Comparative Influence of Steroid Hormones and Immunosuppressive Agents on Autoimmune Expression in Lacrimal Glands of a Female Mouse Model of SJÖGREN’S SYNDROME

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Purpose. Previous research has demonstrated that testosterone therapy causes a profound suppression of autoimmune disease in lacrimal glands of female mouse models of SJÖGREN’S syndrome. The aim of the present study was to determine whether other anabolic androgens, nonandrogenic steroids, or immunosuppressive agents might duplicate this hormonal effect.

For comparative purposes, we also evaluated the influence of these various pharmacologic compounds on the tear volume, the magnitude of lymphocyte infiltration in the submandibular gland, and the extent of mucosal and peripheral lymphadenopathy.

Methods. Female MRL/MpJ-lpr/lpr mice were administered vehicle, steroids, or immunosuppressive compounds for 21 days after the onset of disease. Lacrimal glands and tears, as well as submandibular glands, spleens, and superior cervical and mesenteric lymph nodes were collected immediately before or after treatment and then processed for analysis.

Results. Our results showed that: (1) the immunosuppressive impact of testosterone on lymphocyte infiltration in lacrimal tissue was reproduced by the administration of 19-nortestosterone or cyclophosphamide, but not by therapy with 17β-estradiol, danazol, the experimental steroid Org 4094, cyclosporine A or dexamethasone; (2) treatment with testosterone, 19-nortestosterone, cyclophosphamide, or dexamethasone significantly reduced the extent of inflammation in salivary glands; (3) exposure to cyclophosphamide markedly diminished the size of lymphatic and splenic tissues, whereas glucocorticoid treatment only decreased the weight of superior cervical lymph nodes; and (4) administration of 17β-estradiol, Org 4094, or dexamethasone led to a significant decrease in tear volume.

Conclusions. Overall, these results demonstrate that androgen or cyclophosphamide therapy may successfully ameliorate autoimmune expression in lacrimal and salivary glands of a female mouse model of SJÖGREN’S syndrome. Invest Ophthalmol Vis Sci. 1994;35:2632-2642.

Recent research has demonstrated that systemic androgen therapy causes a profound suppression of autoimmune sequelae in lacrimal glands of female mouse models (MRL/MpJ-lpr/lpr [MRL/lpr] and NZB/NZW F1) of SJÖGREN’S syndrome. Thus, testosterone treatment of these animals after the onset of disease induces a precipitous, time-dependent decline in the area and density of lymphoid foci, a dramatic reduction in the magnitude of lymphocyte infiltration, and an apparent diminution of acinar and ductal tissue disruption. These hormonal effects, which are observed after either physiological or supraphysiological androgen administration, involve a significant decrease in the quantity of various inflammatory cell populations in lacrimal tissue, including helper and suppressor–cytotoxic T cells, B cells, Ia-positive cells, and immature lymphocytes. In addition, testosterone action appears to be site-specific and to be mediated through local microenvironmental processes in the lacrimal gland.
At present, no other hormonal or pharmaceutical agent is known that may duplicate this androgen-related suppression of lacrimal autoimmunity in mouse models of Sjögren’s syndrome. However, it is possible that this androgen effect may not be unique. Other researchers have proposed that glucocorticoid or cyclosporine A therapy might ameliorate the ocular manifestations of this autoimmune disorder. Similarly, various clinicians have speculated that estrogen administration may potentially serve as an effective means to reverse lacrimal dysfunction, and/or the associated keratoconjunctivitis sicca, of patients with perimenopausal dry eye or Sjögren’s syndrome. Moreover, given the ability of cyclophosphamide, danazol, or synthetic nonandrogenic steroids to alleviate sialoadenitis or peripheral immune dyscrasias in MRL/lpr or NZB/NZW F1 mice, it may be that these compounds also curtail lacrimal autoimmune expression.

Therefore, to explore these possibilities and to extend our previous findings, the objectives of the present investigation were fourfold: (1) to determine whether other nonandrogenic, steroid hormones or immunosuppressive agents might reproduce the marked effect of testosterone on lymphocyte infiltration in lacrimal glands of female MRL/lpr mice; (2) to assess whether the anabolic androgen, 19-nortestosterone, also exerts anti-inflammatory activity in lacrimal tissue; (3) to evaluate the comparative impact of these various pharmacologic compounds on the extent of lymphoid infiltration in the submandibular gland; and (4) to examine whether pharmaceutical actions on exocrine gland immunopathology are paralleled by a reduction in the weight of the mesenteric and superior cervical lymph nodes and spleen, which are considerably enlarged in MRL/lpr animals due to lymphocytic accumulation. In addition, although MRL/lpr mice represent a model for the lacrimal inflammation of Sjögren’s syndrome, we also monitored tear volume in case the various drug exposures influenced this parameter.

MATERIALS AND METHODS

Adult, female MRL/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in controlled-temperature rooms with fixed light-dark periods of 12 hours’ duration. When they were 3.4 to 3.7 months old, animals were administered subcutaneous implants of one of the following control-, steroid-, or immunosuppressant-containing pellets in the subcapular region: placebo (i.e., cholesteryl methyl cellulose, lactose), testosterone (10 mg), 19-nortestosterone (10 mg), danazol (50 mg), 17β-estradiol (0.25 mg), Organon International (Oss, The Netherlands) compound 4094E (Org 4094; 2.1 mg), dexamethasone (0.5 mg), cyclosporine A (3.6 mg), or cyclophosphamide (10 mg). These pellets (obtained from Innovative Research of America, Toledo, OH) were either commercially available or, in the case of Org 4094 (synthetic steroid, kindly donated by Dr. Herman Verheul of Organon) and cyclosporine A (a generous gift from Dr. Renee Kaswan, Athens, GA), were custom prepared. The specific dosages of pharmacologic agents were chosen to provide during the 21-day experimental time course: (1) physiological serum levels of testosterone (for males) or its anabolic analogue, 19-nortestosterone; (2) enhanced serum concentrations of estradiol or glucocorticoid (i.e., dexamethasone); (3) quantities of Org 4094 known to suppress sialadenitis in autoimmune NZB/NZW and NOD mice after 13 weeks of exposure; or (4) optimal amounts of danazol, cyclosporine A, or cyclophosphamide, which have been reported to ameliorate significantly the various systemic immune dysfunctions in MRL/lpr mice.

The implantation method of experimental treatment was selected because it has consistently been demonstrated to yield a slow, but continuous, release of designated pharmaceuticals during a 21-day interval. To confirm this finding, several additional tests were conducted. First, the effectiveness of testosterone implants in producing appropriate androgen levels in female MRL/lpr mice was verified by the measurement of serum testosterone concentrations with an RIA kit (ICN Biomedicals, Inc., Costa Mesa, CA). After 0 or 21 days of therapy, serum testosterone levels were calculated <0.1 ng/ml (pretreatment), <0.1 ng/ml (placebo, 21 days), and 5.89 ± 1.53 ng/ml (testosterone, 21 days). Second, analysis by Organon’s Drug Metabolism Department of serum Org 4094 content in MRL/lpr animals after 21 days of exposure showed undetectable levels in the “placebo” group and 3.57 ± 0.37 ng/ml in the “Org 4094” group. This latter amount is analogous to that concentration achieved by the daily, subcutaneous administration of immunosuppressive quantities of the Org 4094 compound (H. Verheul, Oss, The Netherlands, personal communication, 1992). Third, implantation of placebo, testosterone, 19-nortestosterone, or estradiol pellets into castrated, 4-month-old male or female BALB/c mice (7 days after surgery; n = 2 to 4 animals per treatment group) for 9 or 21 days resulted in significant, hormone-induced increases in seminal vesicle or uterine weights.

Immediately before and after the experimental period, tears were collected from both eyes of etherized mice with graded microcapillary pipettes, and tear volumes were measured accurately to within 0.1 μl, according to reported procedures. At the time of sacrifice, blood was aspirated from the heart, allowed to
clot at 4°C, then centrifuged at 10,000g for 4 minutes. Serum was stored at –20°C until determination of steroid concentrations by immunoassay. Exorbital lacrimal and submandibular glands, mesenteric and superior cervical (i.e., single pair adjacent to midline) lymph nodes, and spleen were extirpated, cleared of adherent tissues, and weighed. For histologic purposes, lacrimal and salivary tissues were fixed in 10% buffered formalin overnight, dehydrated, embedded in Historesin (Reichert-Jung, Heidelberg, Germany), cut into 3 μm sections, and stained with hematoxylin and eosin (Fisher, Medford, MA). Sections were obtained from four different tissue areas, all separated by minimal distances of at least 30 μm or 250 μm in the lacrimal and submandibular glands, respectively.

Sections were examined with a Zeiss (Thornwood, NY) light microscope, attached to a Zeiss Videoplan image analysis system, to determine quantitatively the area of tissue sections (4 sections per tissue; X25 magnification) and focal lymphocyte infiltrates (4 sections per tissue; X100 magnification). Lymphoid infiltrates were operationally defined as foci containing 50 or more lymphocytes per focus. In the present study, the smallest lymphoid infiltrates analyzed in lacrimal and submandibular glands of various groups encompassed areas of 1,198 ± 127 μm² and 1,123 ± 45 μm², respectively. To calculate the total extent of infiltration in a given section, the areas of individual foci were added.

To determine the percentage of lymphoid infiltration, the areas of focal infiltrates in a specific section were added, then divided by the entire section area and multiplied by 100. Morphometric evaluations were performed primarily by one individual on masked slides. Results were randomly confirmed on masked slides by two individuals, and findings of all three investigators were essentially identical. Statistical analysis of the data was conducted by using Student’s unpaired, two-tailed t-test, unless otherwise noted.

During the course of this study, which adhered to the ARVO Resolution on the Use of Animals in Research, the mortality rate of MRL/lpr mice equalled 12.4%. Consequently, reported post-treatment results are restricted to only those data from autoimmune animals completing the experimental protocols.

RESULTS

Influence of Steroids and Immunosuppressive Agents on Lymphocyte Infiltration in Lacrimal Glands of Female MRL/lpr Mice

To determine the comparative efficacy of steroids or immunosuppressive agents in alleviating lymphocyte infiltration in lacrimal glands of a mouse model of Sjogren’s syndrome, adult female MRL/lpr mice (n = 9 to 12 animals per treatment group) were administered subcutaneous implants of placebo, testosterone, 19-nortestosterone, danazol, 17β-estradiol, Org 4094, dexamethasone, cyclosporine, or cyclophosphamide after the onset of disease. Lacrimal glands were obtained either immediately before (day 0, pretreatment) or after 21 days of pharmaceutical treatment and processed for the morphometric analysis of various inflammatory parameters.

As shown in Figure 1, lacrimal tissue of pretreatment animals contained substantial lymphocyte infiltration, with an average of 8.9 ± 0.6 lymphoid foci/section and a mean individual infiltrate size of 35,437 ± 2,832 μm²/focus. The magnitude of lymphocyte inflammation equalled 7.47 ± 0.94% of the glandular area, but in specific tissues encompassed up to 26.6% of the entire lacrimal section. Administration of placebo-containing pellets to MRL/lpr mice for a 21-day interval elicited no effect on lacrimal autoimmune expression.

In contrast, a 3-week exposure to testosterone, 19-nortestosterone, or cyclophosphamide resulted in a significant suppression of lymphocyte infiltration in lacrimal glands of MRL/lpr animals (Fig. 1). All three substances dramatically decreased the mean size of lymphoid infiltrates and the percentage of inflamed tissue. In addition, testosterone and cyclophosphamide significantly reduced the number of focal infiltrates in lacrimal glands, compared to those of placebo controls. These immunosuppressive actions could not be accounted for by variations in tissue weight or section area, because both androgens, as well as cyclophosphamide, induced a significant (P < 0.05; nortestosterone effect was one-tail) contraction in the total infiltrate area per section.

In striking contrast to these attenuating effects, treatment of autoimmune female mice with cyclosporine A, 17β-estradiol, or Org 4094 significantly enhanced certain aspects of lacrimal gland inflammation (Fig. 1). Administration of cyclosporine A led to a pronounced increase in the density of lymphocytic foci (P < 0.01), the mean infiltrate size (P < 0.05), the total area of infiltration per section (P < 0.0005), and the percentage inflammation (P < 0.0005) of lacrimal tissue, as compared to corresponding indices in placebo-treated controls. Exposure to 17β-estradiol caused a significant (P < 0.0005) 62% rise in the area of individual infiltrates, but this effect was partially countered by a 25% drop in the number of foci. With regard to Org 4094, treatment with this synthetic steroid was associated with a significant (P < 0.01) augmentation of the percentage lacrimal infiltrate relative to levels in placebo controls.

Regarding the influence of danazol or dexamethasone therapy, neither steroid showed any effect on autoimmune sequelae in lacrimal glands of MRL/lpr mice (Fig. 1).
Impact of Steroid or Immunosuppressant Treatment on Lymphoid Infiltration in Submandibular Glands of Female MRL/lpr Mice

To assess the relative effects of steroids or immunosuppressive agents on immunopathologic lesions in submandibular glands of female MRL/lpr mice (n = 9 to 12/treatment group), salivary tissues were collected either before (pretreatment) or after systemic pharmacologic treatment for 21 days, as described above, and then processed for quantitative image analysis.

As demonstrated in Table 1, the absolute number of lymphocytic foci, the mean infiltrate size, and the total infiltrate area/section in submandibular glands of pretreatment, 3.4- to 3.7-month-old MRL/lpr mice were analogous to those observed in lacrimal glands (compare to Fig. 1). However, given the larger dimensions of submandibular tissue (Table 2), the extent of lymphocyte infiltration averaged 4.3-fold less than found in the lacrimal gland (Table 1, Fig. 1). Thus, the maximum percent infiltration recorded in all submandibular tissue sections of pretreatment controls equalled only 5.57%. This immune profile, depicted by pretreatment submandibular glands, was not altered by a 21-day treatment regimen with placebo-containing compounds (Table 1).

Administration of testosterone, 19-nortestosterone, cyclophosphamide, or dexamethasone for 3 weeks to these female autoimmune mice led to a significant decrease in the percentage of lymphocyte infiltration in submandibular tissue (Table 1). This response was paralleled by varying effects on the number of foci, individual infiltrate area, and the total amount of submandibular infiltration (Table 1). Of the substances evaluated, only cyclophosphamide significantly reduced all immune parameters. In contrast, dexamethasone abrogated the absolute focal density and the magnitude of section infiltrates, whereas 19-nortestosterone diminished the mean size and combined area of lymphocyte infiltration (Table 1). Of interest, the suppressive action of 19-nortestosterone on the percentage of lymphocyte accumulation in submandibular tissue was significantly (P < 0.005) greater than that generated by testosterone.

Treatment of MRL/lpr mice with either danazol or Org 4094 significantly (P < 0.05) lowered the number of lymphocyte foci in submandibular tissue sections, compared to that of placebo controls, but exerted no effect on the overall extent of lymphoid infiltration (Table 1). With respect to 17β-estradiol, this female sex steroid, in a pattern similar to that found in lacrimal tissue, significantly (P < 0.01) elevated the area of individual lymphocyte foci by 55% but significantly (P < 0.0005) lessened the focal density by 40%, relative to placebo measurements. In consequence, es-
TABLE 1. Impact of Steroids and Immunosuppressive Agents on Lymphocyte Infiltration in Submandibular Glands of MRL/lpr Female Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue Section Area (×10^4 μm²)</th>
<th>No. of Focal Infiltrate Foci/Section</th>
<th>Focal Infiltrate Area (×10^4 μm²)</th>
<th>Total Infiltrate Area (×10^4 μm²)</th>
<th>Lymphocyte Infiltration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>11.67 ± 0.55</td>
<td>7.8 ± 0.6</td>
<td>2.75 ± 0.26</td>
<td>21.48 ± 2.99</td>
<td>1.74 ± 0.20</td>
</tr>
<tr>
<td>Placebo</td>
<td>11.49 ± 0.56</td>
<td>9.5 ± 0.7</td>
<td>2.47 ± 0.24</td>
<td>24.43 ± 3.09</td>
<td>2.10 ± 0.24</td>
</tr>
<tr>
<td>Testosterone</td>
<td>14.05 ± 0.70*</td>
<td>8.0 ± 0.8</td>
<td>2.25 ± 0.34</td>
<td>17.94 ± 3.14</td>
<td>1.25 ± 0.19†</td>
</tr>
<tr>
<td>Nortestosterone</td>
<td>14.23 ± 0.67*</td>
<td>7.8 ± 0.6</td>
<td>1.25 ± 0.08†</td>
<td>10.47 ± 1.22†</td>
<td>0.71 ± 0.07‡</td>
</tr>
<tr>
<td>Danazol</td>
<td>12.28 ± 0.56</td>
<td>7.7 ± 0.5†</td>
<td>2.95 ± 0.35</td>
<td>22.65 ± 3.38</td>
<td>1.68 ± 0.24</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10.36 ± 0.50</td>
<td>5.7 ± 0.4†</td>
<td>3.83 ± 0.54*</td>
<td>21.79 ± 3.75</td>
<td>2.17 ± 0.41</td>
</tr>
<tr>
<td>Org 4094</td>
<td>11.31 ± 0.46</td>
<td>7.8 ± 0.5†</td>
<td>2.26 ± 0.22</td>
<td>17.50 ± 2.66</td>
<td>1.57 ± 0.22</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>10.98 ± 0.52</td>
<td>7.0 ± 0.6†</td>
<td>2.18 ± 0.26</td>
<td>15.17 ± 2.42†</td>
<td>1.29 ± 0.19†</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>10.60 ± 0.55</td>
<td>7.9 ± 0.6</td>
<td>3.12 ± 0.34</td>
<td>24.80 ± 3.68</td>
<td>2.21 ± 0.30</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>12.07 ± 0.58</td>
<td>5.5 ± 0.7†</td>
<td>1.26 ± 0.13†</td>
<td>7.14 ± 1.17†</td>
<td>0.55 ± 0.08‡</td>
</tr>
</tbody>
</table>

Submandibular glands were removed from female MRL/lpr mice (n = 9–12/group) immediately before (Pretreatment), or after 21 days of, the administration of placebo, steroid, or immunosuppressive compounds. Tissues were processed for microscopy and the following parameters were examined by image analysis: (1) entire tissue section area (4 sections/gland, 24–44 sections/group), (2) number of lymphocyte foci per tissue section, (3) area of individual lymphocyte infiltrates (n = 191–343 infiltrates/group), (4) total area covered by lymphocyte infiltrates per tissue section, and (5) percentage of lymphocyte infiltration. Values are mean ± SE.

* Significantly greater than value of placebo group (P < 0.01).
† Significantly less than value of placebo group (P < 0.005).
‡ Significantly less than the value of placebo group (P < 0.005).

TABLE 2. Effect of Pharmaceutical Compounds on the Body Weight and Lacrimal and Submandibular Gland Weights of Female MRL/lpr Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Lacrimal Gland</th>
<th>Submandibular Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Weight (mg)</td>
</tr>
<tr>
<td>Placebo</td>
<td>33.9 ± 0.8</td>
<td>38.6 ± 0.9*</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>Testosterone</td>
<td>35.9 ± 0.8</td>
<td>43.8 ± 2.0*§</td>
<td>24.2 ± 1.2§</td>
</tr>
<tr>
<td>Nortestosterone</td>
<td>34.3 ± 1.0</td>
<td>39.2 ± 0.9*</td>
<td>28.3 ± 0.9§</td>
</tr>
<tr>
<td>Danazol</td>
<td>34.3 ± 0.9</td>
<td>37.5 ± 1.0*</td>
<td>12.8 ± 0.5</td>
</tr>
<tr>
<td>Estradiol</td>
<td>35.5 ± 0.7</td>
<td>37.1 ± 1.0</td>
<td>13.0 ± 0.5</td>
</tr>
<tr>
<td>Org 4094</td>
<td>37.0 ± 0.6</td>
<td>35.9 ± 0.3†</td>
<td>10.4 ± 0.5†</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>34.2 ± 1.1</td>
<td>35.5 ± 1.3</td>
<td>11.8 ± 0.3</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>34.4 ± 0.7</td>
<td>36.8 ± 0.9*</td>
<td>11.5 ± 0.4</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>37.4 ± 0.5</td>
<td>34.0 ± 1.2‡</td>
<td>12.6 ± 0.7</td>
</tr>
</tbody>
</table>

Weights were measured before (n = 9–12/group) and/or after (n = 6–11/group) treatment of MRL/lpr mice with various steroids or immunosuppressive agents. The differences between the number of animals in the pre- and post-treatment groups are due to mortality during the course of the experiment. Values are mean ± SE. LGW/BW-% represents the lacrimal gland weight/body weight ratio in terms of percentage, whereas the SGW/BW-% reflects the similar percentage ratio for the submandibular gland.

Significantly greater (P < 0.05*) or less (P < 0.05†) than matched pretreatment value; Significantly less (P < 0.01‡) or greater (P < 0.05§) than placebo control.
FIGURE 2. Impact of steroid or immunosuppressive agent treatment on the weights of the spleen, mesenteric (MLN), and superior cervical (SCLN) lymph nodes in female MRL/lpr mice. Animals (n = 9 to 12/group) were exposed to pharmaceutical compounds, as explained in the legend to Figure 1. For comparative analysis, only the pair of SCLN immediately adjacent to the midline were obtained. Values equal the mean ± SE. Dexamethasone therapy significantly (P < 0.05*) reduced the SCLN weight, compared to that of the placebo-treated group; cyclophosphamide administration significantly (P < 0.005f) decreased the weights of the spleen, MLN and SCLN, relative to those of the placebo group.

With regard to exocrine gland weights (Table 2), both testosterone and 19-nortestosterone significantly (P < 0.005f) increased absolute lacrimal tissue weight, as well as the lacrimal gland:body weight ratio. In contrast, the compound Org 4094 significantly (P < 0.01) decreased the weight of lacrimal glands, but this effect appeared to reflect fluctuations in body weight because the lacrimal gland:body weight ratio did not change. Administration of 17β-estradiol and cyclophosphamide caused no alteration in the size of lacrimal tissue, but associated body weight responses led to an increase in the lacrimal gland:body weight ratio. Regarding submandibular tissue, this gland's weight, as well as its ratio to body weight, were significantly elevated by treatment with testosterone, 19-nortestosterone, 17β-estradiol, and cyclophosphamide.

Exposure of MRL/lpr female mice to various steroids also significantly influenced tear volumes. As shown in Figure 3, administration of 17β-estradiol, Org 4094 or dexamethasone for 3 weeks resulted in a significant (P < 0.05) decline in tear volume, compared to that of placebo-treated controls.

**DISCUSSION**

The present study demonstrates that the anti-inflammatory properties of androgens in autoimmune lacrimal tissue may be somewhat unique and not necessarily shared by other steroids or immunosuppressive compounds. Thus, the androgenic hormone, testosterone, as well as its anabolic analogue, 19-nortestosterone, significantly suppressed lymphocyte infiltration in lacrimal glands of a female mouse model of Sjögren's syndrome. These hormonal effects were duplicated by the administration of cyclophosphamide but not by treatment with dexamethasone, estradiol, danazol, Org 4094, or cyclosporine A. In fact, exposure of MRL/lpr mice to estradiol, Org 4094, or cyclosporine A significantly exacerbated certain aspects of lacrimal gland inflammation.

Androgen therapy also significantly reduced the extent of lymphocyte infiltration in submandibular
glands, but the mechanism(s) underlying hormonal action in salivary tissues may well be different than in lacrimal tissues. Existing evidence indicates that androgens may act through tissue-specific processes to suppress inflammation. Moreover, the potential target cells involved in hormonal effects, as well as the inherent nature, magnitude, and androgen susceptibility (e.g., time course [see also this study]) of autoimmune disease, appear dissimilar in these exocrine glands.

In contrast to its immunosuppressive impact in lacrimal and salivary tissues, androgen treatment did not diminish the size of the mesenteric or superior cervical lymph nodes or spleen, which undergo tremendous enlargement in MRL/lpr mice due to progressive lymphocyte accumulation. This apparent inability of androgens to reduce lymphatic tissue inflammation is not surprising, given the known site specificity associated with endocrine-immune interactions. Hormonal regulation of immune function is often restricted to selected microenvironments or tissues and is not generalized throughout the body. For example, androgens have been shown to control numerous immune parameters in the lacrimal gland but not those of other mucosal or peripheral sites. Furthermore, previous investigations have demonstrated that androgen administration is unable to alleviate various systemic, immune dysfunctions in MRL/lpr mice. These findings would suggest that specific endocrine therapies may not correct all manifestations of multidimensional autoimmune disorders, such as Sjögren’s syndrome. However, appropriate hormone treatment might be targeted to responsive tissues, to alleviate safely and effectively the immunopathologic sequelae in those sites.

Our results showed that cyclophosphamide, like androgens, possessed the capacity to ameliorate inflammation in lacrimal and salivary glands of autoimmune mice. In addition, this immunosuppressive compound significantly decreased the extent of mucosal and peripheral lymphadenopathy. These observations confirm, or extend, the findings of others, who have demonstrated that cyclophosphamide exposure abrogates sialoadenitis, as well as systemic autoantibody production and immune complex deposition in renal glomeruli. The mechanism of action of cyclophosphamide appears to involve hepatic activation, which results in the generation of alkylating metabolites that are cytotoxic for certain lymphocytes. However, the scope or efficacy of cyclophosphamide’s influence on MRL/lpr immunopathology is not unlimited, given that this agent attenuates pulmonary perivascular inflammation in male, but not in female, MRL/lpr mice. Moreover, the degree of immune cell susceptibility to cyclophosphamide may vary in exocrine and lymphatic tissues.

Dexamethasone treatment, known to reduce inappropriate immune expression in certain inflamed glands of MRL/lpr mice, also curtailed the area of lymphocyte infiltration in submandibular tissue and diminished the weight of superior cervical lymph nodes. However, glucocorticoid administration elicited no effect on the magnitude of lacrimal gland inflammation in MRL/lpr animals. This disparate action of dexamethasone on salivary and lacrimal tissues supports the contention that the underlying disease or innate response of lymphocyte populations to pharmacological compounds are different in these glands. It is possible that the absence of glucocorticoid effect on lacrimal infiltration may reflect the advanced state of autoimmunity in these mice, given that glucocorticoids have been proposed as a potential therapy for lacrimal dysfunction during the early stages of Sjögren’s syndrome. However, another possibility is that glucocorticoid exposure is simply ineffective, given that chronic corticosteroid treatment of patients with Sjögren’s syndrome does not appear to mitigate the severity of lacrimal gland disease.

The inability of Org 4094 to decrease lacrimal and salivary gland inflammation, relative to placebo levels, was entirely unexpected. This synthetic steroid, which has very weak estrogenic action and no androgenic, progestational, or glucocorticoid activity, has been demonstrated to suppress significantly lymphocyte infiltration in salivary glands of NZB/NZW F1 and NOD mice. Furthermore, comparative studies have indicated that Org 4094 is twice as effective as tibolone, an immunosuppressive steroid, in reducing sialadenitis in NZB/NZW F1 animals. It may be that the absence of effect of Org 4094 in salivary tissues was due, in part, to an insufficient period of drug exposure. Our experimental design involved a 3-week treatment with various compounds, whereas previous research has shown that Org 4094 may diminish salivary inflammation in other autoimmune mice after 13, but not 6, weeks of administration. Nevertheless, our principle objective in the current studies was to assess the relative ability of pharmaceutical agents in duplicating testosterone’s rapid suppression of lymphocyte infiltration in lacrimal tissue. Consequently, whether an extended interval of Org 4094 treatment may result in an amelioration of MRL/lpr salivary gland inflammation or exert any positive impact on lacrimal autoimmunity remains to be determined.

Administration of danazol to female MRL/lpr mice after the onset of disease did not reduce the extent of inflammation in lacrimal or salivary tissues or alter the magnitude of lymphadenopathy. Danazol, a synthetic 17α-alkyl derivative of ethinyltestosterone, has previously been demonstrated to decrease proteinuria, diminish the serum levels of acute-phase reactants, and prolong survival in female MRL/lpr an-
Moreover, this anabolic hormone is used in the treatment of hereditary angioedema, idiopathic thrombocytopenic purpura, and hemophilia. However, despite displaying some affinity for androgen receptors, danazol appears unable to replace testosterone in the regulation of lacrimal immune function, lacrimal autoimmune expression, or various immunopathologic conditions in murine models of systemic lupus erythematosus.

Cyclosporine A, which blocks the activation of T cells, has been proposed as a possible therapeutic agent for lacrimal gland dysfunction in Sjögren’s syndrome. In support of this hypothesis, investigations have shown that cyclosporine A alleviates certain dry eye symptoms in dogs. In addition, cyclosporine A treatment is known to suppress a wide array of immune disturbances in humans and in animal models. These actions include the apparent elimination of aberrant HLA-DR profiles in inflamed salivary tissue of patients with Sjögren’s syndrome (after 6 months of treatment) and the curtailment of numerous systemic abnormalities in MRL/lpr mice, such as arthritis, glomerulonephritis, lymphoid tissue hyperplasia, and inappropriate T cell proliferation and gene expression (after 14 weeks of treatment, initiated before disease onset). However, our results and those of others suggest that cyclosporine A therapy may be questionable in the treatment of established exocrine gland inflammation in Sjögren’s syndrome. The short-term administration of cyclosporine A to female MRL/lpr mice led to a significant increase in the number and size of lymphocyte infiltrates in lacrimal tissue and to a pronounced rise in the overall magnitude of lacrimal inflammation. Similarly, long-term exposure of patients with Sjögren’s syndrome to cyclosporine A significantly worsened the extent of inflammation in minor salivary glands. Therefore, although cyclosporine A may be effective in ameliorating some disorders of the anterior segment or in diminishing particular immune dyscrasia, the usefulness of this agent for the therapy of immunopathologic lesions in lacrimal or salivary glands of individuals with Sjögren’s syndrome remains to be shown.

Estrogen treatment of female MRL/lpr mice did not lessen the overall magnitude of lymphocyte infiltration in either lacrimal or salivary tissues. Our use of estradiol in the present investigation was prompted by the speculation in the literature that this steroid may serve as an effective treatment for lacrimal gland hyposcrosis and/or keratoconjunctivitis sicca in peri-menopausal women or in patients with Sjögren’s syndrome. The rationale for these propositions, however, is unclear, particularly in regard to Sjögren’s syndrome, given that: (1) estrogens often accelerate and amplify autoimmune diseases and may, in fact, be involved in the etiology of Sjögren’s syndrome; (2) estrogen receptors may or may not exist in lacrimal tissue of different species and are undetectable in the human conjunctiva; (3) estrogens appear to have little or no influence on lacrimal gland structure or function and do not modulate lacrimal mucosal immunity; (4) estrogens, contained within oral contraceptives, have been reported to elicit no effect or to reduce significantly, both tear production and tear film breakup time; and (5) estrogen therapy of patients with Sjögren’s syndrome had no impact on ocular manifestations of the disease. Of interest are several additional observations. First, prenatal estrogen exposure induces the development of autoimmune salivary gland lesions, indistinguishable from those salivary infiltrates evident in patients with Sjögren’s syndrome. Second, lacrimal glands of individuals with prostatic carcinoma contained slight to moderate lymphocyte infiltration after therapy with female hormones. Third, estrogens have been proposed as the trigger for the self-perpetuating autoimmune process in MRL/lpr mice and have been shown to enhance markedly polyclonal B cell activation, antibody formation, and various tissue abnormalities. Fourth, estrogen administration is known to increase serum prolactin levels, which, by apparently binding to the cyclosporine A receptor, may significantly exacerbate autoimmune disease. In contrast, a recent report has indicated that chronic (2 months) estradiol treatment of MRL/lpr mice, if initiated before the onset of disease, may reduce the percentage (i.e., infiltrate area/section area ×100) of salivary gland inflammation. However, whether this effect represents actual immunosuppression or instead reflects the significant, estrogen-induced rise in salivary gland weight is uncertain.

The administration of estradiol, dexamethasone, or Org 4094 led to a significant decrease in tear volume, compared to that of placebo-treated controls. This effect of glucocorticoids or estrogens has previously been observed in non-autoimmune, orchietomized rats, “normal” humans, respectively. However, whether the mechanism of these hormonal actions in MRL/lpr mice is mediated through direct (e.g., fluid secretion by lacrimal epithelial cells) or indirect (e.g., lymphocyte cytokine release, tear clearance rates) effect has yet to be elucidated.

In summary, the present investigation shows that androgen or cyclophosphamide treatment significantly ameliorates autoimmune expression in lacrimal and salivary glands of female MRL/lpr mice. However, whether these findings are applicable to humans remains to be demonstrated. It is of interest that male and female patients with systemic lupus erythematosus, who may also suffer from secondary Sjögren’s syndrome, have reduced serum concentrations of androgen. Moreover, uncontrolled clinical studies
have suggested that systemic androgen therapy may alleviate ocular symptoms and augment tear production in humans with Sjögren's syndrome. Consequently, it is possible that an optimal, nonvirilizing androgen, if delivered through an appropriate route of application, might potentially serve as a safe and effective treatment for lacrimal gland autoimmune disease in Sjögren's syndrome.

**Key Words**
Sjögren's syndrome, lacrimal gland, submandibular gland, androgens, cyclophosphamide, lymphocytes, tears, MRL/Mp-lpr/lpr mouse

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