammation.

Of interest, orchietomy or ovariectomy caused no consistent lacrimal gland regression in rats compared with that in control animals in the sham operation group. This observation, which is different from recently reported results in rabbit models, is in agreement with the findings of a number of other studies. Collectively, these other investigations show that androgens have no consistent effect on the growth of, or acinar complex size or density in, lacrimal tissues of rats, guinea pigs, and rabbits. Moreover, these other studies show that androgen deficiency per se does not cause lacrimal gland atrophy and does not result in acinar cell necrosis or degeneration in lacrimal tissue.

It is important to note that, although androgens regulate many aspects of the lacrimal gland, the nature of this control is quite unlike that found in reproductive tissues, such as the ventral prostate. The prostate, but not the lacrimal gland, is entirely dependent on androgens for size maintenance and undergoes involution and programmed cell death after androgen removal.
TABLE 1. Effect of Androgen Receptor Dysfunction or Androgen Deficiency on the Extent of Inflammation in Lacrimal Glands of Mice, Rats, Guinea Pigs, and Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Mice</th>
<th>Age (Months)</th>
<th>Lacrimal Gland Inflammation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen Receptor Dysfunction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tabby Control</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Tfm</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Tabby Control</td>
<td>9</td>
<td>7-8</td>
<td>0.23 ± 0.16*</td>
</tr>
<tr>
<td>Tfm</td>
<td>10</td>
<td>7-8</td>
<td>1.15 ± 0.45*</td>
</tr>
<tr>
<td>Species</td>
<td>No. of Animals</td>
<td>Gender</td>
<td>Surgical Procedure</td>
</tr>
<tr>
<td>Rat</td>
<td>22</td>
<td>M</td>
<td>Orchiectomy</td>
</tr>
<tr>
<td>Rat</td>
<td>10</td>
<td>M</td>
<td>Orchiectomy and hypophysectomy</td>
</tr>
<tr>
<td>Rat</td>
<td>8</td>
<td>M</td>
<td>Orchiectomy and anterior pituitary ablation</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>10</td>
<td>M</td>
<td>Orchiectomy</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>10</td>
<td>F</td>
<td>Ovariectomy</td>
</tr>
<tr>
<td>Rabbit</td>
<td>11</td>
<td>M</td>
<td>Orchiectomy</td>
</tr>
<tr>
<td>Rabbit</td>
<td>9</td>
<td>F</td>
<td>Ovariectomy</td>
</tr>
</tbody>
</table>

Lacrimal glands were obtained from intact or surgically altered animals and were processed for histology, as described in the Materials and Methods section. Tissue sections (n = 1-4 entire sections/gland) showing evidence of focal lymphocyte infiltration were then evaluated by computer-assisted image analysis (n = 4 entire sections/gland) to determine the extent of lacrimal gland inflammation.

* Values are means ± SEM.

Our findings also showed that androgen insufficiency did not cause aqueous tear deficiency in Tfm mice or in humans with antiandrogen therapy prescribed for prostatic indications. These results are not surprising, considering that androgens do not elicit a consistent, species-independent action on fluid secretion by the lacrimal gland.120 Rather, androgens induce time-, species- and strain-dependent effects, leading to a non-uniform increase or decrease or no impact on the tear volume.121 Recently, it has been suggested that the decline in serum androgen levels that occurs during menopause, pregnancy, lactation, or the use of estrogen-containing oral contraceptives may lead to the development of a type of nonimmune dry eye syndrome, termed primary lacrimal gland deficiency, in women.7 However, if this hypothesis is correct, it may reflect a hormone action that is gender specific.

Finally, our observation that antiandrogen treatment did not induce aqueous tear deficiency in men is of particular interest, given that people with primary or secondary (e.g., that associated with systemic lupus erythematosus, rheumatoid arthritis) Sjögren's syndrome have reduced concentrations of

TABLE 2. Influence of Ovariectomy or Orchiectomy on Weight of Rat Lacrimal Glands

<table>
<thead>
<tr>
<th>Surgical Procedure</th>
<th>No. of Animals per Group</th>
<th>Tissue Collection (Days after Surgery)</th>
<th>Lacrimal Gland Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sham Surgery</td>
</tr>
<tr>
<td>Orchiectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>5</td>
<td>10</td>
<td>84 ± 4</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>7</td>
<td>10</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>8</td>
<td>10</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>5</td>
<td>11</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Experiment 5</td>
<td>5-6</td>
<td>14</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>8</td>
<td>10</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>5</td>
<td>11</td>
<td>106 ± 2</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>5-6</td>
<td>14</td>
<td>102 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SEM. At the end of the designated time period after surgery, lacrimal glands were removed from sham-operated or castrated animals and were weighed.

Significantly greater (*P < 0.05, one-tail; †P < 0.005) or less (‡P < 0.0005) than the value of the sham-operated group.

§ Body weights were also obtained in these experiments, and the mean lacrimal gland weight/body weight ratios were not significantly different (two-tailed t-test) between the sham-operated and castrated groups.
serum androgens.\textsuperscript{2,3} This androgen deficit is believed to contribute to the gender-related incidence and overall development of this disease and to the associated lacrimal gland dysfunction.\textsuperscript{2,3} Moreover, systemic androgen administration to patients with Sjögren’s syndrome appears to increase the tear volume and alleviate the signs and symptoms of dry eye.\textsuperscript{11-13}

In light of the data presented in the current study, it may be that this ocular response to androgens in Sjögren’s syndrome patients is caused by two alternate hormone actions. First, androgens may suppress lacrimal gland inflammation in autoimmune disease, thereby attenuating the immune-related damage to acinar and ductal epithelial cells and allowing increased aqueous tear secretion.\textsuperscript{9} Second, androgens may stimulate meibomian gland function, thus enhancing tear film stability, decreasing tear film evaporation, and consequently, ameliorating dry eye symptoms.\textsuperscript{11} In support of these hypotheses, androgens have been shown to decrease the inflammation and increase the function of lacrimal glands in mouse models of Sjögren’s syndrome.\textsuperscript{9} In addition, recent research has shown that the meibomian gland, which may also be compromised in Sjögren’s syndrome,\textsuperscript{12} is regulated by androgens\textsuperscript{11} and that androgen deficiency in humans is associated with meibomian gland dysfunction, altered lipid profiles in meibomian gland secretions, decreased tear film stability, and apparently, evaporative dry eye.\textsuperscript{11}

In summary, androgen deficiency may promote the progression of Sjögren’s syndrome and its associated lacrimal gland inflammation, meibomian gland dysfunction, and severe dry eye. However, our data show that androgen insufficiency by itself does not cause lacrimal gland inflammation, a Sjögren’s syndrome–like pathology in lacrimal tissue, or aqueous tear deficiency in nonautoimmune animals and humans.

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