Role of Proinflammatory Cytokines in the Impaired Lacrimation Associated with Autoimmune Xerophthalmia

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PURPOSE. To determine the effects of the proinflammatory cytokines interleukin (IL)-1α, IL-1β, and tumor necrosis factor (TNF)-α on neurally mediated lacrimal gland protein secretion and to determine whether the amount of IL-1β protein is upregulated in inflamed lacrimal glands of the MRL/lpr mouse, a murine model of human Sjögren syndrome.

METHODS. Lacrimal gland lobules of BALB/c mice were prepared and incubated for 2 hours in the presence or absence of recombinant human (rh)IL-1α, rhIL-1β (10 ng/mL each), or rhTNFα (50 ng/mL). Peroxidase secretion in response to depolarizing KCl (75 mM) solution was measured by spectrophluorometric assay. In another set of experiments, saline, rhIL-1β (1 μg), or an antibody against IL-1 receptor type 1 (IL-1RI), with or without rhIL-1β, was injected (2 μL) into the lacrimal glands of anesthetized BALB/c mice. Twenty-four hours later, lacrimal gland lobules were prepared and peroxidase secretion was measured. The amount of IL-1β protein in lacrimal gland acinar cell lysates prepared from 3-, 9-, and 13-week-old BALB/c, MRL/lpr, and MRL/lpr mice was determined by ELISA.

RESULTS. KCl-induced peroxidase secretion was inhibited in vitro 62%, 66%, and 53% by rhIL-1α, rhIL-1β, and rhTNFα, respectively. In vivo, rhIL-1β inhibited KCl-induced peroxidase secretion by 72%. This inhibitory effect of IL-1β was completely reversed by an antibody against IL-1RI. Compared with 3-week-old mice, the amount of IL-1β protein was upregulated 15- and 21-fold in lacrimal gland acinar cells isolated from 9- and 13-week-old MRL/lpr mice, respectively.

CONCLUSIONS. Proinflammatory cytokines inhibit neurally mediated lacrimal gland secretion. The amount of IL-1β protein is upregulated in acinar cells prepared from lacrimal glands infiltrated with lymphocytes. These results suggest that elevated levels of IL-1β, as they occur in Sjögren syndrome exocrine glands, may impair the secretory function of these tissues. (Invest Ophthalmol Vis Sci. 2002;43:1429–1436)

The primary function of the lacrimal gland is to secrete proteins, electrolytes, and water onto the ocular surface.¹,² The proteins secreted by the lacrimal gland have antibacterial and growth factor properties that are crucial for the homeostasis of the ocular surface.ª It is therefore not surprising that lacrimal gland fluid secretion is under tight neural control.³ Reflexes from the ocular surface and optic nerve, as well as from higher centers of the brain, stimulate lacrimal gland secretion by means of efferent parasympathetic and sympathetic nerves.⁴ Parasympathetic and sympathetic nerves innervate the acinar cells, duct cells, and blood vessels of the lacrimal gland. The parasympathetic nerves contain the neurotransmitters acetylcholine, which acts through cholinergic muscarinic receptors, and vasoactive intestinal peptide. Sympathetic nerves contain norepinephrine, which acts through adrenergic receptors.²

A decrease in lacrimal gland secretion is a primary cause of aqueous tear-deficient dry eye, and Sjögren syndrome is the leading cause of this type of dry eye.⁵,⁶ Sjögren syndrome is an autoimmune disease that occurs almost exclusively in women (≥90%). The syndrome is associated with extensive lymphocytic infiltration of the lacrimal and salivary glands and destruction of epithelial cells. To date, there is no cure.

The mechanisms responsible for the decreased tear and saliva secretion in Sjögren syndrome are still poorly understood.⁵–⁷ It is believed that immune-mediated destruction of the epithelial cells, due to the progressive lymphocytic infiltration of the lacrimal and salivary glands, is responsible for the decline in tear and saliva production leading to dry eye and dry mouth.⁶–⁷ Recent studies have suggested that production of autoantibodies against acinar cell M₄ muscarinic receptors may be also involved in the impaired production of tears and saliva.¹⁰,¹¹

In previous studies in MRL/lpr mice, a murine model of human Sjögren syndrome, we have found that the lymphocytic infiltration of the lacrimal gland did not alter the parasympathetic, sympathetic, and sensory innervation of the remaining epithelial cells.¹² We also found that acinar cells isolated from lacrimal glands of diseased MRL/lpr animals were responsive to exogenous cholinergic and adrenergic stimulation.¹³ Those findings suggest that neither a decrease in lacrimal gland innervation nor an alteration of the signaling pathways of the remaining epithelial cells could account for the decreased function of the lacrimal gland associated with Sjögren syndrome. In a recent report, we showed that activation with high KCl of nerves of MRL/lpr mouse lacrimal glands infiltrated with lymphocytes does not lead to the release of neurotransmitters that resulted in impaired protein secretion from this gland.¹⁴

Proinflammatory cytokine mediated inhibition of neurotransmitter release is now well documented. For example, several studies have shown that IL-1β, IL-6, and TNFα inhibit acetylcholine and norepinephrine release from sympathetic nerves.¹⁵–¹⁷ In a rat model of acute colitis, an inflammatory disease of the distal colon, IL-1β was implicated in blocking KCl-induced norepinephrine release from the myenteric plexus.¹⁸ In the hippocampus, IL-1β was shown to inhibit glutamate¹⁹ and norepinephrine release and to decrease the acetylcholine content.²⁰ Based on these studies, we hypothesize that concomitant with inflammation of the lacrimal gland in MRL/lpr mice, there is an increase in the production of proinflammatory cytokines, especially IL-1β, that inhibits neurotransmitter release and leads to insufficient lacrimal gland secretion.
secretion. The purpose of the present study was to test this hypothesis.

**MATERIALS AND METHODS**

**Chemicals**

Phenylephrine and hydrogen peroxide were from Sigma (St. Louis, MO), a reagent for peroxidase detection (Amplex Red) was from Molecular Probes (Eugene, OR), and rhIL-1α and rhIL-1β were the generous gift of Craig W. Reynolds (Biological Resources Branch, National Cancer Institute Preclinical Repository, Rockville, MD). Recombinant human TNFα and mouse IL-1β colorimetric sandwich ELISA kits (Quantikine M) were from R&D Systems (Minneapolis, MN), collagenase type CLS III from Worthington Biochemical (Freehold, NJ), purified rat anti-mouse IL-1 receptor type I (IL-1RI, CD121a, clone 35F5) monoclonal antibody from BD PharMingen (San Diego, CA), affinity-purified goat polyclonal antibody against murine IL-1β and affinity-purified rabbit polyclonal antibody against murine IL-1RI from Santa Cruz Biotechnology (Santa Cruz, CA), and nonimmune rat IgG from Jackson ImmunoResearch Laboratories (West Grove, PA). Cytokines, anti-IL-1RI antibody, and nonimmune rat IgG were dissolved in saline.

**Animals**

Female and male MRL/MpJ-Fas-lpr animals (6–12 weeks old) from Taconic (German-town, NY). Animals were maintained in constant-temperature rooms with fixed light-dark intervals of 12 hours length and were fed ad libitum. All experiments were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Schepens Eye Research Institute Animal Care and Use Committee.

**Measurement of Peroxidase Secretion**

Lacrimal glands were removed from female BALB/c mice and cut into small lobules (~2 mm diameter). Lobules were placed in cell striainers, and incubated at 37°C in Krebs-Ringer bicarbonate buffer (KRB; containing, in millimolar: 120 NaCl, 5 KCl, 1 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, and 25 NaHCO₃) supplemented with 10 mM HEPES and 5.5 mM glucose (pH 7.4). The cell striainers containing lobules were transferred into fresh KRB solution every 20 minutes for period of 60 minutes. The lobules were then incubated for 20 minutes in a total volume of 0.8 mL in normal KRB solution (referred to as spontaneous secretion) and then in depolarizing KRB (referred to as evoked secretion) solution, where the concentration of KCl was increased to 75 mM and that of NaCl was decreased to 55 mM to maintain isotonicity. Lacrimal gland lobules were further incubated for 20 minutes in 0.8 mL normal KRB containing phenylephrine (an α₁-adrenergic agonist, 10⁻⁴ M). After incubation, the media were collected and centrifuged to remove debris. The lobules were homogenized in 10 mL Tris-HCl (pH 7.5). The amount of peroxidase in the media and tissue homogenate was determined using peroxidase detection agent (Amplex Red; Molecular Probes). Oxidation of the reagent by peroxidase in the presence of hydrogen peroxide produces a highly fluorescent molecule, resorufin. For the measurement of peroxidase, 0.1 mL medium and 0.01 mL tissue homogenate were spotted in duplicate onto 96-well microplates. To each well was added 0.1 mL assay buffer (50 mM Tris-HCl, pH 7.5) containing 0.2 μL peroxidase detection reagent and 0.2 M hydrogen peroxide. After incubation, the fluorescence was determined in a fluorescence microplate reader (model FL600; Bio-Tek, Winooski, VT) with 530-nm excitation wavelength and 590-nm emission wavelength. The amount of secreted peroxidase was expressed as a percentage of the total: (peroxidase in media/peroxidase in media + peroxidase in tissue) × 100.

**Effect of IL-1β, IL-1α, and TNFα on Peroxidase Secretion**

In one set of experiments, lacrimal gland lobules of BALB/c mice were incubated for 2 hours in the absence or presence of recombinant human (rh)IL-1β, rhIL-1α (10 ng/mL each), or rhTNFα (50 ng/mL). Spontaneous, evoked, and phenylephrine-induced peroxidase secretion was then measured as described. In another set of experiments, rhIL-1α, rhIL-1β (1 μg each), or saline was injected (2 μL) into the lacrimal glands of anesthetized mice. To determine whether the effects of IL-1β were specific and receptor mediated, we used a rat monoclonal antibody against IL-1RI. The specificity of this antibody has been well documented.²²–²³ Mice were injected with saline (vehicle), antibody alone (4 μg), rhIL-1β (1 μg), nonimmune rat IgG (4 μg), or antibody (4 μg), followed 60 minutes later by injection of rhIL-1β (1 μg). Twenty-four hours after injection, the lacrimal glands were removed, the lobules were prepared, and peroxidase secretion was measured as described.

**Preparation of Lacrimal Gland Acini for the Measurement of IL-1β Protein and Western Blot Analysis**

Lacrimal glands were removed from 3-, 9-, and 13-week-old BALB/c, MRL/+/−, and MRL/lpr mice. The glands were minced and incubated in KRB buffer (pH 7.4) supplemented with 10 mM HEPES, 5.5 mM glucose, 0.5% BSA, and collagenase III (150 U/mL). Lobules were subjected to gentle pipetting through tips of decreasing diameter. The preparation was then filtered through nylon mesh (150 μm), and the acini were pelleted by centrifugation (50g, 2 minutes). The pellet was washed through KRB containing 4% BSA by centrifugation (50g, 2 minutes). To remove lymphocytes, acini were subjected to a Ficoll gradient of 2%, 3%, and 4%. We have shown that this treatment successfully removes lymphocytes from the acinar cell preparation.²⁵ Dispersed acini were allowed to recover for 30 minutes in fresh KRB buffer containing 0.5% BSA, after which they were homogenized in 0.3 mL of 10 mM Tris-HCl (pH 7.0). The amount of IL-1β in cell lysates was determined by ELISA and the amount of protein determined using the method of Bradford with BSA as a standard. For Western blot analysis, equal amounts of total protein from acinar cell lysates were separated on 10% sodium dodecyl sulfate-polyacrylamide gels, followed by electrotransfer to nitrocellulose membranes. After Western blot analysis, IL-1RI-immunoreactive bands were visualized using the enhanced chemiluminescence method, and quantitated using NIH Image software (ver. 1.69; provided in the public domain by the National Institutes of Health, Bethesda, MD, and available at http://rsb.info.nih.gov/NIH-image/).

**Immunolocalization of IL-1β and IL-1RI in the Lacrimal Gland**

Lacrimal glands were fixed in 4% formaldehyde in phosphate-buffered saline (PBS, containing in millimolar: 145 NaCl, 7.5 Na₂HPO₄, and 2.7 NaH₂PO₄ (pH 7.2)) overnight at 4°C. After cryopreservation overnight in 30% sucrose in PBS, the tissue was frozen in optimal cutting temperature (OCT) embedding medium. Cryostat sections (6 μm) were placed on gelatin-coated slides and air dried for 1 hour. Sections were incubated with the primary antibody diluted (1:20) in PBS for 2 hours at room temperature. The secondary antibody (1:50) conjugated to FITC, was applied for 30 minutes at room temperature. Coverslips were mounted in medium (Vectashield; Vector Laboratories, Burlin-game, CA). Sections were viewed by a microscope (UFXII; Nikon, Lake Success, NY) equipped for epi-illumination. For negative control, the primary antibody was omitted.

**Data Presentation and Statistical Analysis**

Data were expressed as the mean ± SEM. The data were statistically analyzed using Student’s t-test for paired or unpaired values. P < 0.05 was considered to be significant.
RESULTS

Effect of Overnight Incubation on KCl-Induced Lacrimal Gland Secretion

In a recent study, we described an assay to measure neurotransmitter release from lacrimal gland nerve endings in response to a depolarizing KCl (75 mM) solution. We showed that peroxidase (a protein secreted by the lacrimal gland) secretion in response to a depolarizing KCl solution can be used as an indirect index to monitor lacrimal gland nerve activity. To further validate this assay, we tested the effect of overnight incubation on KCl- and adrenergic agonist-induced peroxidase secretion. If KCl-induced peroxidase secretion is due to nerve activation, then the overnight incubation of lacrimal gland lobules should result in a loss of peroxidase secretion in response to KCl, due to the death of nerve endings. As shown in Table 1, high KCl induced a 3.5-fold increase over basal in peroxidase secretion from freshly isolated lacrimal gland lobules. In contrast, lobules incubated overnight were unresponsive to high KCl stimulation. This was not due to a general unresponsiveness of lacrimal gland tissue after the overnight incubation, because addition of the adrenergic agonist phenylephrine induced a significant increase in peroxidase secretion from both groups of lobules (Table 1).

These results further support our previous report that KCl-induced peroxidase secretion can be used to monitor lacrimal gland nerve activity.

Effect of IL-1α, IL-1β, and TNFα In Vitro on Lacrimal Gland Secretion

Several reports have shown that exogenously added IL-1 or TNFα inhibits neurotransmitter release. Furthermore, elevated levels of IL-1β, occurring with aging or inflammation, have also been shown to inhibit neurotransmitter release. In a recent report, we showed that activation of inflamed lacrimal gland nerve endings with high KCl does not elicit acetylcholine release or peroxidase secretion. We hypothesized that elevated levels of proinflammatory cytokines in inflamed lacrimal glands inhibit neurotransmitter release and hence protein secretion. To test this hypothesis, lacrimal gland lobules were prepared from BALB/c mice and incubated in the presence or absence of rhIL-1α, rhIL-1β (10 ng/mL each), or rhTNFα (50 ng/mL). Peroxidase secretion in response to high KCl (75 mM) or to an adrenergic agonist, phenylephrine (10^−4 M), was then measured. KCl-induced peroxidase secretion was inhibited 62%, 66%, and 53% by rhIL-1α, rhIL-1β, and rhTNFα, respectively (Fig. 1). Phenylephrine-induced peroxidase secretion was also inhibited 40%, 62%, and 36% by rhIL-1α, rhIL-1β, and rhTNFα, respectively.

These results show that proinflammatory cytokines inhibited both neurally and adrenergic agonist-induced lacrimal gland protein secretion.

Effect of IL-1α and IL-1β In Vivo on Lacrimal Gland Secretion

To further substantiate our findings and to study the specificity of the effect of the inflammatory cytokines, rhIL-1α, rhIL-1β (1 μg each), or saline (vehicle) were injected into lacrimal glands of anesthetized BALB/c mice. Twenty-four hours after injection, the lacrimal glands were removed, lobules were prepared, and peroxidase secretion in response to high KCl (75 mM) or to phenylephrine (10^−4 M), was measured. Compared with noninjected animals, saline injection did not have any effect on KCl- or phenylephrine-induced peroxidase secretion (Fig. 2). In contrast, rhIL-1α and rhIL-1β inhibited KCl-induced peroxidase secretion by 65% and 64% and that induced by phenylephrine by 56% and 44%, respectively.

To determine whether the effects of IL-1β were specific and receptor mediated, we used a rat monoclonal antibody against murine IL-1R1 (clone 35F5). Injection of the antibody alone induced a slight, but not statistically significant, increase in KCl- and phenylephrine-induced peroxidase secretion compared with that in saline-injected animals (Fig. 3). Of particular note is that coinjection of this antibody with the cytokine completely abolished the inhibitory effect of rhIL-1β on both KCl- and phenylephrine-induced peroxidase secretion.

The results show that the inhibitory effect of rhIL-1β on lacrimal gland secretion was specific and was mediated by IL-1R1.
and from #evoked peroxidase secretion in the presence of rhIL-1/H9252/H11005 upregulated in biopsy specimens of patients with Sjo/syndrome.26

Several reports have shown that the mRNA for IL-1/H9252/H11005 was upregulated in biopsy specimens of patients with Sjogren syndrome as well as from exocrine tissues of animal models of this syndrome.

Effects of Age and Disease on the Amount of IL-1β Protein

Several reports have shown that the mRNA for IL-1β was upregulated in biopsy specimens of patients with Sjogren syndrome as well as from exocrine tissues of animal models of this syndrome. To determine whether the protein level of IL-1β was increased with age and disease, as suggested by an increase in mRNA, acinar cells were isolated from control BALB/c and MRL/lpr mice and diseased MRL/lpr mice, the amount of IL-1β was then measured with a commercially available ELISA (R&D Systems). MRL/lpr mice undergo spontaneous development in an age-dependent manner of autoimmune disease characterized by lymphoproliferation, autoantibody formation, ocular inflammatory lesions, and lacrimal gland disease, and these mice have been widely used as a model for Sjogren syndrome. MRL/lpr mice are congenic with MRL/lpr in which a later-onset, milder autoimmune disease develops, including lacrimal gland inflammation. MRL/lpr mice can be used as a control for the MRL/lpr animals.

There was a substantial amount of IL-1β protein in lacrimal gland acinar cells of 3-week-old BALB/c, MRL/lpr, and MRL/lpr mice (Fig. 4). The amount of this cytokine increased slightly with age in lacrimal gland acinar cells isolated from BALB/c and MRL/lpr mice. In contrast, there was a dramatic upregulation of IL-1β protein in lacrimal gland acinar cells isolated from 9- and 13-week-old MRL/lpr mice (Fig. 4). Compared with 3-week-old MRL/lpr mice, the amount of IL-1β protein was upregulated 15- and 21-fold in lacrimal gland acinar cells from 9- and 13-week-old MRL/lpr mice, respectively (Fig. 4). The increase in IL-1β protein occurred in a time-dependent manner that seemed to coincide with the kinetics of the lymphocytic infiltration of the lacrimal gland in this murine model of human Sjogren syndrome.

The results show that the amount of IL-1β protein increased in a time-dependent manner in MRL/lpr lacrimal gland acinar cells.

Immunolocalization of IL-1β and the IL-1RI in the Lacrimal Gland

The data shown in Figure 4 suggest that the lacrimal gland acinar cells are the source of IL-1β. To confirm those results, we performed immunofluorescence experiments on lacrimal
glands isolated from 13-week-old MRL/lpr and 18-week-old MRL/+/ mice. IL-1β was present in both the lacrimal gland acinar cells and the infiltrating lymphocytes (Figs. 5A, 5B). IL-1β was present in the cytosol, but a strong immunoreactivity was observed in the plasma membranes of the lacrimal gland acinar cells (Figs. 5A, 5B). Lacrimal gland acinar cells located in areas devoid of lymphocytes also expressed IL-1β (Fig. 5B). In contrast, IL-1β was present only in the infiltrating lymphocytes in lacrimal glands from MRL/+/ mice (Fig. 5C). Omission of the primary antibody led to a loss of immunoreactivity in both MRL/lpr and MRL/+/ lacrimal glands (Figs. 5D, 5E).

There are two receptors for IL-1: a signaling receptor, IL-1RI, and a decoy receptor, IL-1RII. We used both immunofluorescence and Western blot analysis techniques to determine the location and amount of IL-1RI, respectively. Strong immunoreactivity against IL-1RI was identified in both the invading lymphocytes and the lacrimal gland epithelial cells isolated from either MRL/lpr (Figs. 6A, 6B) or MRL/+/ mice (Fig. 6C). As expected, IL-1RI immunoreactivity was mainly membrane-associated. Omission of the primary antibody led to a loss of immunoreactivity in both MRL/lpr and MRL/+/ lacrimal glands (Figs. 6D, 6E).

**Figure 5.** Immunolocalization of IL-1β in the lacrimal gland. Lacrimal glands removed from 13-week-old female MRL/lpr (A, B, D) and 18-week-old female MRL/+/ (C, E) mice were fixed and frozen and cryostat sections prepared. The slides were then incubated with an antibody against murine IL-1β (A, B, C; diluted 1:20) followed by an FITC-conjugated secondary antibody (diluted 1:50). Sections were viewed by epi-illumination microscope. For the negative control, the primary antibody was omitted (D, E). (☆) Areas of lymphocytic infiltration. Similar results were obtained in two other experiments.

**Figure 6.** Immunolocalization of IL-1RI in the lacrimal gland. Lacrimal glands removed from 13-week-old female MRL/lpr (A, B, D) and 18-week-old female MRL/+/ (C, E) mice were fixed and frozen and cryostat sections prepared. The slides were then incubated with an antibody against murine IL-1RI (A, B, C; diluted 1:20) followed by an FITC-conjugated secondary antibody (diluted 1:50). Sections were viewed by epi-illumination microscope. For the negative control, the primary antibody was omitted (D, E). (☆) Areas of lymphocytic infiltration. Similar results were obtained in two other experiments.
After Western blot analysis, IL-1RI was upregulated in lacrimal glands of female MRL/lpr mice, we showed that the hyperresponsiveness of salivary glands isolated from both male and female MRL/lpr mice was not due to the increased amount of this receptor in diseased glands. However, we did not have direct evidence, we hypothesize that the elevated levels of IL-1β in the MRL/lpr murine model of human Sjögren syndrome inhibit neurotransmitter release, leading to insufficient lacrimal gland secretion.

In another important finding of our study, the lacrimal gland epithelial cells were found to be the source of IL-1β. Until recently, it has been thought that this cytokine is only made by bone marrow-derived cells. However, a growing number of reports have shown that IL-1β can be synthesized by somatic cells, including microglia,32 neurons,33,34 pancreatic β-cells,35,36 and parotid gland acinar cells.37 As a consequence, the somatic cells can no longer be considered as passive victims of inflammatory cells, but rather as active participants in maintaining the disease state.

As stated in the Results section, there are two receptors for IL-1, a signaling receptor (IL-1RI) and a decoy receptor (IL-1RII).38 The expression of IL-1 receptors has been shown to be regulated by IL-1 and other cytokines.39,40 Our results show that the lacrimal gland epithelial cells expressed IL-1RI and that the amount of this receptor increased in diseased lacrimal glands.

In our study, proinflammatory cytokines inhibited neurally as well as adrenergic agonist-induced lacrimal gland secretion. Thus, it seems that these cytokines have a dual target in the lacrimal gland: the nerve endings (i.e., inhibition of neurotransmitter release) and the epithelial cells (i.e., inhibition of agonist-induced protein secretion).

In parietal cells, it has been shown that IL-1β and TNFα inhibit agonist-induced acid secretion by multiple pathways.41 The inhibition occurs at postreceptor level and involves pertussis toxin and tyrosine kinase-dependent and independent pathways.42 In another study, IL-1β has been shown to directly inhibit cholinergic agonist-stimulated parietal cell acid secretion. This action occurs distal to cholinergic muscarinic receptor activation and elevations in intracellular calcium and requires protein kinase C.43 In pancreatic β cells, inhibition by IL-1β of glucose-stimulated insulin and glucagon secretion involves multiple pathways including nitric oxide and prostanoids.44-46

Similarly, IL-1β-mediated inhibition of neurotransmitter release has been shown to include multiple pathways. Several elegant studies by Lynch39 and Verker et al.47 show that concomitant with inhibition of glutamate release in the hippocampus by IL-1β, both in vitro and in vivo, there is increased activity of the stress-activated protein kinases c-Jun NH₂-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). Another group has shown that inhibition of p38 MAPK antagonizes the inhibitory effect of IL-1β on long-term potentiation in the rat dentate gyrus.48 After application of IL-1β, it has been found that there is a parallel increase in lipid peroxidation, through the production of reactive oxygen spe-

**DISCUSSION**

Proinflammatory cytokine-mediated inhibition of neurotransmitter release is now well documented. For example, several studies have shown that IL-1β, IL-6, and TNFα inhibit myenteric nerve activity.15-17 In a rat model of acute colitis, IL-1β was implicated in blocking norepinephrine release from the myenteric plexus.18 In the hippocampus, IL-1β has been shown to inhibit glutamate19 and norepinephrine release20 and to decrease acetylcholine content.21 TNFα has also been shown to alter neurotransmitter release in cultured sympathetic neurons.29 Recent reports have shown that IL-1β and IL-6 act synergistically to suppress cholinergic and adrenergic neurotransmission in the myenteric plexus.30,31 The results in the present study add lacrimal gland nerve endings as a target of proinflammatory cytokines.

To further confirm the expression of IL-1RI, we performed Western blot analyses on lacrimal gland acinar cell (devoid of lymphocytes) homogenates prepared from male and female MRL/lpr and MRL/+ mice. Acinar cells were then prepared and lymphocytes removed. Acini were homogenized and proteins separated by SDS-PAGE followed by electrophoresis to nitrocellulose membranes. After Western blot analysis, IL-1RI-immunoreactive bands were visualized by the enhanced chemiluminescence method, and quantitated by computer. (A) Actual blots. Left: molecular mass (in kilodaltons) of protein standards. Data in (B) are expressed as the mean ± SEM, n = 3. Significant difference between female MRL/lpr and female MRL/+ mice.
cies, and a decrease in membrane arachidonic acid concentration. The investigators proposed that the decrease in membrane arachidonic acid concentration, by changing membrane fluidity, could alter the activity of membrane-associated proteins such as Ca\(^{2+}\) channels and protein kinases. Neurotransmitter release, similar to protein exocytosis from exocrine tissues, is tightly regulated by changes in Ca\(^{2+}\) concentration and the activity of several protein kinases and phosphatases.

In summary, in our study proinflammatory cytokines inhibited neurally as well as agonist-mediated lacrimal gland secretion. The amount of IL-1\(\beta\) and IL-1RI proteins were upregulated in acinar cells prepared from lacrimal glands infiltrated with lymphocytes. Taken together, our results suggest that elevated levels of IL-1\(\beta\), as occurs in Sjögren syndrome exocrine glands, may impair the secretory function of these tissues, leading to dry eye and dry mouth.

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