Androgen Influence on Lacrimal Gland Apoptosis, Necrosis, and Lymphocytic Infiltration

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PURPOSE. Previous studies have shown that ovariectomy and hypophysectomy cause regression of the lacrimal gland and have implicated androgens as trophic hormones that support the gland. The purposes of this study were to test the hypothesis that glandular regression after ovariectomy is due to apoptosis, to identify the cell type or types that undergo apoptosis, to survey the time course of the apoptosis, and to determine whether ovariectomy-induced apoptosis could be prevented by dihydrotestosterone (DHT) treatment.

METHODS. Groups of sexually mature female New Zealand White rabbits were ovariectomized and killed at various time periods up to 9 days. Additional groups of ovariectomized rabbits were treated with 4 mg/kg DHT per day. At each time period, sham-operated rabbits were used as controls. Lacrimal glands were removed and processed for analysis of apoptosis as assessed by DNA fragmentation and for morphologic examination. DNA fragmentation was determined using the TdT-dUTP terminal nick-end labeling assay and by agarose gel electrophoresis. Labeled nuclei were quantified by automated densitometry. Sections were also stained for RTLA (rabbit thymic lymphocyte antigen), rabbit CD18, and La antigen. Morphology was evaluated by both light and electron microscopy.

RESULTS. The time course of apoptosis exhibited two phases, a rapid and transient phase and a second prolonged phase. A transient phase peaked at approximately 4 to 6 hours after ovariectomy. The values for degraded DNA as a percentage of total nuclear area were 4.29% ± 0.79% and 4.26% ± 0.54%, respectively. The values for sham-operated controls examined at the same time periods were 1.77% ± 0.08% and 0.82% ± 0.21%, respectively. The percentage of degraded DNA at 24 hours after ovariectomy was not different from controls examined at the same interval after sham operation. The percentage of degraded DNA 6 days after ovariectomy was significantly increased (8.5% ± 2.4%), compared with sham-operated animals at the same time period (0.68% ± 0.03%). DNA laddering was more pronounced after ovariectomy. Dihydrotestosterone treatment in ovariectomized rabbits suppressed the increase in DNA degradation. Morphologic examination of lacrimal gland sections indicated that ovariectomy caused apoptosis of interstitial cells rather than acinar or ductal epithelial cells. Tissue taken 4 hours and 6 days after ovariectomy showed nuclear chromatin condensation principally in plasma cells. Increased numbers of macrophages were also evident. Significant levels of cell degeneration and cell debris, characteristic of necrosis, were observed in acinar regions 6 days after ovariectomy. Dihydrotestosterone prevented this necrosis. Increased numbers of RTLA+, CD18+, and La+ interstitial cells were also evident 6 days after ovariectomy. In addition, ovariectomy increased La expression in ductal cells. Dihydrotestosterone treatment prevented the increase in numbers of lymphoid cells and La expression. Dihydrotestosterone also promoted the appearance of mitotic figures in acinar cells and increased the sizes of acini by 43% (P < 0.05).

CONCLUSIONS. Glandular atrophy observed after ovariectomy is likely to proceed by necrosis of acinar cells rather than apoptosis. This process begins with an apparent time lag after a rapid phase of interstitial cell apoptosis. These processes are accompanied by increased lymphocytic infiltration. These results suggest that a critical level of androgen is necessary to maintain lacrimal gland structure and function and that a decrease in available androgen below this level could trigger lacrimal gland apoptosis and necrosis, and an autoimmune response. Because apoptotic and necrotic cell fragments may be sources of autoantigens that can be processed and presented to initiate an autoimmune reaction, we surmise that cell death triggered by androgen withdrawal may trigger an autoimmune response such as that encountered in Sjögren’s syndrome. Therefore, replacement of androgens in states of low androgen levels, such as after menopause, might help to cure primary lacrimal deficiency and prevent Sjögren’s autoimmunity. The possibility of the role of an autocrine factor, a paracrine factor, or both, promoted by androgens, and the action of other hormones, such as gonadotropin releasing factor, on lacrimal gland cells needs to be investigated.

Recent research has suggested that androgens play a major role in supporting lacrimal gland cell number and lacrimal gland secretory function. Lacrimal glands rapidly regressed when female rats were hypophysectomized and when rabbits were ovariectomized. In these experimental animal models, decreases in DNA and total protein were observed, together with decreases in catalytic activities or receptor numbers related to the capacity of the gland to secrete in response to autonomic stimulation. Dihydrotestosterone (DHT) partially or totally reversed or prevented the decreases of most of these parameters. In addition, DHT increased the volume of fluid produced by the lacrimal gland in response to cholinergic stimulation.

Hormone withdrawal has been shown to promote apoptosis in certain differentiated mammalian organs that are hormone-dependent. Thus, prostatic cells deprived of testosterone, adrenal cortical cells deprived of adrenocorticotropic hormone, renal tubular epithelial cells after removal of renal growth factors such as epidermal growth factor, and insulin-like growth factor-1, and endometrial cells deprived of progesterone all show apoptosis.

These observations led us to postulate that a decline of androgen levels, below the minimum value needed to support normal function, triggers glandular regression through the process of programmed cell death (apoptosis). Therefore, the purpose of this study was to test the hypothesis that glandular regression after ovariectomy is due to apoptosis, to identify the cell type or types that undergo apoptosis, to survey the time course of the apoptosis, and to determine whether ovariectomy-induced apoptosis could be prevented by DHT treatment.

Materials and Methods

Materials

The in situ apoptosis detection kit ApopTag was obtained from Oncor (Gaithersburg, MD), and the Easy-DNA kit for extraction of DNA was obtained from Invitrogen (San Diego, CA). Mouse monoclonal antibody (MAB) against rabbit CD18 (MAB L13/64) was obtained from Spring Valley Laboratories (Sykesville, MD). A mouse MAB that is directed to the RNP consensus motif of human Lα protein and cross-reacts with rabbit was used (M. Bachmann, unpublished observations). This antibody recognizes an N-terminal epitope similar to the anti-Lα MAB SW5 that was previously described by Smith et al. A goat polyclonal antibody to rabbit thymic lymphocyte antigen (RTLA) was obtained from Accurate Chemical and Scientific (Westbury, NY). Biotinylated goat anti-mouse antiserum was obtained from Chemicon International (Temecula, CA), and biotinylated donkey-anti-goat antiserum was obtained from The Binding Site (San Diego, CA). The Vectastain Elite ABC kit was obtained from Vector Laboratories (Burlingame, CA). Other reagents were obtained from standard suppliers.

Animals and Treatments

Sexually mature female New Zealand white rabbits (4–4.5 kg) were obtained from Irish Farms (Norco, CA). They were used in accordance with the National Institute of Health Guiding Principles for the Care and Use of Animals and the ARVO Statement on the Use of Animals in Ophthalmic Research. Rabbits were anesthetized with 40 mg/kg ketamine and 10 mg/kg xylazine and ovariectomized through a midline abdominal incision. Short- and long-term ovariectomy experiments were performed; after the experimental period the animals were killed with an overdose of pentobarbital (120 mg/kg). In the short-term experiments, rabbits were ovariectomized and killed after 1, 2, 4, 6, and 24 hours. In the long-term experiments, animals were killed at 3, 6, and 9 days after ovariectomy. Once the time course of apoptosis was clarified, these experiments were repeated, and an extra group of ovariectomized rabbits was treated subcutaneously at the same time with 4 mg/kg of DHT dissolved in corn oil. They were killed at the times when nuclear DNA degradation was the greatest (i.e., at 4 hours and 6 days). Each animal in the long-term experiment received 0.02 mg/kg of the analgesic buprenorphine hydrochloride intramuscularly 24 hours after surgery. In addition, animals were monitored daily for eating, drinking, wound healing, and incision status. Lacrimal glands from both eyes were removed, and each was divided into small pieces that were processed for morphology, immunocytochemistry, and DNA analysis as described below. At each time period, sham-operated rabbits were used as controls. Control and ovariectomized groups were injected with corn oil only.

DNA Degradation

Apoptosis is characterized by an extensive cleavage of the cell’s DNA into oligonucleosome-sized fragments (300 kb, 50 kb, and, eventually, 200 bp) by a Ca2+-dependent endonuclease, resulting in typical ladder patterns after DNA agarose gel electrophoresis. This DNA fragmentation generates 3′-OH DNA ends to which digoxigenin-nucleotide can be bound, and these tagged nucleotides can be recognized microscopically by anti-digoxigenin antibodies conjugated to horseradish peroxidase.

DNA Extraction and Agarose Gel Analysis. A piece of lacrimal tissue from each animal was snap-frozen in liquid nitrogen and stored at −70°C until processed. The frozen tissues were pulverized with a mortar, and DNA was extracted using the Easy-DNA kit. DNA was quantitated with spectrophotometry at 260 nm. Aliquots of DNA (20 μg) from each sample were loaded onto 1.8% agarose gels containing ethidium bromide (0.45 μg/ml) and separated by electrophoresis for 4 hours at 70 V using 0.5X TBE solution (0.045 M Tris–borate and 0.001 M EDTA, pH 8.0) as running buffer. DNA was visualized by a UV (302 nm) transilluminator, and the gels were photographed with a Polaroid camera (Polaroid Corp., Cambridge, MA).

In Situ DNA Degradation Assay. Fresh portions of lacrimal tissue from each animal were frozen with ornithine carbamoyltransferase in liquid nitrogen. Frozen sections
FIGURE 1. TUNEL assay showing nuclei with degraded DNA (brown staining) from 4-hour sham-operated rabbit (A), 4 hours after ovariectomy (B), and 4 hours after ovariectomy in DHT-treated rabbit (C). A magnified picture (40X) of the TUNEL assay from animals 4 hours after ovariectomy shows that DNA degradation occurs in nuclei from interstitial cells (cells between the rings) rather than in acinar cells (cells forming the rings; D). Scale bar, (A, B, C) 90 μm; (D) 50 μm. Sections from five animals from each group were examined.

μm) were cut on a Zeiss Microm Cryostat (HM 500 OM; Microm, Woeldorf, Germany) and picked up on Superfrost-Plus (Fisher Scientific, Pittsburgh, PA) glass slides. The nuclei containing degraded DNA were stained by using the in situ apoptosis detection kit ApopTag (Oncor), which is a TdT-dUTP terminal nick-end labeling (TUNEL) assay, and counter-stained with methylene green, dehydrated, and mounted with Permount (Fisher Scientific, Pittsburgh, PA). The areas containing labeled nuclei were quantified as described below.

Heart and thymus were used as negative and positive controls for apoptosis.

Morphologic Assays

Light Microscopy. Lacrimal gland tissue was immediately fixed for a minimum of 2 hours in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (half-strength Karnovsky's fixative, pH 7.2). Primary fixative was removed, and tissues were briefly rinsed in 0.1 M cacodylate buffer and postfixed for 2 hours in 1% OsO₄ on ice. The tissues were dehydrated in a series of alcohols and embedded in Epon. Semi-thin sections (1-2 μm) were cut with a glass knife, stained with methylene blue-azure II-basic fuchsin, and examined and photographed with an Olympus Vanox microscope (Olympus, Tokyo, Japan).

Electron Microscopy. Portions of the same blocks used for light microscopy were thin-sectioned using a diamond knife, stained with lead citrate and uranyl acetate, and examined with an electron microscope (JEOL 1200 EX; Japanese Electronic and Optics Ltd., Tokyo, Japan).

Necrosis

One-micron-thick plastic sections were stained as described above under light microscopy. Acinar necrotic cells, characterized by swollen and empty cytoplasm, were counted in lacrimal gland sections obtained from rabbits 6 days after sham operation, 6 days after ovariectomy, or 6 days after ovariectomy treated simultaneously with DHT, using an Olympus Vanox microscope with a 40X objective lens. The area of the lacrimal gland sections occupied by acinar cells that was used to count the necrotic cells was quantified using an Olympus Vanox microscope equipped with the Image Pro analysis system (Scientific Instrument, Sunnyvale, CA). Areas containing ducts, blood vessels, and connective tissue were excluded. Lacrimal gland tissues from five or six animals per group were used, and one section per animal was analyzed. Total acinar area, examined using a 4X plano objective lens, was approximately $8 \times 10^5 \mu m^2$ for the sham-operated group, $5 \times 10^5 \mu m^2$ for the ovariectomized groups, and $7 \times 10^5 \mu m^2$ for the ovariectomized group treated with DHT. Data were expressed as number of necrotic cells per $2 \times 10^6 \mu m^2$ of acinar area.

Immunohistochemistry

Tissue was placed in ornithine carbamoyltransferase and rapidly frozen in liquid nitrogen as discussed above for the in situ DNA degradation assay. Cryostat sections were cut at 8 μm, fixed in cold acetone for 5 minutes, blocked with 5% bovine serum albumin, and incubated for 1 hour at room temperature with the primary antibodies diluted in 50 mM Tris buffer...
containing 150 mM NaCl plus 0.1% bovine serum albumin. After thorough rinsing, sections were incubated for 30 minutes with goat-anti-mouse-biotin 1:750 or donkey anti-goat-biotin 1:500 in the same medium described above. Sections were then quenched in 0.3% H2O2 in 40% methanol for 15 minutes, incubated in ABC reagent for 30 minutes, and developed 3 minutes in a solution of 0.05% diaminobenzidine containing 0.03% H2O2. The sections were rinsed in tap water and mounted in “Brite” floor polish (S. C. Johnson & Son, Inc., Racine, WI) for viewing and photographed with the Olympus Vanox microscope. Immunohistochemistry controls were obtained by omitting primary antibody.

Quantitation of Staining

The areas containing nuclei with degraded DNA were quantified using a Nikon Microphot-FXA microscope (Nikon Inc. Instrument Group, Melville, NY) equipped with a Metamorph Image Analysis System (A.G. Heinze, Irvine, CA). Measurement consisted of two sections per animal and an average of eight separate fields on each section. With the 10× plano objective used, the area for each field analyzed was approximately 245,000 μm². Comparable sections from experimental and control groups were measured for areas of brown (containing apoptotic nuclei) and green (nonapoptotic nuclei) stain, and area of apoptotic nuclei was expressed as a percentage of the total nuclear area. Sample micrographs were taken with an Olympus Vanox-T microscope.

Acinar Size Measurement

One-micron-thick plastic sections were stained as described above for light microscopy. Acinar areas were measured using a Leica Quantimet 570 Image Analyzer (Leica Cambridge, Ltd., Cambridge, England) and a software program customized for this purpose. We measured two slides per animal and eight fields per slide. With the 16× objective used, each field had a calibrated area of 62,953 μm².

Statistical Analysis

The significance of differences among groups was determined by ANOVA and Duncan’s new multiple range test. In time course experiments, in which ovariectomized groups were compared with their respective control group, Student’s t-test for unpaired samples was used. Significance was set at \( P < 0.05 \).

RESULTS

DNA Degradation

The TUNEL assay showed very low levels of nuclear DNA degradation in the lacrimal glands of sham-operated rabbits after 4 hours (Fig. 1A) or 6 days (data not shown). Ovariectomy increased nuclear DNA degradation after 4 hours (Fig. 1B) and 6 days (data not shown). Dihydrotestosterone treatment at the time of ovariectomy suppressed the increase in DNA degradation observed at 4 hours (Fig. 1C) and 6 days (data not shown).

Quantitation of the TUNEL assay verified that DNA degradation increased after ovariectomy, reaching a peak at 4 to 6 hours (4.29% ± 0.79% and 4.26% ± 0.54% of total nuclear area, compared with control values of 1.77% ± 0.08% and 0.82% ± 0.21% of nuclear area), and returning to normal levels by 24 hours (0.40% ± 0.21% compared with control values 0.37% ± 0.14%; Fig. 2). A second phase of increased DNA degradation was observed between 3 and 9 days after ovariectomy. DNA degradation significantly increased from 3 to 9 days compared with control values (Fig. 3). The increases in DNA degradation at 3, 6, and 9 days after ovariectomy were not significantly different from each other (Fig. 3). Dihydrotestosterone treatment suppressed the increase in DNA degradation observed at 4 hours and 6 days after ovariectomy (Fig. 4 and Fig. 5).

For control studies, the TUNEL assay showed no apoptotic nuclei in the heart and many apoptotic nuclei in the thymus (data not shown).

Analysis of DNA by gel electrophoresis showed that the ladder pattern characteristic of oligonucleosome chains was evident in all groups but was substantially more pronounced in the ovariectomized, nontreated groups (Fig. 6).
FIGURE 3. Extended time course of apoptosis after ovariectomy. Values are mean ± SE. *P < 0.05, comparing the two groups at the same time point by Student's t-test. The ovariectomized animals at each point, although significantly different from the sham-operated controls, were not significantly different from each other (by ANOVA and Duncan's new multiple range test). Numbers of animals per group are given inside bars. OVX, ovariectomized; sham, sham-operated.

Morphologic examination showed that cells undergoing nuclear DNA degradation were interstitial cells (Fig. 1D), mainly plasma cells. These cells were observed under the light and electron microscopes and recognized by their eccentrically located nuclei and basophilic cytoplasm and by their prominent rough endoplasmic reticulum with no secretory vesicles. Electron microscopy also showed the typical nuclear chromatin condensation characteristic of apoptosis in the plasma cells of ovariectomized rabbits after 4 hours (data not shown) or 6 days (Fig. 7). Chromatin condensation was not evident in acinar cells or interstitial cells in lacrimal glands of sham-operated animals at the time periods examined (data not shown).

Lymphocytic Infiltration

Immunocytochemical staining revealed that the cells populating the lacrimal glands of sham-operated animals included, in addition to acinar and ductal epithelial cells, T lymphocytes, indicated by RTLA staining (Fig. 8A), and bone marrow–de-
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Sham
OVX
OVX+DHT

FIGURE 5. Effect of DHT treatment on apoptosis observed 6 days after ovariectomy. Values (mean ± SE) with different letter superscripts differ from each other at \( P < 0.05 \) (by ANOVA and Duncan's new multiple range test). Numbers of animals per group are given inside bars. OVX, ovariectomized; sham, sham-operated; OVX + DHT, ovariectomized and treated with DHT.

Dihydrotestosterone decreased the number of interstitial cells expressing La to 31% below control values (Fig. 9C), and it prevented the expression of La by the ductal cells observed 6 days after ovariectomy (Fig. 8).

Necrosis

In addition to the apoptosis observed in interstitial cells, a significant increase in the number of necrotic acinar cells was observed in the lacrimal tissue 6 days after ovariectomy (Fig. 11). Necrosis was distinguished from apoptosis by signs of cell degeneration, such as empty cytoplasm due to plasma membrane breakdown and cellular debris in the absence of nuclear fragmentation (Fig. 8). Dihydrotestosterone treatment of ovariectomized rabbits reduced the number of necrotic cells to a number not significantly different from that of control cells (Fig. 11).

Acinar Size

Although ovariectomy appeared to decrease the size of the acini, the overall change in size was found not to be statistically significant (Fig. 12).

Six days of DHT treatment significantly increased the size of acini (1194 ± 46 \( \mu \text{m}^2 \)) compared with sham-operated (881 ± 47 \( \mu \text{m}^2 \)) and ovariectomized animals (807 ± 59 \( \mu \text{m}^2 \)) (Fig. 12). Dihydrotestosterone treatment also promoted acinar cell division, indicated by the appearance of mitotic figures (not shown).

FIGURE 6. DNA fragmentation assay using frozen lacrimal gland tissue. DNA was isolated and electrophoresed (20 \( \mu \text{g/sample} \)) on a 1.8% agarose gel containing 0.45 \( \mu \text{g/ml ethidium bromide.} \) DNA was visualized by UV light and photographed. (A) Lane 1: DNA from a representative 4-hour sham-operated rabbit; lane 2: DNA from a representative 4-hour ovariectomized rabbit; lane 3: DNA from a representative 4-hour ovariectomized rabbit treated simultaneously with 4 mg/kg DHT. (B) Lane 1: DNA from a representative 6-day sham-operated rabbit; lane 2: DNA from a representative 6-day ovariectomized rabbit; lane 3: DNA from a representative 6-day ovariectomized rabbit treated simultaneously with 4 mg/kg DHT. Left lane in both gels: 123-bp DNA ladder. DNA ladder was more pronounced in lane 2 in both gels. DNA fragmentation from lacrimal glands from three animals per group was assessed.

FIGURE 7. Representative electron micrograph of lacrimal glands from a rabbit 6 days after ovariectomy, showing nuclear chromatin condensation in plasma cells, characteristic of apoptosis. N, normal plasma cell; AP, plasma cell with condensed chromatin; AC, acinar cells. Scale bar, 1 \( \mu \text{m.} \) Sections from six animals per group were examined.
DISCUSSION

Previous experiments have shown that ovariectomy causes functional and biochemical atrophy of the lacrimal gland in rabbits. In addition, ovariectomy decreases the levels of testosterone and androstenedione by 88.5% and 35.9%, respectively, in these animals. Dihydrotestosterone (which unlike testosterone cannot be converted to estrogens) partially or totally prevents the functional and biochemical changes caused by ovariectomy. These observations suggest that lacrimal gland secretory function depends on the circulating levels of androgens and that the decline of androgen levels below the minimum value needed to support normal function triggers glandular regression.

It has been documented in many organs that withdrawal of so-called survival factors could trigger programmed cell death. The survival factors have been shown to be different hormones or growth factors specific for the various tissues. Thus, we postulated that lacrimal gland regression occurred through the process of apoptosis. In the present experiments, ovariectomy caused two surges of nuclear DNA degradation, a characteristic of apoptosis. A rapid and transient phase, reaching peak values at approximately 4 to 6 hours and a second more steady phase reaching a peak value 6 days after ovariectomy. However, the cells undergoing apoptosis were interstitial cells, principally plasma cells, rather than acinar or ductal epithelial cells. Both phases of nuclear DNA degradation were suppressed by treatment with DHT. These observations clearly suggest that a decrease in circulating androgen levels triggered a sequence of events leading to programmed cell death.
Although there was no evidence for apoptosis in the acinar or ductal epithelial cells, morphologic studies of lacrimal gland samples from animals 6 days after ovariectomy revealed considerable cell debris in the acinar region, together with some empty acinar cell cytoplasm. These features are characteristic of a loss of functional and structural integrity of the plasma membrane. Because acinar cells are the largest population of cells in the lacrimal gland, the loss of these cells by necrosis could account for the gland atrophy observed after ovariectomy. The cause of necrosis in the acinar areas is not known. Some possible explanations include a secondary response to loss of trophic paracrine factors produced by interstitial cells eliminated by apoptosis, loss of other factor or factors from the ovary, and increased gonadotropin secretion in response to ovariectomy. Alternatively, necrosis could be a delayed direct response to androgen loss, because androgen receptors have been reported in acinar and ductal epithelial cells in lacrimal tissue of MRL/lpr mice.13

The fact that both apoptosis and necrosis are observed in the lacrimal gland is not unique, because it has been shown that in some other experimental systems cell death is atypical, either exhibiting only some features of apoptosis or mixed features of apoptosis and necrosis. It has been suggested that in these instances the factors triggering cell death also block particular metabolic events of apoptosis. In other instances, certain cells cannot execute all the metabolic events of apoptosis because they may lack one or more effectors needed to carry out these processes.

A greater number of macrophages were observed in the glands at 4 hours and 6 days after ovariectomy. It seems reasonable to assume that their role is to ingest and degrade the apoptotic bodies, necrotic fragments, or both. Although no evidence of phagocytic activity by macrophages or the surrounding epithelial cells was observed, the removal of apoptotic fragments is so rapid that it may be difficult to detect on histologic sections. It is claimed that even if fewer than 1% of cells are identified as apoptotic in a tissue section, the overall cellular loss due to apoptosis occurring over a few days can be substantial. We should also consider the possibility that macrophages play a perhaps inadvertent compensatory role, by increasing the levels of active adrenal androgens, which in turn can be metabolized to more potent androgens, such as androstenedione and testosterone. It has been shown that a high activity of the enzyme dehydroepiandrosterone (DHEA) sulfatase, which converts DHEA-sulfate (inactive) to the active hormone DHEA, exists in murine macrophages.

Our experiments confirm previous reports that lacrimal gland acinar area significantly increases after administration of androgens. Acinar area in lacrimal tissue of males of several species, including rats, mice, guinea pigs, rabbits, and humans, has been shown to be significantly larger than in the females. The mechanism by which androgens prevent apoptosis in plasma cells and promote the growth of acinar cells in the lacrimal gland is not clear. Androgens could be acting independently in the two kinds of cells, but the two cell populations

![Figure 9](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933212/)
FIGURE 10. Representative light micrographs of lacrimal glands from rabbits 6 days after sham operation (A), 6 days after ovariectomy (B), and 6 days after ovariectomy with simultaneous DHT treatment (C). (B) Macrophages (arrowheads), plasma cells (thin arrow), and necrotic acinar cells (thick arrows). (B, inset) A macrophage (M; arrowhead) and a plasma cell (P; thin arrow). Scale bar, (A, B, C, inset) 20 µm. Sections from six animals per each group were examined.

also could act in concert to favor and maintain optimal lacrimal gland function.

One possibility is that androgens exert a direct action on the interstitial cells that will release a factor necessary to maintain the function and structure of the acinar cells. Although Ono et al.,13 found no detectable androgen receptors in the lymphocytic population of the lacrimal tissue of MRL/lpr mice, androgen receptors have been reported in primary cultures of macrophages from human synovial tissues24 and in all classes of thymocytes defined by surface markers CD4 and CD8 from normal and autoimmune disease animal models.25 In addition, a direct action of testosterone on T cells, which is not mediated through the classic androgen receptor but rather through unconventional plasma membrane receptors, has been suggested.26

One factor released by the interstitial cells necessary to maintain acinar cell function and structure could be immunoglobulin A. Plasma cells are the source of the main immunoglobulin of the tear film, IgA, and the polymeric immunoglobulin receptor, plgR, expressed in high levels by acinar cells. The concentration of both IgA and the secretory component (SC), which is a fragment of plgR, are well known to be androgen dependent. In rat tears, the levels of both IgA and SC decrease significantly after androgen removal by castration27,28 whereas treatment of orchietomized rats with testosterone reverses this decrease and produces a three- to five-fold increase in their concentration.27,28

Other candidates could be growth factors and/or cytokines present in the lacrimal gland. Androgen has been shown to enhance prostatic epithelial cell growth only when epithelial cells are cocultured with prostatic fibroblasts,29 suggesting that a paracrine growth factor from the fibroblasts plays an important role in androgen-induced growth of the epithelial cells. Keratinocyte growth factor has been proposed as the paracrine growth factor.30 Androgens could also induce growth of the acinar cells by an autocrine action. Thus, it has been shown that androgen can induce growth of mouse mammary carcinoma cells (SC-3) through an autocrine mechanism mediated by androgen-induced growth factor and its receptor.31

The present experiments support the thesis that lacrimal gland integrity and secretory function depend significantly on the action of androgens and that periods of insufficient androgen levels could be the cause for primary lacrimal deficiency.32 In fact, recent studies have demonstrated correlations between dry eye and decreased testosterone levels in women.33,34

Androgens have also been shown to protect from autoimmune diseases. Thus, women have a higher incidence of most autoimmune diseases, including SLE,35 rheumatoid arthritis,36 and Sjögren's syndrome.37 Androgen deficiency has been reported in both men and women with rheumatoid arthritis and SLE.38 Patients with SLE and rheumatic disease have been

FIGURE 11. Number of necrotic acinar cells per 2 × 10⁶ µm² of acinar area from rabbit lacrimal glands 6 days after sham operation (sham), 6 days after ovariectomy (OVX), and 6 days after ovariectomy with simultaneous DHT treatment (OVX + DHT). Values (mean ± SE) with different letter superscripts differ from each other at P < 0.05 (by ANOVA and Duncan's new multiple range test). Numbers of animals per group are given inside bars.
found to have elevated oxidation of androgens at C-17 with P < 0.05 (by ANOVA and Duncan's new multiple range test). Numbers of animals per group are given inside bars.

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