Testosterone-Induced Suppression of Autoimmune Disease in Lacrimal Tissue of a Mouse Model (NZB/NZW F1) of Sjögren’s Syndrome

Ana Cristina L.M. Vendramini, Cynthia Soo, and David A. Sullivan

The current investigation was designed to examine whether androgen administration might suppress autoimmune disease in lacrimal glands of a mouse model (NZB/NZW F1) of Sjögren’s syndrome. Autoimmune, female mice were treated with vehicle or varying concentrations of testosterone for 0, 17, 34, or 51 days, and tears, lacrimal glands, as well as submandibular tissue, were collected from killed mice after androgen exposure. Glands were histologically processed and evaluated with a computer-assisted image analysis system. Results showed that testosterone administration induced a significant, time-dependent decrease in the extent of lymphocytic accumulation in the lacrimal gland. After 34–51 days of androgen therapy, the magnitude of lymphocyte infiltration had been suppressed 22- to 46-fold, compared with that in placebo-treated tissue. This hormone effect was associated with significant reductions in the number of focal infiltrates, the area of individual foci, and the total quantity of lymphocyte infiltration per lacrimal section. Testosterone exposure also stimulated an increase in lacrimal gland weight and a rise in tear volumes, relative to those measured in the same mice before treatment. In addition, androgens significantly diminished the extent of lymphocyte accumulation in submandibular tissue. In summary, our results demonstrate that androgen administration may inhibit the progression of autoimmune disease in lacrimal and submandibular glands of NZB/NZW F1 mice. Invest Ophthalmol Vis Sci 32:3002–3006, 1991

Sjögren’s syndrome is a chronic, inflammatory autoimmune disease that predominately afflicts females and is associated with progressive, pathological alterations in glandular and extraglandular tissues. The ocular manifestations of this syndrome include a pronounced accumulation of extensive, multifocal lymphocyte infiltrates in the lacrimal gland, which appear to be composed primarily of B and helper T cells. This mononuclear cell infiltration is paralleled by a degeneration of acinar and ductal cells in lacrimal tissue and the development of persistent keratoconjunctivitis sicca.

No medical therapy appears to be available to cure Sjögren’s syndrome. However, during the last 45 years, clinical reports have indicated that androgen, but not estrogen, exposure may significantly reduce ocular symptoms and increase tear flow of Sjögren’s patients (reports cited in Ariga et al). These studies, which were not pair-controlled, suggest that androgen administration may ameliorate autoimmune expression in lacrimal tissue and promote gland secretion.

To test this hypothesis, the objective of the current investigation was to assess the efficacy of androgen treatment for lacrimal disease by using an autoimmune, animal model (NZB/NZW F1 mouse) of Sjögren’s syndrome. As in humans, lacrimal glands of this mouse strain, which contains a fundamental B-cell defect, harbor dense, lymphocytic aggregates, that contain a prevalence of B and helper T cells. Moreover, this murine disease is accompanied by focal destruction of acinar and ductal tissue and apparent ocular surface dryness. As a positive control in these experiments, we also monitored the impact of androgen therapy on immunological sequelae in submandibular tissue because long-term androgen administration to NZB/NZW F1 mice is known to markedly diminish the magnitude of this gland’s lymphocytic infiltration, which is qualitatively different in composition than that observed in lacrimal tissue.

Materials and Methods. Age-matched, female NZB/NZW F1 mice (6 weeks old) were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in constant temperature rooms with fixed light/dark periods of 12 hr duration. Animals were maintained without hormone treatment until after the onset of autoimmune disease: symptoms are typically manifested after 4–5 months of age and were demonstrated in the current study by the presence of lymphocytic infiltrates in lacrimal glands of randomly selected, 5-month-old mice. At 5.7 months of age, mice received subcutaneous implants of placebo (cholesterol, methyl cellulose, lactose) or testosterone-containing (10 or 25 mg) pellets (Innovative Research of America, Toledo, OH) in the subcapsular region. These pellets were designed for a 3-week release of physiological (for a male; 10 mg) or supra-physiological (25 mg) amounts of testosterone. When indicated, pellets were reimplanted at 17-day intervals to provide continuous hormone exposure. The efficacy of androgen treatment was verified by the analysis of serum testosterone levels with a radioimmunoassay (RIA) kit (ICN Biomedicals, Inc., Costa Mesa, CA). Testosterone concentrations on day 17...
were <0.1 ng/ml (placebo), 6.93 ± 0.84 ng/ml (10 mg dose), or 21.84 ± 4.03 ng/ml (25 mg dose). Over the time course of these experiments, mortality in NZB/NZW F1 mice was 7.3%; the cause of death in 28.6% of these animals could be directly attributed to anesthetic complications during surgery. Studies adhered to the ARVO Resolution on the Use of Animals in Research.

Tears were collected from both eyes of anesthetized mice with graded microcapillary pipettes, and volumes were measured, with an accuracy of ±0.1 µl, according to reported methods. The precision of this procedure, which drained the entire available tear volume, was 0.1 µl tears. When the animals were killed, blood was obtained by aspiration from the heart, allowed to clot at room temperature, and centrifuged at 10,000 × g for 4 min. Serum was stored at −20°C until determination of testosterone concentrations by RIA. Lacrimal (exorbital) and submandibular glands were removed and successively cleared of adherent debris, weighed, fixed overnight in 10% buffered formalin, dehydrated, embedded in Historesin (LKB, Bromma, Sweden), cut into 3-µm sections and stained with hematoxylin and eosin (Fisher, Medford, MA). Tissue sections were obtained from four different gland regions, all separated by distances of at least 30 µm in the lacrimal and submandibular tissues, respectively.

Fig. 1. Influence of testosterone therapy on lymphocyte infiltration in lacrimal tissue of NZB/NZW F1 mice. Lacrimal glands were obtained from age-matched, female mice (n = 8–10/group) immediately before hormone administration (day 0) or after 17, 34, or 51 days of treatment with vehicle (Placebo) or physiologic (10 mg; T10) or supraphysiologic (25 mg; T25) doses of testosterone. Following histologic processing, tissue sections (32–40 sections/group) were evaluated microscopically for the: (A) number of focal lymphocyte infiltrates; (B) area of individual lymphoid infiltrates (n = 9–120 infiltrates/group); (C) total area encompassed by lymphocyte infiltrates per section; and (D) percentage of lymphocyte infiltration, as described in the Materials and Methods. Symbols and bars represent the mean ± SE. * Significantly (P < 0.05) less than value of pretreatment group; † significantly (P < 0.05) less than value of age-matched, control group; ‡ significantly (P < 0.05) greater than mean of pretreatment group.
Sections were evaluated with a Zeiss light microscope, coupled to a Zeiss Videoplan 2 image analysis system, to quantitatively measure the area of tissue sections (4 sections/tissue; 25× magnification) and individual lymphoid infiltrates (4 sections/tissue; 100× magnification). Lymphocyte infiltrates were arbitrarily defined as lymphoid foci harboring 50 or more lymphocytes/focus. In these studies, the smallest lymphoid infiltrates examined in lacrimal and submandibular glands of experimental groups encompassed areas of 5,524 µm² and 6,577 µm², respectively. To determine the percentage of lymphocyte infiltration, the areas of individual foci in a specific section were summed, divided by the total section area, and multiplied by 100. Histologic analyses were performed by two individuals, and comparative results were identical, regardless of whether masked or unmasked slides were used. Results were randomly confirmed by a third individual under masked conditions. Statistical evaluations of the data were conducted by using Student’s unpaired, two-tailed t-test.

Results. To examine whether androgen treatment might ameliorate lymphocyte accumulation in lacrimal glands of NZB/NZW F1 mice, 5.7-month-old female animals (n = 8-10/group) were administered subcutaneous implants of testosterone (10 mg [physiological] or 25 mg [supraphysiological])- or vehicle-containing pellets in the subscapular region. When indicated, pellets were reimplanted at 17-day intervals to ensure maintained androgen exposure. Lacrimal, as well as submandibular, tissue, serum, and tears were obtained either immediately before (pretreatment group), or 17, 34, or 51 days after hormone therapy; glands were processed for morphometric evaluation by image analysis.

As shown in Figure 1, androgen administration was started after the initiation of autoimmune disease: lacrimal glands of pretreatment controls contained definitive lymphocyte infiltrates, which covered 0.86 ± 0.20% of the total tissue area. The extent of this lymphocyte infiltration increased dramatically in control mice during the experimental time course. Thus, by day 51, the magnitude of lymphocyte accumulation had risen significantly (P < 0.005) by a six-fold amount in lacrimal glands of placebo-treated animals. This lymphocytic expansion involved distinct elevations in the number of focal infiltrates, the size of individual infiltrates (Fig. 1).

In contrast to controls, testosterone treatment in NZB/NZW F1 mice induced a significant (P < 0.001), time-dependent decrease in the extent of lymphocytic accumulation in the lacrimal gland (Fig. 1). After 17 days of hormone exposure, the percentage of glandular tissue infiltrated by lymphocyte foci had been re-
Influence of testosterone exposure on tear volumes in NZB/NZW F1 mice

Table 1. Influence of testosterone exposure on tear volumes in NZB/NZW F1 mice

| Treatment | Day 17 | | Day 34 | | Day 51 | |
|-----------|--------|--------|--------|--------|--------|
|            | Pre    | Post   | Pre    | Post   | Pre    | Post   |
| Placebo    | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 | 0.3 ± 0.1 |
| TEST 10    | 0.3 ± 0.1 | 0.5 ± 0.2 | 0.4 ± 0.1 | 0.6 ± 0.1 | 0.3 ± 0.1 | 0.7 ± 0.2 |
| TEST 25    | 0.3 ± 0.1 | 0.6 ± 0.1* | 0.6 ± 0.1* | 1.1 ± 0.2* | 0.4 ± 0.1 | 1.0 ± 0.1* |

Tears were collected from the eyes of the same NZB/NZW F1 mice (n = 8-10/group) immediately before (Pre), or 17, 34, or 51 days after (Post) subcutaneous implantation of pellets containing vehicle (Placebo) or testosterone (TEST; 10 or 25 mg). Tear volumes were measured accurately with graded capillary micropipets. Numbers equal the mean ± SE.

* Significantly (P < 0.05) higher than matched, pretreatment value.
† Significantly (P < 0.01) greater than value of placebo-treated control.

Androgen administration stimulated a significant (P < 0.0001), threefold to fourfold increase in lacrimal gland weight (day 51: placebo = 7.9 ± 0.2 mg; T10 = 29.6 ± 1.1 mg; T25 = 30.2 ± 1.4 mg) and the lacrimal gland/body weight ratio (Fig. 2). This action coincided with a slight, but significant (P < 0.05) 8-10% rise in body weight of androgen-treated mice on days 34 and 51. Of interest, supraphysiological testosterone therapy also induced a significant elevation in tear volume, compared with that measured in the same mice before treatment (Table 1). However, these hormone-related volumes were not consistently different from those measured in placebo-treated mice (Table 1).

With regard to submandibular tissue, testosterone exposure significantly diminished the size of focal lymphocyte infiltrates and the percentage of lymphoid infiltration compared with that found in "placebo" glands; however, this hormone action was not associated with a decline in the number of lymphocyte foci (Table 2). Androgen treatment also elicited a 100% increase in submandibular gland weight, regardless of whether this parameter was expressed in terms of absolute weight or standardized to body weight (Fig. 2).

Discussion. This investigation demonstrates that androgen therapy may significantly suppress the expression, as well as effectively prevent the progression, of autoimmune disease in lacrimal glands of a mouse model (NZB/NZW F1) of Sjögren's syndrome. Endocrine action induced a precipitous decrease in the number and size of focal infiltrates, thereby causing a pronounced decline in the area encompassed by inflammatory cells in lacrimal tissue. Of interest, the extent of androgen influence on immunopathology was greater in lacrimal than in submandibular glands (eg, compare data in Fig. 1 with that in Table 2). This discrepancy may represent variations in hormone susceptibility by resident lymphocyte populations, which are qualitatively dissimilar in these tissues. Alteratively, differences in immune response between lacrimal and salivary glands may reflect the higher potency of androgens on the immunological architecture and function of lacrimal tissue.

Table 2. Testosterone-induced suppression of lymphocyte infiltration in submandibular glands of NZB/NZW F1 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue section area (×10⁶ µm²)</th>
<th>Number of lymphocyte foci/section</th>
<th>Focal infiltrate area (×10⁶ µm²)</th>
<th>Total infiltrate area (×10⁶ µm²)/section</th>
<th>Lymphocyte infiltration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>21.23 ± 1.48</td>
<td>2.0 ± 0.3</td>
<td>7.92 ± 1.30</td>
<td>1.57 ± 0.37</td>
<td>0.64 ± 0.13</td>
</tr>
<tr>
<td>Placebo</td>
<td>19.59 ± 1.45</td>
<td>4.2 ± 0.6†</td>
<td>26.47 ± 3.88‡</td>
<td>12.62 ± 2.98§</td>
<td>6.15 ± 1.58§</td>
</tr>
<tr>
<td>TEST 10</td>
<td>30.06 ± 1.64*</td>
<td>2.9 ± 0.5</td>
<td>8.93 ± 1.46</td>
<td>2.56 ± 0.60</td>
<td>0.73 ± 0.18</td>
</tr>
<tr>
<td>TEST 25</td>
<td>31.46 ± 1.93*</td>
<td>3.5 ± 0.5†</td>
<td>10.20 ± 1.26</td>
<td>3.53 ± 0.80‖</td>
<td>0.94 ± 0.19</td>
</tr>
</tbody>
</table>

Submandibular glands were obtained from age-matched NZB/NZW F1 mice (n = 8-10/group) immediately before (Pretreatment) or 51 days after exposure to vehicle (Placebo) or testosterone (TEST; 10 or 25 mg). Tissues were processed for light microscopy and the following indices were examined by image analysis: (1) total tissue section area (four sections/gland, 32-40 sections/group); (2) number of lymphoid infiltrate foci per tissue section; (3) area of individual lymphocyte infiltrates (n = 71-135 infiltrates/group); (4) total lymphocyte infiltrate area per tissue section; and (5) extent (%) of lymphocyte infiltration, calculated as described in Materials and Methods. Numbers represent the mean ± SE.

* Significantly (P < 0.0005) greater than section area of pretreatment and placebo groups.
† Significantly (P < 0.05) higher than number of foci than in pretreatment group.
‡ Significantly (P < 0.001) greater than focal infiltrate area of pretreatment and testosterone groups.
§ Significantly (P < 0.005) higher than infiltrate area of pretreatment and testosterone groups.
‖ Significantly (P < 0.05) greater than pretreatment area.
* Significantly (P < 0.0005) higher than percent infiltrate in pretreatment and testosterone groups.
Testosterone administration also stimulated a rise in lacrimal and submandibular gland weight, which previously has been observed in either NZB/NZW F1 or other mouse strains (Sullivan, unpublished data). In addition, supraphysiological androgen concentrations induced an increase in tear volume relative to pretreatment, but not placebo, levels. This specific hormone action may be species- and strain-dependent (Sullivan, unpublished data), yet given the reported ocular dryness in NZB/NZW F1 mice, the action may be caused by androgen-associated contraction of inflammatory cell infiltrates and potential enhancement of secretory cell activity in mouse lacrimal tissue.

Overall, our findings show that androgen therapy dramatically curtails the incidence and severity of lymphocyte infiltration in lacrimal glands of NZB/NZW F1 mice. Moreover, this hormone effect appears to mirror the immunosuppressive action of androgens on autoimmune sequelae in lacrimal tissue of MRL/Mp-1pr/1pr mice, which suffer primarily from an immunoregulatory disorder of T cells. This androgen-related diminution of lacrimal autoimmunity may serve as a reasonable explanation for the success of uncontrolled, clinical studies which demonstrated that androgen administration alleviated dry eye symptoms in Sjögren’s patients (Ariga et al11). Clearly, further research is required to determine whether androgens, or more importantly their non-virilizing analogues, may provide a safe and effective treatment for Sjögren’s syndrome.

Key words: Sjögren’s syndrome, lacrimal gland, tears, androgen, lymphocyte, mouse

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