

# Sex Effects on Gene Expression in Lacrimal Glands of Mouse Models of Sjögren Syndrome

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**PURPOSE.** Sjögren syndrome is an autoimmune disease that occurs primarily in women, and is associated with lacrimal gland inflammation and aqueous-deficient dry eye. We hypothesize that sex-associated differences in lacrimal gland gene expression are very important in promoting lymphocyte accumulation in this tissue and contribute to the onset, progression, and/or severity of the inflammatory disease process. To test our hypothesis, we explored the nature and extent of sex-related differences in gene expression in autoimmune lacrimal glands.

**METHODS.** Lacrimal glands were collected from age-matched, adult, male and female MRL/MpJ-Tnfrsf6<sup>lpr</sup> (MRL/lpr) and nonobese diabetic/LtJ (NOD) mice. Glands were processed for the analysis of differentially expressed mRNAs by using CodeLink Bioarrays and Affymetrix GeneChips. Data were evaluated with bioinformatics and statistical software.

**RESULTS.** Our results show that sex significantly influences the expression of thousands of genes in lacrimal glands of MRL/lpr and NOD mice. The immune nature of this glandular response is very dependent on the Sjögren syndrome model. Lacrimal glands of female, as compared with male, MRL/lpr mice contain a significant increase in the expression of genes related to inflammatory responses, antigen processing, and chemokine pathways. In contrast, it is the lacrimal tissue of NOD males, and not females, that presents with a significantly greater expression of immune-related genes.

**CONCLUSIONS.** These data support our hypothesis that sex-related differences in gene expression contribute to lacrimal gland disease in Sjögren syndrome. Our findings also suggest that factors in the lacrimal gland microenvironment are critically important in mediating these sex-associated immune effects.

**Keywords:** sex differences, Sjögren syndrome, lacrimal gland, gene expression, MRL/lpr-lpr/lpr mice, nonobese diabetic mice

Sjögren syndrome is an autoimmune disease often accompanied by chronic and extensive inflammation of the lacrimal glands.<sup>1,2</sup> This lymphocyte infiltration may severely damage acinar and ductal epithelial cell function, resulting in a significantly diminished output of aqueous tears.<sup>1</sup> In consequence, Sjögren syndrome is a leading cause of aqueous-deficient dry eye disease.<sup>1</sup>

One of the most compelling features of Sjögren syndrome is that it affects predominantly females.<sup>3-5</sup> In fact, female sex is a significant risk factor for the development of Sjögren syndrome, given that 93% of the patient population is female.<sup>3-5</sup> This sexual dichotomy is frequently linked to fundamental sex-related differences in the immune system.<sup>4,6,7</sup> Women have a more potent and competent systemic immune capability than men, and this heightened immunological activity is believed to contribute to the much greater incidence of many autoimmune diseases in females.<sup>3,4,6,7</sup> Indeed, women constitute almost 80%

of the 20 million people in the United States with autoimmune disease.<sup>8</sup>

We hypothesize that sex-associated differences in lacrimal gland gene expression are also very important in promoting lymphocyte accumulation in this tissue and contribute to the onset, progression, and/or severity of the inflammatory disease process. Consistent with this hypothesis is our discovery that the expression of a number of proto-oncogenes and apoptotic genes are significantly increased in the inflamed lacrimal tissues of female, as compared with male, MRL/lpr mice.<sup>9</sup>

To continue to test our hypotheses, we sought to explore further the nature and extent of sex-related differences in gene expression in autoimmune lacrimal glands. Toward that end, we examined and compared the gene expression in lacrimal glands of female and male MRL/MpJ-Tnfrsf6<sup>lpr</sup> (MRL/lpr) and nonobese diabetic/LtJ (NOD) mice, respectively. The extent of lacrimal and salivary gland inflammation in MRL/lpr mice is, as in humans, far greater in females as compared with males.<sup>10</sup> In



contrast, although the salivary gland immunopathology in NOD mice is more extensive in females, the magnitude of lacrimal gland inflammation is far worse in NOD males (Toda I, et al. *IOVS* 1997;34:ARVO Abstract 434).<sup>10,11</sup> We believe that this differential autoimmune expression in lacrimal glands of MRL/lpr and NOD mice reflects, in large part, the influence of local tissue, as compared with systemic, factors.

## MATERIALS AND METHODS

### Animals and Tissue Collections

Adult male and female MRL/lpr and NOD mice were obtained from the Jackson Laboratories (Bar Harbor, ME, USA). Mice ( $n = 15$  to  $18$ /sex/strain) were housed in constant temperature rooms with fixed light/dark intervals of 12 hours' length. When indicated, mice were killed by CO<sub>2</sub> inhalation and exorbital lacrimal glands were removed for molecular biological procedures. Lacrimal gland samples were prepared by combining tissues from five to six mice/sex/group. Three different sample preparations were made for each tissue/sex/group and then processed for the analysis of gene expression.

All research experiments with mice were approved by the Institutional Animal Care and Use Committee of The Schepens Eye Research Institute and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Molecular Biological Procedures

Total RNA was extracted from lacrimal glands by using TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) and purified with RNAqueous spin columns (Ambion, Austin, TX, USA). The lacrimal gland RNA samples were treated with RNase-free DNase (Invitrogen), analyzed spectrophotometrically at 260 nm to determine concentration, and evaluated with an RNA 6000 Nano LabChip and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) to confirm RNA integrity. The RNA samples were then stored at  $-80^{\circ}\text{C}$  until further processing.

Gene expression was examined by the use of two procedures. One involved the processing of RNA samples for hybridization to CodeLink UniSet Mouse 20K I Bioarrays ( $n \sim 20,000$  genes/array; Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA), according to detailed methods.<sup>12</sup> cDNA was synthesized from RNA (2  $\mu\text{g}$ ) with a CodeLink Expression Assay Reagent Kit (Amersham) and purified with a QIAquick purification kit (Qiagen, Valencia, CA, USA). Samples were dried, and cRNA was generated with a CodeLink Expression Assay Reagent Kit (Amersham), recovered with an RNeasy kit (Qiagen) and quantitated with an UV spectrophotometer. Fragmented, biotin-labeled cRNA was then incubated and shaken at 300 rpm on a CodeLink Bioarray at  $37^{\circ}\text{C}$  for 18 hours. After this time period, the Bioarray was washed, exposed to streptavidin-Alexa 647, and scanned by using ScanArray Express software and a ScanArray Express HT scanner (Packard BioScience, Meriden, CT, USA) with the laser set at 635 nm, laser power at 100%, and photomultiplier tube voltage at 60%. Scanned image files were evaluated by using CodeLink image and data analysis software (Amersham), which yielded both raw and normalized hybridization signal intensities for each array spot. The intensities of the approximately 20,000 spots on the Bioarray image were standardized to a median of 1. Normalized data, with signal intensities greater than 0.50, were analyzed with bioinformatic software (Geospiza, Seattle, WA, USA). This sophisticated software also created gene ontology, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and z-score reports. The ontologies

encompassed biological processes, molecular functions, and cellular components and were organized according to the recommended guidelines of the Gene Ontology Consortium (<http://www.geneontology.org/GO.doc.html>).<sup>13</sup>

The second method to examine differential gene expression involved the hybridization of each cRNA (20  $\mu\text{g}$ ) sample to a GeneChip Mouse Genome 430A 2.0 Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. Reagents for the fragmentation and hybridization steps were from a GeneChip HT One-Cycle Target Labeling and Control Kit, and materials for the washing and staining steps came from a GeneChip HWS kit (Affymetrix). Hybridized GeneChips were scanned with an Affymetrix Model 700 Scanner and expression data files were created from array images by using Affymetrix Microarray Suite 4.0 software. GeneChip data were standardized by choosing the default scaling in Affymetrix GeneChip Operating Software, which yields a trimmed mean intensity of 500 for each GeneChip microarray. Normalized data with a quality value of 1.0 were then analyzed with Geospiza GeneSifter software (Geospiza).

Counts of unique mappings of probes to gene identifications in the CodeLink and Affymetrix arrays showed that there were 15,711 and 13,265 unique genes, respectively, in these arrays. Analysis of the intersection of these lists demonstrated that there was an overlap of 11,299 genes.

Gene expression data were examined without log transformation and statistical analyses of these data were performed with Student's *t*-test (two-tailed, unpaired) by using the GeneSifter software. Our statistical approach was not tailored for multiple comparisons. Genes that were expressed in the same direction in different groups were identified by using GenBank accession numbers and an intersector program (Geospiza). Data used for these CodeLink and Affymetrix arrays are accessible for free download through the National Center for Biotechnology Information's Gene Expression Omnibus via series accession number GSE5876.

## RESULTS

### Influence of Sex on Gene Expression in Lacrimal Glands of MRL/lpr and NOD Mice

To determine the influence of sex on gene expression in lacrimal glands of autoimmune mice, tissues were obtained after disease onset<sup>10</sup> from MRL/lpr ( $n = 18$  mice/sex; age = 19.8  $\pm$  0.3 weeks old) and NOD ( $n = 15$  mice/sex; age = 21.4 weeks old) mice. Glands were pooled according to sex and group ( $n = 10$ – $12$  glands/sex/sample;  $n = 3$  samples/sex/group), processed for the isolation of total RNA, and examined for differentially expressed mRNAs by using CodeLink Bioarrays and Affymetrix GeneChips. Microarray data were analyzed with Geospiza bioinformatics software.

Our findings demonstrate that sex has a significant impact on the expression of thousands of genes in lacrimal glands of MRL/lpr and NOD mice (Table 1). Non-sex chromosome genes with the greatest differences in terms of expression ratios in MRL/lpr mice are shown in Table 2. Genes, such as pancreatic lipase-related protein 1, asialoglycoprotein receptor, S100 calcium-binding proteins A8 and A9, and growth differentiation factor 5, were increased in females, and lymphocyte antigen 6 complex, locus F and cytochrome P450, family 2, subfamily j, polypeptide 13 in were higher in males, and the results were similar with both CodeLink and Affymetrix microarrays.

Additional genes of interest included that for cathepsin S, which is significantly increased in the tears of Sjögren syndrome patients,<sup>14</sup> and is more highly expressed in lacrimal

**TABLE 1.** Number of Genes With Significant, Sex-Related Differences in Expression in Lacrimal Glands of MRL/lpr and NOD Mice

| Mouse Strain/Array | Genes F>M | Genes M>F | Total Genes |
|--------------------|-----------|-----------|-------------|
| MRL/lpr            |           |           |             |
| CodeLink           | 2674      | 1880      | 4554        |
| Affymetrix         | 1316      | 1237      | 2553        |
| NOD                |           |           |             |
| CodeLink           | 3292      | 1721      | 5013        |
| Affymetrix         | 1531      | 1569      | 3100        |

The expression of listed genes was significantly ( $P < 0.05$ ) upregulated between the groups.

tissues of female MRL/lpr mice (CodeLink = 2.85-fold; Affymetrix = 3.03-fold). Also notable were the increased expression of X-chromosome genes, such as X inactive specific transcript (Xist) (CodeLink = 32.0-fold), domesticus antisense RNA from the Xist locus (Affymetrix = 27.7-fold), and moesin (Affymetrix = 3.45-fold) in females, and the X (androgen receptor; CodeLink = 1.7-fold) and Y (eukaryotic translation initiation factor 2, subunit 3; CodeLink = 60.1-fold; Affymetrix = 205.2-fold) chromosome genes in males.

Genes with many of the highest expression differences in terms of ratios in NOD mice are shown in Table 3. Some of these genes (e.g., female [F] > male [M], pancreatic lipase-related protein 1 and asialoglycoprotein receptor; M>F, cytochrome P450, family 2, subfamily j, polypeptide 13, and neuromedin U) showed analogous degrees of difference in both the CodeLink and Affymetrix microarrays. Elevated levels

of Y chromosome genes, including gene eukaryotic translation initiation factor 2, subunit 3 (CodeLink = 48.8-fold; Affymetrix = 10.1-fold) and DEAD box polypeptide 3 (Affymetrix = 115.1-fold) were also found in lacrimal glands of males, whereas the expression of the X-chromosome gene, androgen receptor (Affymetrix = 3.06-fold), was greater in female lacrimal tissues. In contrast to the results with MRL/lpr mice, the expression of cathepsin S (CodeLink = 3.85-fold; Affymetrix = 6.06-fold) and the X-linked gene moesin (Affymetrix = 6.32-fold) were significantly higher in male lacrimal glands, as compared with those of females.

Most of the lacrimal gland genes in MRL/lpr and NOD female and male mice, respectively, which were identified as differentially expressed by the CodeLink and Affymetrix microarrays, were unique to each platform. As shown in Table 4, relatively few genes displaying sex-related differences were expressed by both microarrays. These findings are consistent with our previous investigations,<sup>15-17</sup> as well as those of others,<sup>18-21</sup> which discovered little agreement between CodeLink and Affymetrix microarrays in the detection of differential gene expression. Although these platforms seem to measure different things,<sup>20</sup> most gene expression changes revealed by each of the platforms are thought to be biologically correct.<sup>19,20</sup>

Comparison of gene expression between the inflamed lacrimal glands of MRL/lpr (F>M) and NOD (M>F) mice showed that 465 genes were common (CodeLink). The alternate comparison (i.e., MRL/lpr, M>F; NOD, F>M) revealed 187 genes in common (CodeLink).

**TABLE 2.** Influence of Sex on Gene Expression in Lacrimal Glands of MRL/lpr Mice

| Accession No.   | Gene   | Ratio | P      | Ontology   |
|-----------------|--|-------|--------|--|
| F>M, CodeLink   |  |       |        |  |
| NM_018874       | Pancreatic lipase-related protein 1                    | 960.8 | 0.0183 | Lipid metabolic process                          |
| NM_009714       | Asialoglycoprotein receptor 1                          | 67.6  | 0.0011 | Endocytosis                                      |
| NM_009114       | S100 calcium-binding protein A9                        | 37.0  | 0.0059 | Chemotaxis                                       |
| NM_011105       | Polycystin and REJ                                     | 28.0  | 0.0012 | Transport  |
| NM_013650       | S100 calcium-binding protein A8                        | 27.2  | 0.0039 | Chemotaxis                                       |
| NM_008109.1     | Growth differentiation factor 5                        | 16.4  | 0.0013 | Cell differentiation                             |
| F>M, Affymetrix |  |       |        |  |
| NM_018874       | Pancreatic lipase-related protein 1                    | 629.9 | 0.0015 | Lipid metabolic process                          |
| U09362          | Asialoglycoprotein receptor 1                          | 66.6  | 0.0048 | Endocytosis                                      |
| NM_013650       | S100 calcium-binding protein A8                        | 24.5  | 0.0119 | Chemotaxis                                       |
| NM_009114       | S100 calcium-binding protein A9                        | 21.3  | 0.0054 | Chemotaxis                                       |
| NM_008109       | Growth differentiation factor 5                        | 11.8  | 0.0128 | Cell differentiation                             |
| M93428          | Endothelial ligand for L-selectin                      | 11.8  | 0.0095 | Cell adhesion                                    |
| M>F, CodeLink   |  |       |        |  |
| NM_145548       | Cytochrome P450, family 2, subfamily j, polypeptide 13 | 462.2 | 0.0005 | Monooxygenase activity                           |
| NM_146592       | Olfactory receptor 1086                                | 130.3 | 0.0004 | Signal transduction                              |
| NM_008530       | Lymphocyte antigen 6 complex, locus F                  | 105.3 | 0.0000 | Intrinsic to membrane                            |
| NM_133221       | Solute carrier family 24, member 6                     | 70.9  | 0.0002 | Transport  |
| NM_153419       | Glutamate-rich WD repeat containing 1                  | 69.5  | 0.0004 | Ribosome biogenesis                              |
| NM_145967.1     | V-set and transmembrane domain containing 2A           | 64.1  | 0.0010 | Cell differentiation                             |
| M>F, Affymetrix |  |       |        |  |
| AY079153        | Melanocortin 2 receptor accessory protein              | 244.1 | 0.0002 | Positive regulation of cAMP biosynthetic process |
| M16360          | Major urinary protein V                                | 202.7 | 0.0015 | Transport  |
| NM_008530       | Lymphocyte antigen 6 complex, locus F                  | 171.0 | 0.0000 | Intrinsic to membrane                            |
| NM_008644       | Mucin 10   | 146.0 | 0.0420 | Negative regulation of peptidase activity        |
| BC016446        | Cytochrome P450, family 2, subfamily j, polypeptide 13 | 84.6  | 0.0001 | Monooxygenase activity                           |
| NM_010232       | Flavin-containing monooxygenase 5                      | 57.7  | 0.0041 | Monooxygenase activity                           |

Non-sex chromosome genes with the greatest differences in terms of expression ratios in MRL/lpr mice are listed. Relative ratios were calculated from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from female and male MRL/lpr mice.

**TABLE 3.** Effect of Sex on Gene Expression in Lacrimal Glands of NOD Mice

| Accession No.             | Gene   | Ratio  | P      | Ontology   |
|---------------------------|--|--------|--------|--|
| <b>F&gt;M, CodeLink</b>   |  |        |        |  |
| NM_018874                 | Pancreatic lipase-related protein 1                                | 3757.0 | 0.0001 | Lipid metabolic process                          |
| NM_011857                 | ODZ3   | 29.1   | 0.0003 | Signal transduction                              |
| NM_019752                 | HtrA serine peptidase 2  | 25.4   | 0.0009 | Proteolysis                                      |
| NM_145561                 | Transmembrane protease, serine 11d                                 | 22.4   | 0.0101 | Proteolysis                                      |
| AK002477                  | Plasma membrane proteolipid  | 20.9   | 0.0005 | Transport  |
| NM_009714                 | Asialoglycoprotein receptor 1                                      | 19.2   | 0.0003 | Endocytosis                                      |
| <b>F&gt;M, Affymetrix</b> |  |        |        |  |
| NM_018874                 | Pancreatic lipase-related protein 1                                | 3679.0 | 0.0001 | Lipid metabolic process                          |
| M16360                    | Major urinary protein V  | 30.6   | 0.0327 | Pheromone binding                                |
| NM_007814                 | Cytochrome P450, family 2, subfamily b, polypeptide 19             | 29.6   | 0.0002 | Epoxygenase P450 pathway                         |
| AY061807                  | Calmodulin-like 4, transcript variant 1                            | 22.1   | 0.0015 | Calcium ion binding                              |
| NM_009349                 | Indolethylamine N-methyltransferase                                | 21.2   | 0.0009 | Metabolic process                                |
| U09362                    | Asialoglycoprotein receptor 1                                      | 15.3   | 0.0005 | Endocytosis                                      |
| <b>M&gt;F, CodeLink</b>   |  |        |        |  |
| NM_145548                 | Cytochrome P450, family 2, subfamily j, polypeptide 13             | 533.7  | 0.0002 | Oxidation-reduction process                      |
| NM_019515                 | Neuromedin U   | 206.7  | 0.0005 | Neuropeptide signaling pathway                   |
| NM_008957                 | Patched homolog 1  | 53.9   | 0.0002 | Branching involved in ureteric bud morphogenesis |
| BC012259                  | Major urinary protein 2  | 46.4   | 0.0002 | Pheromone binding                                |
| NM_145967                 | V-set and transmembrane domain containing 2A                       | 40.3   | 0.0001 | Cell differentiation                             |
| NM_020277                 | Transient receptor potential cation channel, subfamily M, member 5 | 39.1   | 0.0002 | Signal transduction                              |
| <b>M&gt;F, Affymetrix</b> |  |        |        |  |
| BC016446                  | Cytochrome P450, family 2, subfamily j, polypeptide 13             | 341.4  | 0.0008 | Oxidation-reduction process                      |
| NM_133997                 | Apolipoprotein F   | 105.4  | 0.0010 | Lipid metabolic process                          |
| NM_008599                 | Chemokine (C-X-C motif) ligand 9                                   | 81.0   | 0.0001 | Inflammatory response                            |
| NM_019515                 | Neuromedin U   | 77.7   | 0.0000 | Neuropeptide signaling pathway Process:          |
| BC025936                  | Cytochrome P450, family 4, subfamily a, polypeptide 12a            | 45.6   | 0.0001 | Alkane 1-monooxygenase activity                  |
| NM_010232                 | Flavin-containing monooxygenase 5                                  | 45.3   | 0.0000 | Metabolic process                                |

Genes with many of the highest expression differences in terms of ratios in NOD mice are shown. Relative ratios were determined from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from female and male NOD mice.

### Impact of Autoimmune Disease on Immune-related Biological Process, Molecular Function, and Cellular Component Ontologies in Lacrimal Glands of MRL/lpr Female and NOD Male Mice

Autoimmune disease had a dramatic impact on the expression of numerous immune-related gene ontologies in the lacrimal

glands of female MRL/lpr and male NOD mice. Many of these ontologies were identified by both CodeLink and Affymetrix platforms.

As shown in Tables 5 and 6, the expression of immune-related ontologies in lacrimal tissues of female MRL/lpr and male NOD mice was significantly increased in all three major gene function areas, including biological processes (e.g.,

**TABLE 4.** Comparison of Gene Expression Data Between CodeLink and Affymetrix Microarrays

|  | Genes M>F | Genes F>M | Total Genes |
|--|-----------|-----------|-------------|
| <b>MRL/lpr</b>                                     |           |           |             |
| CodeLink   |           |           |             |
| Unique CodeLink genes, not expressed by Affymetrix | 1683      | 2364      | 4047        |
| Affymetrix   |           |           |             |
| Unique Affymetrix genes, not expressed by CodeLink | 1025      | 979       | 2004        |
| CodeLink versus Affymetrix                         |           |           |             |
| Genes changed in same direction                    | 181       | 307       |             |
| Genes changed in opposite direction                | 8         |           |             |
| <b>NOD</b>   |           |           |             |
| CodeLink   |           |           |             |
| Unique CodeLink genes, not expressed by Affymetrix | 1454      | 2923      | 4377        |
| Affymetrix   |           |           |             |
| Unique Affymetrix genes, not expressed by CodeLink | 1256      | 1161      | 2417        |
| CodeLink versus Affymetrix                         |           |           |             |
| Genes changed in same direction                    | 265       | 318       |             |
| Genes changed in opposite direction                | 4         |           |             |

Data were analyzed without log transformation. Genes labeled as “unique” were significantly ( $P < 0.05$ ) increased on one, but not the other, microarray. The phrase “Genes changed in the same (or opposite) direction” means that the findings were significant ( $P < 0.05$ ) on both platforms.

TABLE 5. Immune Gene Ontologies Upregulated in Lacrimal Glands of Female MRL/lpr Mice

| Ontology                                     | CodeLink Genes ↑ | Affymetrix Genes ↑ | CodeLink z-Score | Affymetrix z-Score |
|--|------------------|--------------------|------------------|--------------------|
| <b>Biological process</b>                    |                  |                    |                  |                    |
| Immune system process                        | 228              | 147                | 6.26             | 8.24               |
| Defense response                             | 141              | 85                 | 6.12             | 6.37               |
| Immune response                              | 133              | 84                 | 5.89             | 7                  |
| Leukocyte activation                         | 100              | 67                 | 5.51             | 6.93               |
| Immune effector process                      | 74               | 46                 | 5.38             | 5.87               |
| Cytokine production                          | 72               | 33                 | 5.12             | 2.9                |
| Leukocyte proliferation                      | 47               |                    | 4.96             |                    |
| Lymphocyte proliferation                     | 46               |                    | 4.95             |                    |
| Lymphocyte activation                        | 84               | 57                 | 4.84             | 6.32               |
| Inflammatory response                        | 71               | 44                 | 4.8              | 5.12               |
| Regulation of response to stress             | 98               | 53                 | 4.73             | 3.84               |
| Regulation of cytokine production            | 62               |                    | 4.65             |                    |
| Response to stress                           |                  | 166                |                  | 4.6                |
| Regulation of immune response                | 77               | 44                 | 4.38             | 4.33               |
| Cellular response to stress                  | 142              | 65                 | 4.35             | 2.04               |
| Regulation of immune system process          | 119              | 76                 | 4.29             | 5.59               |
| Regulation of lymphocyte activation          | 51               |                    | 4.12             |                    |
| Regulation of leukocyte activation           | 56               |                    | 4.11             |                    |
| Regulation of immune effector process        | 42               |                    | 4                |                    |
| Positive regulation of immune system process | 85               | 57                 | 3.99             | 5.36               |
| Regulation of defense response               | 60               | 33                 | 3.79             | 3.29               |
| Leukocyte mediated immunity                  | 43               |                    | 3.78             |                    |
| Positive regulation of immune response       | 59               | 39                 | 3.72             | 4.94               |
| T-cell activation                            | 55               | 42                 | 3.53             | 6.03               |
| Response to cytokine stimulus                | 49               |                    | 3.34             |                    |
| Innate immune response                       | 56               | 40                 | 3.15             | 5.15               |
| Positive regulation of defense response      | 39               |                    | 3.14             |                    |
| Hemopoietic or lymphoid organ development    |                  | 47                 |                  | 2.82               |
| Immune system development                    |                  | 48                 |                  | 2.56               |
| Activation of immune response                | 41               |                    | 2.52             |                    |
| Leukocyte differentiation                    | 50               |                    | 2.22             |                    |
| <b>Molecular function</b>                    |                  |                    |                  |                    |
| Immunoglobulin G binding                     | 5                |                    | 5.04             |                    |
| Chemokine activity                           | 14               | 9                  | 4.18             | 4.19               |
| Chemokine receptor binding                   | 16               | 11                 | 4.15             | 4.54               |
| Immunoglobulin binding                       | 5                |                    | 3.17             |                    |
| Antigen binding                              | 7                | 7                  | 2.95             | 5.03               |
| Cytokine activity                            | 34               |                    | 2.12             |                    |
| <b>Cellular components</b>                   |                  |                    |                  |                    |
| MHC class II protein complex                 | 6                | 4                  | 4.04             | 3.95               |
| MHC protein complex                          | 9                | 7                  | 3.81             | 4.74               |
| MHC class I protein complex                  |                  | 3                  |                  | 3.01               |
| Immunological synapse                        |                  | 4                  |                  | 2.77               |
| B-cell receptor complex                      | 3                |                    | 2.61             |                    |
| T-cell receptor complex                      | 4                |                    | 2.54             |                    |

Biological process ( $\geq 50$  genes/ontology), molecular function ( $\geq 5$  genes/ontology) and cellular component ( $\geq 4$  genes/ontology) immune ontologies were identified following the analysis of nontransformed CodeLink and Affymetrix data. A z-score is a statistical rating of the relative expression of genes, and demonstrates how greatly they are over- or underrepresented in a given gene list.<sup>22</sup> Positive z-scores reflect a higher number of genes meeting the criterion than is expected by chance, and values  $>2.0$  are significant. These immune ontologies were not upregulated in lacrimal gland samples from male MRL/lpr mice. Terms: CodeLink Genes ↑ - number of genes upregulated in female lacrimal tissues, as determined with a CodeLink Bioarray; Affymetrix Genes ↑ - number of genes upregulated in female lacrimal glands, as calculated with Affymetrix GeneChips; z-score: specific score for the upregulated genes in the CodeLink and Affymetrix tissues.

inflammatory response), molecular functions (e.g., chemokine activity), and cellular components (e.g., major histocompatibility complex [MHC] protein complex). These aspects, as defined by the Gene Ontology Consortium (<http://www.geneontology.org/page/ontology-documentation>), address the biological programs accomplished by multiple molecular activities (i.e., biological processes), the molecular-level activities performed by gene products (i.e., molecular functions), and the locations relative to cellular structures in which a gene product performs a function (i.e., cellular components).

An example of the degree of autoimmune influence was demonstrated by analysis of biological process ontologies in male NOD lacrimal glands, which showed that 41 of the 53 most highly significant ontologies ( $\geq 50$  genes/ontology; z-score  $\geq 6.0$ ) were all immune-related. One such ontology, inflammatory response, displayed a significant increase in multiple inflammatory genes by both CodeLink and Affymetrix microarrays in female MRL/lpr (Table 7) and male NOD (Table 8) mouse lacrimal tissues. Twenty-six of these inflammatory

TABLE 6. Immune Gene Ontologies Significantly Increased in Lacrimal Glands of Male NOD Mice

| Ontology  | CodeLink<br>Genes ↑ | Affymetrix<br>Genes ↑ | CodeLink<br>z-score | Affymetrix<br>z-score |
|---|---------------------|-----------------------|---------------------|-----------------------|
| Biological process  |                     |                       |                     |                       |
| Immune response   | 152                 | 137                   | 15.16               | 13.98                 |
| Immune system process   | 227                 | 215                   | 14.62               | 14.34                 |
| Positive regulation of immune system process  | 102                 | 93                    | 12.13               | 10.93                 |
| Regulation of immune response   | 90                  | 75                    | 11.75               | 9.72                  |
| Defense response  | 134                 | 112                   | 11.7                | 9.14                  |
| Regulation of immune system process   | 128                 | 114                   | 11.66               | 10.27                 |
| Immune effector process   | 81                  | 73                    | 11.65               | 10.82                 |
| Positive regulation of immune response  | 75                  | 64                    | 11.48               | 9.81                  |
| Leukocyte activation  | 99                  | 97                    | 10.89               | 11.19                 |
| Innate immune response  | 69                  | 61                    | 10.11               | 9.08                  |
| Leukocyte mediated immunity   | 53                  | 50                    | 10.1                | 10.02                 |
| Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin | 48                  | 51                    | 10.08               | 11.67                 |
| Adaptive immune response  | 48                  | 51                    | 9.88                | 11.32                 |
| Lymphocyte activation   | 83                  | 85                    | 9.72                | 10.71                 |
| Lymphocyte-mediated immunity  | 45                  |                       | 9.6                 |                       |
| Activation of immune response   | 55                  | 47                    | 9.56                | 8.44                  |
| Regulation of immune effector process   | 47                  |                       | 8.99                |                       |
| Regulation of lymphocyte activation   | 54                  | 49                    | 8.81                | 8.02                  |
| T-cell activation   | 60                  | 60                    | 8.74                | 9.35                  |
| Immune response-regulating signaling pathway  | 46                  |                       | 8.71                |                       |
| Regulation of leukocyte activation  | 58                  | 50                    | 8.71                | 7.34                  |
| Immune response-activating signal transduction  | 45                  |                       | 8.6                 |                       |
| Regulation of lymphocyte proliferation  | 37                  |                       | 8.23                |                       |
| Positive regulation of lymphocyte activation  | 39                  |                       | 8.19                |                       |
| Regulation of mononuclear cell proliferation  | 37                  |                       | 8.16                |                       |
| Positive regulation of leukocyte activation   | 41                  |                       | 8.11                |                       |
| Regulation of leukocyte proliferation   | 37                  |                       | 8.03                |                       |
| Cytokine production   | 63                  | 53                    | 7.94                | 6.51                  |
| Lymphocyte proliferation  | 43                  | 42                    | 7.93                | 7.99                  |
| Mononuclear cell proliferation  | 43                  |                       | 7.88                |                       |
| Leukocyte proliferation   | 43                  | 42                    | 7.72                | 7.78                  |
| Positive regulation of response to stimulus   | 125                 |                       | 7.53                |                       |
| Regulation of T-cell activation   | 39                  |                       | 7.47                |                       |
| B-cell activation   | 38                  |                       | 7.34                |                       |
| Regulation of cytokine production   | 54                  | 46                    | 7.21                | 6.02                  |
| Regulation of defense response  | 55                  | 43                    | 6.95                | 4.77                  |
| Inflammatory response   | 58                  | 45                    | 6.75                | 4.41                  |
| Response to stress  | 218                 | 217                   | 6.64                | 7.63                  |
| Response to cytokine stimulus   | 45                  | 45                    | 6.21                | 6.52                  |
| T-cell differentiation  | 32                  |                       | 6.06                |                       |
| Positive regulation of defense response   | 37                  |                       | 6.02                |                       |
| Molecular function  |                     |                       |                     |                       |
| Cytokine binding  | 30                  | 22                    | 7.37                | 4.64                  |
| Antigen binding   | 9                   | 10                    | 6.2                 | 7.06                  |
| Peptide antigen binding   | 6                   | 6                     | 6.15                | 5.95                  |
| Chemokine activity  | 13                  | 12                    | 5.81                | 5.53                  |
| Chemokine receptor binding  | 14                  | 14                    | 5.43                | 5.63                  |
| Chemokine binding   | 9                   | 7                     | 5.05                | 3.51                  |
| Cytokine activity   | 33                  |                       | 5.01                |                       |
| Chemokine receptor activity   | 8                   | 7                     | 4.69                | 3.82                  |
| G-protein chemoattractant receptor activity   | 8                   |                       | 4.69                |                       |
| Immunoglobulin binding  | 5                   | 7                     | 4.58                | 5.87                  |
| C-C chemokine receptor activity   | 6                   |                       | 4.12                |                       |
| MHC protein binding   | 5                   |                       | 3.94                |                       |
| C-C chemokine binding   | 6                   |                       | 3.89                |                       |
| Cytokine receptor binding   | 29                  | 27                    | 3.87                | 3.46                  |
| MHC class I protein binding   | 4                   |                       | 3.79                |                       |
| Cytokine receptor activity  | 12                  | 9                     | 3.5                 | 2.35                  |
| CCR chemokine receptor binding  |                     | 4                     |                     | 3.11                  |

TABLE 6. Continued

| Ontology                           | CodeLink<br>Genes ↑ | Affymetrix<br>Genes ↑ | CodeLink<br>z-score | Affymetrix<br>z-score |
|------------------------------------|---------------------|-----------------------|---------------------|-----------------------|
| Cellular components                |                     |                       |                     |                       |
| MHC protein complex                | 12                  | 13                    | 8                   | 9.15                  |
| MHC class II protein complex       | 8                   | 8                     | 7.87                | 8.18                  |
| T-cell receptor complex            | 7                   | 4                     | 7.29                | 3.94                  |
| Alpha-beta T-cell receptor complex | 4                   |                       | 5.2                 |                       |
| B-cell receptor complex            | 4                   | 5                     | 5.2                 | 6.95                  |
| Immunoglobulin complex             | 4                   | 5                     | 5.2                 | 6.95                  |
| MHC class I protein complex        |                     | 4                     |                     | 3.94                  |
| Immunological synapse              | 5                   | 6                     | 3.64                | 4.3                   |
| CD40 receptor complex              |                     | 4                     |                     | 3.32                  |

Immune ontologies related to biological processes ( $\geq 50$  genes/ontology,  $\geq 6.0$  CodeLink z-score), molecular functions ( $\geq 5$  genes/ontology,  $\geq 2.0$  z-score) and cellular component ( $\geq 4$  genes/ontology,  $\geq 2.0$  z-score) were identified after the evaluation of nontransformed CodeLink and Affymetrix data. These immune ontologies were not significantly increased in lacrimal tissue samples from female NOD mice. Terms are described in the Table 5 legend.

genes were the same in both female MRL/lpr and male NOD mice.

### Effects of Autoimmune Disease on Immune-related KEGG Pathways in Lacrimal Glands of MRL/lpr Female and NOD Male Mice

Lacrimal gland samples from female MRL/lpr and male NOD mice also showed a significant increase in the expression of immune-related KEGG pathways (Tables 9 and 10). These included pathways related to antigen processing (Tables 11 and 12), chemokines (Tables 13 and 14), and Fc $\gamma$  R-mediated phagocytosis (Table 10), as well as those linked to type 1 diabetes mellitus and systemic lupus erythematosus (SLE) (Tables 9 and 10). Inflammation in these tissues also significantly enhanced the expression of lysosome pathways (Affymetrix; MRL/lpr female, 19 genes upregulated  $\uparrow$ , z-score = 2.43; NOD male, 25 genes  $\uparrow$ , z-score = 3.68).

Of interest, an average of more 95% of the ribosome KEGG pathways were significantly increased in lacrimal glands of female MRL/lpr (CodeLink, 47 genes  $\uparrow$ , z-score = 9.64; Affymetrix, 17 genes  $\uparrow$ , z-score = 3.03) and NOD (CodeLink, 53 genes  $\uparrow$ , z-score = 10.78; Affymetrix, 59 genes  $\uparrow$ , z-score = 17.5) mice. Similarly, more than 81% of the proteasome KEGG pathways were significantly higher in lacrimal tissues of female MRL/lpr (CodeLink, 22 genes  $\uparrow$ , z-score = 5.87; Affymetrix, 10 genes  $\uparrow$ , z-score = 2.77) and NOD (CodeLink, 21 genes  $\uparrow$ , z-score = 5.09; Affymetrix, 20 genes  $\uparrow$ , z-score = 7.4) mice.

## DISCUSSION

Our results demonstrate that sex significantly influences the expression of thousands of genes in lacrimal glands of MRL/lpr and NOD mice. The nature of this sex-related expression, especially with regard to immune-associated genes, is very dependent on the specific mouse model of Sjögren syndrome. Lacrimal glands of female, as compared with those of male, MRL/lpr mice contain a significant increase in the expression of genes related to inflammatory responses, antigen processing, and chemokine pathways. In contrast, it is the lacrimal tissue of NOD males, and not NOD females, that presents with a significantly greater expression of immune-related genes. These findings support our hypothesis that sex-related differences in gene expression contribute to the onset, progression, and/or severity of the lacrimal gland inflammatory disease process. Our results also suggest that factors in the

lacrimal gland microenvironment are critically important in mediating these sex-associated immune effects.

Our finding that significant sex-related differences exist in lacrimal gland gene expression in MRL/lpr and NOD mice was not unexpected. Significant, sex-associated differences are known to be present in the anatomy, physiology, and pathophysiology of the lacrimal gland. These differences are found in multiple species and include variations between males and females in the mean area and density of acinar complexes; the quantity of intercalated, intralobular, and interlobular ducts; the membrane contours, cytoplasmic appearance, vesicular content, and turnover of acinar epithelial cells; the position, size, and shape of acinar epithelial cell nuclei; the number of intranuclear inclusions; the prominence of nucleoli; the frequency of intercellular channels; the quantity of capillary endothelial pores; the expression of numerous genes; the synthesis, activity, phosphorylation, and affinity of many proteins, enzymes, and receptors; the population of lymphocytes; the expression of secretory immunity; the response to neural stimulation and drugs; the secretion of specific proteins; and the susceptibility to focal adenitis, fibrosis, atrophy, viral replication, and autoimmune disease.<sup>5</sup>

Three genes of particular interest that showed sexual dimorphism are those encoding ASGPR1, tripartite motif-containing 21 (TRIM21), and major urinary protein V (MUPV). First, expression of the ASGPR1 gene was many-fold greater in lacrimal glands of female, as compared with male, MRL/lpr mice. This receptor mediates the intracellular uptake of hepatitis C virus (HCV),<sup>23</sup> thereby facilitating viral infection and increasing glandular inflammation.<sup>23-25</sup> Chronic HCV infection, in turn, is linked to an enhanced prevalence of keratoconjunctivitis sicca<sup>26</sup> and mimics the clinical manifestations of Sjögren syndrome.<sup>24,25,27,28</sup> In addition, ASGPR is an autoantigenic target of both T and B cells.<sup>29</sup> However, the ASGPR1 gene was also upregulated in lacrimal tissues of female NOD mice, which indicates that it is not a strain-independent inducer of inflammation.

Second, TRIM21, also known as Ro52/SSA, is a prominent antigen in Sjögren syndrome. Expression of TRIM21 was higher in lacrimal glands of female MRL/lpr mice (Affymetrix = 2.12-fold; CodeLink = 1.72-fold) and male NOD mice (Affymetrix = 2.71-fold; CodeLink = 1.45-fold). Antibodies targeting TRIM21/Ro52 are common in Sjögren syndrome patients and may be present years before diagnosis.<sup>30</sup> Anti-TRIM21/Ro52 autoantibodies have also been detected in MRL/lpr and NOD mice.<sup>31,32</sup> TRIM21/Ro52 is a ubiquitin E3 ligase that may be induced by interferons (type I or type II) and has immunomodulatory

TABLE 7. Increased Expression of Genes in Inflammatory Response Ontology in Lacrimal Glands From Female MRL/lpr Mice

| Gene  | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|---|----------------|------------------|------------------|--------------------|
| Indoleamine 2,3-dioxygenase 1   | 9.82           |                  | 0.0014           |                    |
| <i>Chemokine (C-X-C motif) ligand 13</i>                                  | 7.29           | 5.5              | 0.0021           | 0.0060             |
| Chitinase 3-like 3  | 6.43           |                  | 0.0015           |                    |
| <i>Serine (or cysteine) peptidase inhibitor, clade A, member 1B</i>       | 5.59           | 8.53             | 0.0002           | 0.0267             |
| Strain SJL/J small inducible cytokine A4                                  | 5.47           |                  | 0.0028           |                    |
| Tachykinin 1  | 4.88           | 3.88             | 0.0146           | 0.0142             |
| Calcitonin receptor-like  |                | 4.48             |                  | 0.0049             |
| <i>Complement component 3</i>   | 4.38           | 3                | 0.0050           | 0.0255             |
| <i>Interleukin 4 receptor, <math>\alpha</math></i>                        | 4.35           |                  | 0.0039           |                    |
| Amine oxidase, copper containing 3  |                | 4.26             |                  | 0.0377             |
| <i>Chemokine (C-X-C motif) ligand 9</i>                                   | 4.21           | 3.45             | 0.0053           | 0.0243             |
| Elastase 2, neutrophil  | 3.96           |                  | 0.0088           |                    |
| Tryptase $\beta$ 2  | 3.9            |                  | 0.0067           |                    |
| Serum amyloid A 3   | 3.66           |                  | 0.0174           |                    |
| CD14 antigen  | 3.51           | 3.44             | 0.0025           | 0.0195             |
| Adiponectin, C1Q and collagen domain containing                           | 3.43           | 2.32             | 0.0022           | 0.0166             |
| <i>Chemokine (C-C motif) receptor 1</i>                                   | 3.4            |                  | 0.0025           |                    |
| <i>Toll-like receptor 2</i>   | 3.31           | 2.54             | 0.0033           | 0.0044             |
| Integrin $\beta$ 2  | 3.24           | 2.2              | 0.0334           | 0.0167             |
| <i>Acid phosphatase 5</i>   | 3.14           |                  | 0.0004           |                    |
| <i>C-type lectin domain family 7, member a</i>                            | 3.11           | 3.63             | 0.0025           | 0.0092             |
| <i>Mediterranean fever</i>  | 3.05           |                  | 0.0483           |                    |
| <i>Phospholipase A2, group VII</i>  | 3.02           |                  | 0.0120           |                    |
| Interleukin 23 receptor   | 3              |                  | 0.0001           |                    |
| <i>Lymphocyte antigen 86</i>  | 2.99           | 2.65             | 0.0081           | 0.0047             |
| Lipopolysaccharide binding protein  |                | 2.93             |                  | 0.0104             |
| Transglutaminase 2, C polypeptide   |                | 2.91             |                  | 0.0014             |
| Phospholipase A2, group IVA   | 2.9            | 2.05             | 0.0040           | 0.0472             |
| Peroxisome proliferator activated receptor $\gamma$                       | 2.87           |                  | 0.0080           |                    |
| <i>Fc receptor, IgG, high affinity I</i>                                  | 2.85           |                  | 0.0299           |                    |
| Yamaguchi sarcoma viral oncogene homolog                                  |                | 2.83             |                  | 0.0082             |
| <i>Fc receptor, IgG, low affinity IIb</i>                                 | 2.79           | 2.63             | 0.0101           | 0.0207             |
| Fatty acid binding protein 4, adipocyte                                   | 2.73           | 3.16             | 0.0055           | 0.0182             |
| <i>Fc<math>\gamma</math> receptor III</i>                                 | 2.73           | 3.03             | 0.0174           | 0.0080             |
| Complement component factor h   |                | 2.6              |                  | 0.0008             |
| Orosomucoid 1   | 2.56           |                  | 0.0237           |                    |
| <i>Chemokine (C-C motif) ligand 8</i>                                     | 2.44           | 2.03             | 0.0071           | 0.0285             |
| AXL receptor tyrosine kinase  | 2.39           |                  | 0.0107           |                    |
| Lysosomal acid lipase A, transcript variant 1                             |                | 2.38             |                  | 0.0131             |
| <i>CD47 antigen</i>   | 2.35           |                  | 0.0432           |                    |
| <i>Complement component 4B</i>  |                | 2.35             |                  | 0.0021             |
| <i>Toll-like receptor 1</i>   | 2.34           |                  | 0.0083           |                    |
| <i>Chemokine (C-C motif) receptor 2</i>                                   | 2.31           | 2.02             | 0.0063           | 0.0419             |
| Arachidonate 5-lipoxygenase activating protein                            | 2.3            |                  | 0.0019           |                    |
| <i>CD55 antigen</i>   | 2.29           |                  | 0.0035           |                    |
| Interleukin 33  | 2.29           | 6.12             | 0.0253           | 0.0468             |
| E74-like factor 3   |                | 2.25             |                  | 0.0003             |
| IkappaBNS   | 2.22           |                  | 0.0023           |                    |
| Purinergic receptor P2X, ligand-gated ion channel, 1                      | 2.22           |                  | 0.0027           |                    |
| <i>Cytochrome b-245, polypeptide</i>                                      | 2.21           |                  | 0.0057           |                    |
| <i>Annexin A1</i>   | 2.18           |                  | 0.0209           |                    |
| <i>Janus kinase 2</i>   | 2.13           |                  | 0.0062           |                    |
| CD44 antigen  |                | 2.12             |                  | 0.0196             |
| INAP for IL-1 inducible nuclear ankyrin-repeat protein                    |                | 2.11             |                  | 0.0034             |
| <i>Heme oxygenase 1</i>   | 2.09           |                  | 0.0027           |                    |
| Chemokine (C-X-C motif) ligand 11   | 2.07           |                  | 0.0112           |                    |
| <i>Chemokine (C-X-C motif) ligand 10</i>                                  | 2.04           |                  | 0.0324           |                    |
| Chemokine (C-C motif) ligand 5  |                | 2.05             |                  | 0.0286             |
| UDP-Gal: $\beta$ -GlcNAc $\beta$ 1,4-galactosyltransferase, polypeptide 1 | 2.03           | 2.28             | 0.0459           | 0.0185             |

Relative ratios were calculated from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from female and male MRL/lpr mice. Listed genes were increased  $\geq 2$ -fold. Italicized genes were also found to be upregulated in lacrimal glands of male NOD mice (Table 8).



TABLE 8. Increased Expression of Genes in Inflammatory Response Ontology in Lacrimal Glands From Male NOD Mice

| Gene  | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|---|----------------|------------------|------------------|--------------------|
| Regenerating islet-derived 3 $\gamma$                               | 24.92          |                  | 0.0008           |                    |
| <i>Chemokine (C-X-C motif) ligand 9</i>                             | 15.74          | 81.01            | 0.0000           | 0.0001             |
| Chemokine (C-C motif) ligand 20                                     | 13.04          | 44.52            | 0.0001           | 0.0001             |
| <i>Chemokine (C-X-C motif) ligand 10</i>                            | 10.13          | 6.79             | 0.0001           | 0.0055             |
| CD28 antigen  |                | 8.44             |                  | 0.0009             |
| <i>Serine (or cysteine) peptidase inhibitor, clade A, member 1B</i> | 6.9            | 17.57            | 0.0004           | 0.0013             |
| <i>Chemokine (C-C motif) receptor 1</i>                             | 6.88           |                  | 0.0013           |                    |
| <i>Chemokine (C-X-C motif) ligand 13</i>                            | 6.69           | 8.03             | 0.0007           | 0.0028             |
| Forkhead box P3   | 6.56           |                  | 0.0007           |                    |
| <i>Lymphocyte antigen 86</i>  | 6.45           | 9.55             | 0.0001           | 0.0003             |
| <i>Chemokine (C-C motif) ligand 8</i>                               | 5.52           | 7.81             | 0.0001           | 0.0000             |
| <i>C-type lectin domain family 7, member a</i>                      | 5.45           | 6.08             | 0.0024           | 0.0000             |
| <i>Complement component 4B</i>                                      | 5.38           | 6.94             | 0.0053           | 0.0002             |
| <i>Cytochrome b-245, <math>\alpha</math> polypeptide</i>            | 5.23           | 6.84             | 0.0007           | 0.0002             |
| Chemokine (C-C motif) ligand 5                                      | 5.03           | 9.05             | 0.0012           | 0.0103             |
| Sodium channel, voltage-gated, type IX, $\alpha$                    | 4.92           |                  | 0.0016           |                    |
| <i>Fc receptor, IgG, low affinity IIb</i>                           | 4.83           | 6.17             | 0.0005           | 0.0003             |
| Adenosine A2b receptor  | 4.73           |                  | 0.0023           |                    |
| <i>Toll-like receptor 1</i>   | 4.28           | 5.62             | 0.0094           | 0.0027             |
| Chemokine (C-C motif) ligand 1                                      | 4.27           | 4.04             | 0.0052           | 0.0009             |
| Tumor necrosis factor receptor superfamily, member 4                | 4.14           | 3.83             | 0.0029           | 0.0065             |
| Tumor necrosis factor receptor superfamily, member 4                | 4.14           | 3.83             | 0.0029           | 0.0065             |
| Integrin $\beta$ 2  | 4.07           | 7.53             | 0.0002           | 0.0011             |
| Transforming growth factor, $\beta$ 1                               | 3.94           |                  | 0.0008           |                    |
| <i>Interleukin 4 receptor, <math>\alpha</math></i>                  | 3.91           | 2.22             | 0.0025           | 0.0449             |
| V-rel reticuloendotheliosis viral oncogene homolog A                | 3.71           |                  | 0.0023           |                    |
| Fc receptor, IgE, high affinity I, $\gamma$ polypeptide             | 3.6            | 4.94             | 0.0038           | 0.0014             |
| Nucleotide-binding oligomerization domain containing 2              | 3.54           |                  | 0.0014           |                    |
| <i>Chemokine (C-C motif) receptor 2</i>                             | 3.38           |                  | 0.0028           |                    |
| <i>CD55 antigen</i>   | 3.36           | 2.96             | 0.0052           | 0.0264             |
| Tumor necrosis factor receptor superfamily, member 1b               |                | 3.31             |                  | 0.0246             |
| <i>Fc receptor, IgG, high affinity I</i>                            | 3.21           | 4.57             | 0.0006           | 0.0082             |
| <i>Phospholipase A2, group VII</i>                                  | 3.2            | 4.45             | 0.0044           | 0.0003             |
| <i>Complement component 3</i>                                       | 3.14           | 3.52             | 0.0050           | 0.0035             |
| <i>Toll-like receptor 2</i>   | 3.04           |                  | 0.0003           |                    |
| Toll-like receptor 7  | 3.03           |                  | 0.0052           |                    |
| Chemokine (C-X-C motif) ligand 1                                    | 3.01           | 4.4              | 0.0068           | 0.0002             |
| Coagulation factor XII  | 2.99           |                  | 0.0012           |                    |
| Chemokine (C-C motif) receptor 5                                    |                | 2.9              |                  | 0.0145             |
| Arachidonate 5-lipoxygenase activating protein                      | 2.83           | 2.77             | 0.0064           | 0.0101             |
| Interleukin 1 $\beta$   | 2.72           | 2.78             | 0.0001           | 0.0027             |
| Neutrophil cytosolic factor 1                                       | 2.71           |                  | 0.0468           |                    |
| <i>Acid phosphatase 5</i>   |                | 2.66             |                  | 0.0007             |
| <i>Serine (or cysteine) peptidase inhibitor, clade B, member 9</i>  |                | 2.53             |                  | 0.0054             |
| Mitogen-activated protein kinase 8                                  | 2.48           |                  | 0.0198           |                    |
| <i>Fc<math>\gamma</math> receptor III</i>                           | 2.46           | 2.48             | 0.0001           | 0.0060             |
| <i>Janus kinase 2</i>   |                | 2.4              |                  | 0.0003             |
| <i>Mediterranean fever</i>  | 2.39           |                  | 0.0103           |                    |
| Chemokine (C-C motif) ligand 7                                      | 2.32           |                  | 0.0092           |                    |
| Interleukin 10  | 2.32           |                  | 0.0443           |                    |
| Carbohydrate sulfotransferase 2                                     | 2.26           |                  | 0.0051           |                    |
| Toll-like receptor 6  | 2.26           |                  | 0.0155           |                    |
| <i>Heme oxygenase 1</i>   |                | 2.26             |                  | 0.0257             |
| <i>CD47 antigen</i>   |                | 2.24             |                  | 0.0015             |
| Unc-13 homolog D  | 2.17           |                  | 0.0033           |                    |
| Solute carrier family 11  |                | 2.12             |                  | 0.0160             |
| <i>Annexin A1</i>   |                | 2.11             |                  | 0.0003             |
| Phosphatidylinositol 3-kinase $\gamma$ isoform                      | 2.01           |                  | 0.0038           |                    |

Relative ratios were determined from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from male and female NOD mice. Listed genes were increased  $\geq 2$ -fold. Italicized genes were also found to be upregulated in lacrimal glands of female MRL/lpr mice (Table 7).

**TABLE 9.** Immune KEGG Pathways Upregulated in Lacrimal Glands of Female MRL/lpr Mice

| KEGG Pathway                              | CodeLink Genes ↑ | Affymetrix Genes ↑ | CodeLink z-Score | Affymetrix z-Score |
|---|------------------|--------------------|------------------|--------------------|
| Antigen processing and presentation       | 30               | 17                 | 6.38             | 4.34               |
| Systemic lupus erythematosus              | 27               | 18                 | 4.96             | 3.95               |
| Graft-versus-host disease                 | 16               |                    | 4.2              |                    |
| Phagosome                                 | 44               | 27                 | 4.17             | 3.41               |
| Natural killer cell-mediated cytotoxicity | 36               | 20                 | 3.94             | 2.53               |
| Allograft rejection                       | 15               |                    | 3.76             |                    |
| B-cell receptor signaling pathway         | 24               | 14                 | 3.47             | 2.83               |
| Primary immunodeficiency                  |                  | 9                  |                  | 3.19               |
| Type I diabetes mellitus                  | 15               |                    | 3.14             |                    |
| Chemokine signaling pathway               | 43               | 26                 | 2.98             | 2.43               |
| Autoimmune thyroid disease                | 14               |                    | 2.52             |                    |
| Cell adhesion molecules                   | 30               |                    | 2.23             |                    |

Immune-related KEGG pathways that were increased in female, as compared with male, MRL/lpr mice are listed.

functions including regulation of proliferation and cell death, regulation of inflammatory cytokine production, and modulation of antiviral responses.<sup>33-37</sup> Although these roles were largely described in immune cells, additional studies have detected an increase in TRIM21/Ro52 protein in salivary gland epithelial cell lines or salivary gland ductal epithelial cells from Sjögren syndrome patients.<sup>38,39</sup> Expression of TRIM21/Ro52 has not, to our knowledge, been reported in lacrimal gland epithelial cells. Our findings of increased expression of TRIM21/Ro52 in lacrimal glands of MRL/lpr and NOD mice in the context of inflammation suggests this may contribute to the role of TRIM21/Ro52 as an autoantigen in Sjögren syndrome.

The third gene of particular interest is MUPV. This gene is one of the most highly upregulated genes in lacrimal glands of male MRL/lpr (202-fold) and female NOD (31-fold) mice. Hence, MUPV expression is inversely correlated with inflammation, and may possibly serve a protective function. Major urinary proteins are pheromone-binding lipocalins<sup>40-43</sup> and implied effects include sexual attraction, aggression, hormone modulation, individual recognition, and spatial learning.<sup>41,44,45</sup>

Little is known about the relation of MUPV to sex and the immune system. However, considering that major urinary proteins function as pheromone-binding proteins, the pheromones themselves may play a role.

Such pheromones could be exocrine gland secreting peptides (ESPs), which are found in mice and exhibit sex-specific expression.<sup>43,46-48</sup> ESP1 is male-specific, and its expression increases in response to androgen administration.<sup>46</sup> In contrast, ESP36 is female-specific and is negatively regulated by androgen.<sup>46</sup> Further, it has been suggested that the reception of ESPs in the vomeronasal system differs according to sex.<sup>49</sup> The vomeronasal system is an accessory olfactory system, and pheromones also can be detected by the anatomically distinct main olfactory system.<sup>46</sup> Of note, our CodeLink results showed that olfactory receptor 1086 is significantly upregulated in male lacrimal glands in MRL/lpr mice. This supports the concept of pheromone perception as an important factor in sexually dimorphic responses.<sup>50</sup>

Research has also provided evidence that the olfactory system may be inextricably linked to immunological function.<sup>51</sup> For example, it has been shown that pheromone

**TABLE 10.** Immune KEGG Pathways Upregulated in Lacrimal Glands of Male NOD Mice

| KEGG Pathway                                 | CodeLink Genes ↑ | Affymetrix Genes ↑ | CodeLink z-Score | Affymetrix z-Score |
|--|------------------|--------------------|------------------|--------------------|
| Graft-versus-host disease                    | 23               | 17                 | 9.9              | 6.21               |
| Antigen processing and presentation          | 30               | 27                 | 9.21             | 7.73               |
| Natural killer cell-mediated cytotoxicity    | 43               | 28                 | 8.95             | 4.31               |
| Allograft rejection                          | 21               | 16                 | 8.85             | 5.84               |
| Autoimmune thyroid disease                   | 21               | 16                 | 7.69             | 4.57               |
| Type I diabetes mellitus                     | 20               | 16                 | 7.5              | 4.99               |
| Phagosome                                    | 43               | 44                 | 7.13             | 7.24               |
| Intestinal immune network for IgA production | 17               | 14                 | 6.45             | 4.37               |
| Cytokine-cytokine receptor interaction       | 54               | 45                 | 6.01             | 3.88               |
| Primary immunodeficiency                     | 15               | 15                 | 6                | 6.04               |
| Systemic lupus erythematosus                 | 22               | 24                 | 5.62             | 5.57               |
| Cell adhesion molecules                      | 32               | 32                 | 5.43             | 5.09               |
| Chemokine signaling pathway                  | 40               | 50                 | 5.38             | 7.75               |
| B-cell receptor signaling pathway            | 20               | 23                 | 4.37             | 5.79               |
| Jak-STAT signaling pathway                   | 30               | 24                 | 4.07             | 2.54               |
| Leukocyte transendothelial migration         |                  | 24                 |                  | 3.53               |
| NOD-like receptor signaling pathway          | 14               | 13                 | 3.39             | 2.91               |
| Toll-like receptor signaling pathway         | 21               | 19                 | 3.35             | 2.68               |
| Hematopoietic cell lineage                   | 19               |                    | 3.33             |                    |
| T-cell receptor signaling pathway            | 22               | 19                 | 3.23             | 2.43               |
| Complement and coagulation cascades          | 16               |                    | 3.01             |                    |
| Fc $\gamma$ R-mediated phagocytosis          | 16               | 25                 | 2.23             | 5.39               |

Immune-related KEGG pathways that were increased in male, as compared with female, NOD mice are listed.

**TABLE 11.** Upregulated Genes in the Antigen Processing KEGG Pathway in Lacrimal Glands From Female MRL/lpr Mice

| Ontology  | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|---|----------------|------------------|------------------|--------------------|
| Interferon- $\gamma$                                    | 3.46           |                  | 0.0071           |                    |
| Histocompatibility 2, O region $\beta$ locus            | 3.14           |                  | 0.0057           |                    |
| Cathepsin S   | 2.85           | 3.03             | 0.0166           | 0.0050             |
| Histocompatibility 2, M region locus 3                  | 2.75           |                  | 0.0134           |                    |
| Histocompatibility 2, class II antigen A, $\alpha$      | 2.64           | 2.87             | 0.0142           | 0.0096             |
| Killer cell lectin-like receptor, subfamily D, member 1 | 2.44           | 2.15             | 0.0012           | 0.0151             |
| Histocompatibility 2, class II, locus DMA               | 2.36           |                  | 0.0215           |                    |
| Similar to histocompatibility 2, D region locus 1       |                | 2.35             |                  | 0.0117             |
| Histocompatibility 2, class II antigen A, $\beta$ 1     |                | 2.33             |                  | 0.0209             |
| Bactrianus MHC class II antigen H-2E $\alpha$ precursor | 2.32           |                  | 0.0382           |                    |
| Histocompatibility 2, class II antigen E $\beta$        | 2.30           | 1.98             | 0.0429           | 0.0195             |
| CD8 antigen, $\alpha$ chain 1                           | 2.27           |                  | 0.0087           |                    |
| Cathepsin B   | 2.23           | 3.00             | 0.0164           | 0.0012             |
| Histocompatibility 2, T region locus 10                 | 2.20           |                  | 0.0261           |                    |
| Histocompatibility 2, Q region locus 8                  | 2.13           |                  | 0.0000           |                    |
| Calnexin  | 2.11           |                  | 0.0086           |                    |
| Heat shock protein 90, $\alpha$ , class A member 1      | 2.10           |                  | 0.0008           |                    |
| Preprolegumain  | 2.00           | 2.25             | 0.0209           | 0.0144             |
| Interferon $\gamma$ inducible protein 30                | 1.87           | 2.25             | 0.0161           | 0.0062             |
| Heat shock protein                                      | 1.78           | 2.21             | 0.0145           | 0.0088             |

Relative ratios were determined from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from female MRL/lpr to those of male MRL/lpr mice. Listed genes were increased  $\geq 2.0$ -fold in either the CodeLink or Affymetrix platform.

**TABLE 12.** Upregulated Genes in the Antigen Processing KEGG Pathway in Lacrimal Glands From Male NOD Mice

| Ontology  | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|---|----------------|------------------|------------------|--------------------|
| Histocompatibility 2, class II, locus Mb2               | 12.17          | 8.13             | 0.0000           | 0.0021             |
| Histocompatibility 2, K1, K region                      |                | 11.16            |                  | 0.0001             |
| MHC I=H-2Kd homolog                                     |                | 10.48            |                  | 0.0000             |
| Histocompatibility 2, class II, locus Mb1               | 9.25           | 7.13             | 0.0000           | 0.0003             |
| Histocompatibility 2, Q region locus 7                  | 7.21           |                  | 0.0007           |                    |
| Similar to histocompatibility 2, D region locus 1       | 6.53           | 8.67             | 0.0027           | 0.0016             |
| Histocompatibility 2, blastocyst                        | 6.44           |                  | 0.0001           |                    |
| Histocompatibility 2, Q region $\alpha$ locus 8         | 6.20           | 6.52             | 0.0003           | 0.0004             |
| Histocompatibility 2, class II antigen A, $\beta$ 1     | 5.87           | 5.45             | 0.0002           | 0.0001             |
| Histocompatibility 2, O region $\beta$ locus            | 5.83           |                  | 0.0000           |                    |
| Histocompatibility 2, class II, locus DMA               |                | 5.42             |                  | 0.0002             |
| Histocompatibility 2, O region $\alpha$ locus           | 5.38           | 5.05             | 0.0019           | 0.0001             |
| CD74 antigen  | 5.19           | 6.44             | 0.0041           | 0.0002             |
| MHC class Ib antigen Qa-1                               | 5.00           | 3.52             | 0.0224           | 0.0025             |
| Histocompatibility 2, class II antigen E $\beta$        | 4.87           | 6.30             | 0.0001           | 0.0000             |
| Histocompatibility 2, M region locus 3                  | 4.79           | 6.80             | 0.0003           | 0.0002             |
| Natural killer cell protein group 2-A2                  | 4.76           |                  | 0.0037           |                    |
| $\beta$ 2 microglobulin, segment 1, clones PBRCB-(1-3)  | 4.53           | 5.43             | 0.0002           | 0.0004             |
| Histocompatibility 2, class II antigen A, $\alpha$      | 4.22           | 6.05             | 0.0017           | 0.0000             |
| MHC class II transactivator CIITA form IV               | 4.16           | 3.19             | 0.0026           | 0.0412             |
| Histocompatibility 2, T region locus 10                 | 4.13           | 4.53             | 0.0001           | 0.0004             |
| Cathepsin S   | 3.85           | 6.06             | 0.0002           | 0.0001             |
| Transporter 1   | 3.69           | 6.42             | 0.0007           | 0.0004             |
| Proteasome 28 subunit, $\beta$                          | 3.51           | 3.09             | 0.0148           | 0.0017             |
| CD8 antigen, $\beta$ chain 1                            | 3.33           |                  | 0.0069           |                    |
| Zinc finger and BTB domain containing 22                |                | 3.25             |                  | 0.0001             |
| Histocompatibility 2, Q region locus 1                  | 2.85           | 7.16             | 0.0056           | 0.0002             |
| CD8 antigen, $\alpha$ chain                             | 2.84           | 4.06             | 0.0012           | 0.0016             |
| Killer cell lectin-like receptor, subfamily D, member 1 | 2.81           | 8.71             | 0.0094           | 0.0110             |
| Natural killer cell protein group 2-C2                  | 2.39           |                  | 0.0208           |                    |
| Interferon $\gamma$ inducible protein 30                | 2.00           | 1.80             | 0.0035           | 0.0002             |
| Proteasome 28 subunit, $\alpha$                         | 1.93           | 2.60             | 0.0022           | 0.0001             |
| Preprolegumain  | 1.89           | 2.08             | 0.0041           | 0.0016             |
| Transporter 2, ATP-binding cassette, subfamily B        | 1.76           | 4.00             | 0.0234           | 0.0003             |

Relative ratios were determined from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from male NOD to those of female NOD mice. Listed genes were increased  $\geq 2.0$ -fold in either the CodeLink or Affymetrix microarray.

**TABLE 13.** Heightened Gene Expression in the Chemokine KEGG Pathway in Lacrimal Glands of Female MRL/lpr Mice

| Gene   | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|--|----------------|------------------|------------------|--------------------|
| Chemokine (C-X-C motif) ligand 13                                | 7.29           | 5.5              | 0.0021           | 0.0060             |
| Chemokine (C-X-C motif) ligand 16                                | 6.95           | 2.49             | 0.0116           | 0.0350             |
| Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 |                | 5.76             |                  | 0.0102             |
| Strain SJL/J small inducible cytokine A4                         | 5.47           |                  | 0.0028           |                    |
| Chemokine (C-C motif) ligand 19                                  | 5.29           | 3.04             | 0.0047           | 0.0409             |
| G protein-coupled receptor kinase 5                              | 4.57           |                  | 0.0037           |                    |
| Chemokine (C-X-C motif) ligand 9                                 | 4.21           | 3.45             | 0.0053           | 0.0243             |
| Chemokine (C-C motif) ligand 6                                   | 3.8            | 4.5              | 0.0005           | 0.0097             |
| Strain SJL/J small inducible cytokine A10                        | 3.67           | 4.27             | 0.0120           | 0.0254             |
| Chemokine subfamily B Cys-X-Cys                                  | 3.62           |                  | 0.0036           |                    |
| Chemokine (C-C motif) receptor 1                                 | 3.4            |                  | 0.0025           |                    |
| RAS-related C3 botulinum substrate 2                             | 3.09           | 2.41             | 0.0035           | 0.0044             |
| Chemokine (C motif) ligand 1                                     | 2.98           |                  | 0.0018           |                    |
| Yamaguchi sarcoma viral oncogene homolog                         |                | 2.83             |                  | 0.0082             |
| Inhibitor of kappaB kinase $\gamma$                              | 2.78           |                  | 0.0379           |                    |
| Vav 1 oncogene   | 2.78           |                  | 0.0008           |                    |
| Arrestin, $\beta$ 2  | 2.77           | 1.55             | 0.0025           | 0.0466             |
| Dedicator of cyto-kinesis 2                                      |                | 2.68             |                  | 0.0210             |
| RAS-related protein 1b   |                | 2.68             |                  | 0.0176             |
| Chemokine (C-C motif) ligand 8                                   | 2.44           | 2.03             | 0.0071           | 0.0285             |
| MIP2 $\gamma$  | 2.39           |                  | 0.0111           |                    |
| Chemokine (C-X3-C) receptor 1                                    | 2.34           |                  | 0.0002           |                    |
| Chemokine (C-X-C motif) receptor 6                               | 2.32           |                  | 0.0226           |                    |
| Chemokine (C-C motif) receptor 2                                 | 2.31           | 2.02             | 0.0063           | 0.0419             |
| Janus kinase 2, transcript variant 1                             | 2.13           |                  | 0.0062           |                    |
| Chemokine (C-X-C motif) ligand 11                                | 2.07           | 1.98             | 0.0112           | 0.0177             |
| Chemokine (C-C motif) ligand 5                                   |                | 2.05             |                  | 0.0286             |
| Chemokine (C-X-C motif) ligand 10                                | 2.04           |                  | 0.0324           |                    |
| Cell division cycle 42 homolog                                   | 2.01           |                  | 0.0073           |                    |
| Chemokine (C-C motif) ligand 11                                  | 1.88           | 2                | 0.0306           | 0.0278             |

Relative ratios were calculated from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from female MRL/lpr mice with those of male MRL/lpr mice. Listed genes were increased  $\geq 2.0$ -fold in either the CodeLink or Affymetrix platform.

treatment suppresses hepatic inflammation in mice.<sup>52</sup> Whether this effect has relevance to humans has not yet been determined, but it indicates that pheromone-sensing organs may have an underestimated value that warrants further investigation. Thus, it has been shown that patients with SLE have disturbances in olfactory function.<sup>50</sup> The possible link between smell and autoimmunity may be due to gene location, considering that olfactory receptor gene clusters are located in close proximity to key loci of susceptibility for autoimmune disease, such as the MHC.<sup>50</sup>

In our study, a number of immune-related genes were upregulated in the lacrimal glands of female MRL/lpr and/or male NOD mice that may be important in the pathogenesis of Sjögren syndrome. These include the following: many interleukins, interferons, and their related proteins; the damage-associated molecular pattern proteins S100A8 and S100A9, which are expressed by neutrophils, monocytes, dendritic and epithelial cells, act as Toll-like receptor (TLR) ligands, and stimulate the production of multiple proinflammatory cytokines; myeloid differentiation primary response 88, which is used by most TLRs to activate nuclear factor- $\kappa$ B; B-cell linker, which regulates B-cell receptor signaling and development; the chemokines CXCL12, CXCL13, and CCL19, which promote the formation and perpetuation ectopic lymphoid structures; and the enzymes indoleamine 2,3-dioxygenase and kynurenine 3-monooxygenase, which ultimately may lead to immune system activation, inflammation, and the accumulation of potentially neurotoxic compounds.<sup>53-57</sup>

Numerous ontologies and KEGG pathways that were significantly upregulated in lacrimal tissues of female MRL/lpr

and/or male NOD mice have also been linked to Sjögren syndrome. These ontologies encompass such immune system processes as antigen binding, T- and B-cell activation, signaling pathways, cytokine production, chemokine activity, and inflammatory responses, all of which appear to play a role in Sjögren syndrome pathogenesis.<sup>4,58,59</sup> The increased expression of KEGG pathways related to lysosomes and Fc $\gamma$  R-mediated phagocytosis was of particular interest, because they have been reported as the only pathways common to the development of the four autoimmune diseases type 1 diabetes mellitus, SLE, multiple sclerosis, and rheumatoid arthritis.<sup>60</sup>

A major question in our research is what triggers the sex-related inflammation in female MRL/lpr and male NOD lacrimal glands. There are a number of possibilities, some of which may be associated with sex chromosomes (i.e., X) and/or sex steroids (i.e., androgens).<sup>5,55</sup> Thus, several recent studies suggest that the female prevalence of Sjögren syndrome is due to an X-chromosome dose effect, and that individuals with X-chromosome abnormalities like triple X syndrome (47 XXX) and Klinefelter syndrome (47 XXY) have an increased risk for developing the disease.<sup>61-63</sup> In fact, attention has been drawn to X-chromosome vulnerability as a possible explanation for the high female prevalence of autoimmune diseases in general.<sup>64-67</sup> Therefore, the genes located on the X-chromosome are especially intriguing. One such gene is moesin, which is significantly upregulated in female MRL/lpr and in male NOD lacrimal tissues. Moesin is a membrane organizing protein that plays a role in immunologic synapse formation, lymphoid cell regulation, and T regulatory cell (Treg) differentiation.<sup>68,69</sup> In this last regard, there is evidence that a shift in the T helper cell

TABLE 14. Increased Gene Expression in the Chemokine KEGG Pathway in Lacrimal Glands of Male NOD Mice

| Ontology  | CodeLink Ratio | Affymetrix Ratio | CodeLink P Value | Affymetrix P Value |
|---|----------------|------------------|------------------|--------------------|
| Chemokine (C-C motif) receptor 7  |                | 15.88            |                  | 0.0011             |
| Chemokine (C-X-C motif) ligand 9  | 15.74          | 81.01            | 0.00003          | 0.0001             |
| Chemokine (C-C motif) ligand 20   | 13.04          | 44.52            | 0.0001           | 0.0001             |
| Chemokine (C-C motif) receptor 6  | 11             |                  | 0.0038           |                    |
| chemokine (C-X-C motif) receptor 4  |                | 10.24            |                  | 0.0043             |
| Chemokine (C-X-C motif) receptor 5  |                | 10.14            |                  | 0.0001             |
| Chemokine (C-X-C motif) ligand 10   | 10.13          | 6.79             | 0.0001           | 0.0055             |
| Gardner-Rasheed feline sarcoma viral oncogene homolog   | 9.32           |                  | 0.0015           |                    |
| Chemokine (C-X-C motif) receptor 3  |                | 8.85             |                  | 0.0055             |
| P21 (CDKN1A)-activated kinase 1   |                | 8.66             |                  | 0.0033             |
| RAS-related C3 botulinum substrate 2  | 7.52           | 6.19             | 0.0000           | 0.0005             |
| Chemokine (C-C motif) receptor 1  | 6.88           |                  | 0.0013           |                    |
| Chemokine (C-X-C motif) ligand 13   | 6.69           | 8.03             | 0.0007           | 0.0028             |
| Protein kinase C, $\beta$   | 6.28           | 10.96            | 0.0136           | 0.0007             |
| Chemokine (C motif) ligand 1  | 6.12           |                  | 0.0054           |                    |
| Wiskott-Aldrich syndrome protein  |                | 5.93             |                  | 0.0305             |
| Chemokine (C-C motif) ligand 19   | 5.58           | 9.83             | 0.0001           | 0.0020             |
| Chemokine (C-C motif) ligand 8  | 5.52           | 7.81             | 0.0001           | 0.0000             |
| Vav 1 oncogene  | 5.52           |                  | 0.0000           |                    |
| Protein kinase B $\gamma$   | 5.51           |                  | 0.0168           |                    |
| Signal transducer and activator of transcription 1  | 5.36           | 7.89             | 0.0042           | 0.0001             |
| Chemokine (C-C motif) ligand 5  | 5.03           | 9.05             | 0.0012           | 0.0103             |
| Hemopoietic cell kinase   | 4.74           | 4.87             | 0.0005           | 0.0055             |
| Chemokine (C-C motif) ligand 12   | 4.27           | 4.04             | 0.0052           | 0.0009             |
| V-rel reticuloendotheliosis viral oncogene homolog A  | 3.71           |                  | 0.0023           |                    |
| Dedicator of cyto-kinesis 2   | 3.5            | 3.92             | 0.0051           | 0.0170             |
| Chemokine (C-X-C motif) receptor 6  | 3.43           | 13.81            | 0.0056           | 0.0001             |
| Chemokine (C-C motif) receptor 2  | 3.38           |                  | 0.0028           |                    |
| Strain SJL/J small inducible cytokine A10   |                | 3.3              |                  | 0.0000             |
| Chemokine (C-C motif) receptor 6  |                | 3.17             |                  | 0.0137             |
| P21 (CDKN1A)-activated kinase 1   |                | 3.15             |                  | 0.0008             |
| Chemokine (C-X-C motif) ligand 1  | 3.01           | 4.4              | 0.0068           | 0.0002             |
| Adenylate cyclase 7, transcript variant 1   |                | 2.93             |                  | 0.0031             |
| Chemokine (C motif) receptor 1  | 2.81           |                  | 0.0009           |                    |
| Guanine nucleotide-binding protein, $\alpha$ inhibiting 2   | 2.81           | 4.57             | 0.0005           | 0.0004             |
| Neutrophil cytosolic factor 1   | 2.71           |                  | 0.0468           |                    |
| Signal transducer and activator of transcription 2  | 2.6            |                  | 0.0093           |                    |
| Guanine nucleotide-binding protein, $\gamma$ transducing activity polypeptide 2 (Gngt2), transcript variant 1 | 2.42           |                  | 0.0017           |                    |
| Janus kinase 2, transcript variant 1  |                | 2.4              |                  | 0.0003             |
| Chemokine (C-X3-C) receptor 1   | 2.38           |                  | 0.0244           |                    |
| Chemokine (C-C motif) ligand 28   |                | 2.37             |                  | 0.0053             |
| Chemokine (C-C motif) ligand 7  | 2.32           |                  | 0.0092           |                    |
| C-src tyrosine kinase   |                | 2.28             |                  | 0.0004             |
| Arrestin, $\beta$ 2   | 2.26           |                  | 0.0242           |                    |
| Signal transducer and activator of transcription 2  |                | 2.1              |                  | 0.0200             |
| G protein-coupled receptor kinase 6, transcript variant 2   | 2.08           | 2.43             | 0.0031           | 0.0018             |
| Phosphatidylinositol 3-kinase $\gamma$ isoform  | 2.01           |                  | 0.0038           |                    |
| Chemokine (C-C motif) receptor 5  | 1.77           | 2.9              | 0.0254           | 0.0145             |
| Chemokine (C-X-C motif) ligand 16   | 1.75           | 4.52             | 0.0142           | 0.0000             |
| Growth factor receptor bound protein 2  | 1.73           | 2.07             | 0.0214           | 0.0022             |
| Guanine nucleotide-binding protein, $\gamma$ 10   | 1.55           | 3.14             | 0.0279           | 0.0031             |

Relative ratios were determined from CodeLink and Affymetrix data by comparing the degree of gene expression in lacrimal glands from male NOD mice with those of female NOD mice. Listed genes were increased  $\geq 2.0$ -fold in either the CodeLink or Affymetrix microarray.

17 (Th17)/Treg balance toward the proinflammatory Th17 axis contributes to the development of Sjögren syndrome and other autoimmune disorders.<sup>70-73</sup> The reasons for this shift are not completely known, but may be due, at least in part, to moesin activity and other microenvironmental stimuli.<sup>52</sup>

Another gene of particular interest is the X-chromosome-linked androgen receptor, the expression of which is increased in male MRL/lpr and female NOD lacrimal glands. Androgen

receptors are members of the nuclear receptor superfamily of ligand-inducible transcription factors and appear to mediate almost all of the biological actions of androgens.<sup>74,75</sup> Androgens, in turn, appear to be very important in Sjögren syndrome. For example, testosterone treatment of female MRL/lpr mice causes a dramatic suppression of the inflammation in, and a significant increase in the function of, the lacrimal gland.<sup>5,76</sup> These effects are analogous to those found

in humans, wherein topical or systemic androgen administration significantly decreases dry eye disease signs and symptoms, and stimulates tear flow, in patients with Sjögren syndrome.<sup>5,76</sup> Indeed, androgen deficiency seems to be a risk factor for the development of lacrimal gland inflammation in women with Sjögren syndrome.<sup>5,76</sup> In contrast, androgens induce lymphocyte infiltration into the lacrimal glands of NOD mice.<sup>5,77,78</sup> This anomalous effect appears to be mediated through the lacrimal gland microenvironment,<sup>11</sup> as well as male-specific factors that cause CD4(+) CD25(+) Foxp3(+) regulatory T-cell dysfunction.<sup>78</sup> Further, this androgen response differs markedly from the androgen-induced decrease of inflammation in NOD salivary and pancreatic tissues.<sup>11,79,80</sup>

It is noteworthy that acinar and ductal epithelial cells contain the androgen receptors that are the target for androgen activity in lacrimal tissue.<sup>81</sup> In addition, these cells are thought to be the primary cells involved in the initiation and perpetuation of glandular autoimmune reactivity in Sjögren syndrome.<sup>82</sup> We hypothesize that this androgen-epithelial cell interaction induces the altered activity of specific genes in lacrimal glands, and leads to the reduction of pathological lesions and an improvement in glandular function in MRL/lpr, and the opposite effects in NOD, mice. Further research is required to test this hypothesis, and to identify those genes that may underlie the sex- and hormonal-regulation of the lacrimal gland in Sjögren syndrome.

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### References

- Bron AJ, de Paiva CS, Chauhan S, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15:438-510.
- Psianou K, Panagoulas I, Papanastasiou AD, et al. Clinical and immunological parameters of Sjögren's syndrome [published online ahead of print August 10, 2018]. *Autoimmun Rev*. doi:10.1016/j.autrev.2018.05.005.
- Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol*. 2014;35:347-369.
- Brandt JE, Priori R, Valesini G, Fairweather D. Sex differences in Sjögren's syndrome: a comprehensive review of immune mechanisms. *Biol Sex Differ*. 2015;6:19.
- Sullivan DA, Rocha EM, Aragona P, et al. TFOS DEWS II sex, gender, and hormones report. *Ocul Surf*. 2017;15:284-333.
- Whitacre CC. Sex differences in autoimmune diseases. *Nat Immunol*. 2001;2:777-780.
- Klein SL, Flanagan KL. Sex differences in immune response. *Nat Rev Immunol*. 2016;16:626-638.
- Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. *Am J Pathol*. 2008;173:600-609.
- Toda I, Wickham LA, Sullivan DA. Gender and androgen treatment influences the expression of proto-oncogenes and apoptotic factors in lacrimal and salivary tissues of MRL/lpr mice. *Clin Immunol Immunopath*. 1998;86:59-71.
- Toda I, Sullivan BD, Rocha EM, Da Silveira LA, Wickham LA, Sullivan DA. Impact of gender on exocrine gland inflammation in mouse models in Sjögren syndrome. *Exp Eye Res*. 1999;69:355-366.
- Hunger RE, Carnaud C, Vogt I, Mueller C. Male gonadal environment paradoxically promotes dacryoadenitis in non-obese diabetic mice. *J Clin Invest*. 1998;101:1300-1309.
- Richards SM, Jensen RV, Liu M, et al. Influence of sex on gene expression in the mouse lacrimal gland. *Exp Eye Res*. 2006;82:13-23.
- Ashburner M, Ball CA, Blake JA, et al. Gene Ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. 2000;25:25-29.
- Hamm-Alvarez SF, Janga SR, Edman MC, et al. Tear cathepsin S as a candidate biomarker for Sjögren's syndrome. *Arthritis Rheumatol*. 2014;66:1872-1881.
- Suzuki T, Richards SM, Liu S, Jensen RV, Sullivan DA. Impact of sex on gene expression in human corneal epithelial cells. *Mol Vis*. 2009;15:2554-2569.
- Darabad RR, Suzuki T, Richards SM, et al. Influence of aromatase absence on the gene expression and histology of the mouse meibomian gland. *Invest Ophthalmol Vis Sci*. 2013;54:987-998.
- Rahimi Darabad R, Suzuki T, Richards SM, et al. Does estrogen deficiency cause lacrimal gland inflammation and aqueous-deficient dry eye in mice? *Exp Eye Res*. 2014;127:153-160.
- Tan PK, Downey TJ, Spitznagel EL Jr, et al. Evaluation of gene expression measurements from commercial microarray platforms. *Nucleic Acids Res*. 2003;31:5676-5684.
- Yauk CL, Berndt ML, Williams A, Douglas GR. Comprehensive comparison of six microarray technologies. *Nucleic Acids Res*. 2004;32:e124.
- Hollingshead D, Lewis DA, Mirnics K. Platform influence on DNA microarray data in postmortem brain research. *Neurobiol Dis*. 2005;18:649-655.
- MAQC Consortium. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol*. 2006;24:1151-1161.
- Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklyn BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol*. 2003; 4:R.
- Saunier B, Triyatni M, Ulianich L, Maruvada P, Yen P, Kohn LD. Role of the asialoglycoprotein receptor in binding and entry of hepatitis C virus structural proteins in cultured human hepatocytes. *J Virol*. 2003;77:546-559.
- De Vita S, Damato R, De Marchi G, Sacco S, Ferraccioli G. True primary Sjögren's syndrome in a subset of patients with hepatitis C infection: a model linking chronic infection to chronic sialadenitis. *Isr Med Assoc J*. 2002;4:1101-1105.
- Toussiro E, Le Huede G, Mouglin C, Balblanc JC, Bettinger D, Wendling D. Presence of hepatitis C virus RNA in the salivary glands of patients with Sjögren's syndrome and hepatitis C virus infection. *J Rheumatol*. 2002;29:2382-2385.
- Siagris D, Pharmakakis N, Christofidou M, et al. Keratoconjunctivitis sicca and chronic HCV infection. *Infection*. 2002; 30:229-233.
- Loustaud-Ratti V, Riche A, Liozon E, et al. Prevalence and characteristics of Sjögren's syndrome or Sicca syndrome in chronic hepatitis C virus infection: a prospective study. *J Rheumatol*. 2001;28:2245-2251.
- Ramos-Casals M, Garcia-Carrasco M, Brito Zeron MP, Cervera R, Font J. Viral etiopathogenesis of Sjögren's syndrome: role of the hepatitis C virus. *Autoimmun Rev*. 2002;1:238-243.
- Villalta D, Mytilinaiou MG, Elsner M, et al. Autoantibodies to asialoglycoprotein receptor (ASGPR) in patients with autoimmune liver diseases. *Clin Chim Acta*. 2015;450:1-5.

30. Theander E, Jonsson R, Sjöström B, Brokstad K, Olsson P, Henriksson G. Prediction of Sjögren's syndrome years before diagnosis and identification of patients with early onset and severe disease course by autoantibody profiling. *Arthritis Rheumatol.* 2015;67:2427-2436.
31. Wahren M, Skarstein K, Blange I, Pettersson I, Jonsson R. MRL/lpr mice produce anti-Ro 52,000 MW antibodies: detection, analysis of specificity and site of production. *Immunology.* 1994;83:9-15.
32. Skarstein K, Wahren M, Zaura E, Hattori M, Jonsson R. Characterization of T cell receptor repertoire and anti-Ro/SSA autoantibodies in relation to sialadenitis of NOD mice. *Autoimmunity.* 1995;22:9-16.
33. Espinosa A, Zhou W, Ek M, et al. The Sjogren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death. *J Immunol.* 2006;176:6277-6285.
34. Espinosa A, Dardalhon V, Brauner S, et al. Loss of the lupus autoantigen Ro52/Trim21 induces tissue inflammation and systemic autoimmunity by disregulating the IL-23-Th17 pathway. *J Exp Med.* 2009;206:1661-1671.
35. Yoshimi R, Chang TH, Wang H, Atsumi T, Morse HC III, Ozato K. Gene disruption study reveals a nonredundant role for TRIM21/Ro52 in NF-kappaB-dependent cytokine expression in fibroblasts. *J Immunol.* 2009;182:7527-7538.
36. Kong HJ, Anderson DE, Lee CH, et al. Cutting edge: autoantigen Ro52 is an interferon inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. *J Immunol.* 2007;179:26-30.
37. Yang K, Shi HX, Liu XY, et al. TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. *J Immunol.* 2009;182:3782-3792.
38. Aqrabi LA, Kvarnström M, Brokstad KA, Jonsson R, Skarstein K, Wahren-Herlenius M. Ductal epithelial expression of Ro52 correlates with inflammation in salivary glands of patients with primary Sjögren's syndrome. *Clin Exp Immunol.* 2014;177:244-252.
39. Kyriakidis NC, Kapsogeorgou EK, Gourzi VC, Konsta OD, Baltatzis GE, Tzioufas AG. Toll-like receptor 3 stimulation promotes Ro52/TRIM21 synthesis and nuclear redistribution in salivary gland epithelial cells, partially via type I interferon pathway. *Clin Exp Immunol.* 2014;178:548-560.
40. Flower DR. The lipocalin protein family: structure and function. *Biochem J.* 1996;318:1-14.
41. Halpern M, Martínez-Marcos A. Structure and function of the vomeronasal system: an update. *Prog Neurobiol.* 1993;70:245-318.
42. Hurst JL, Payne CE, Nevison CM, et al. Individual recognition in mice mediated by major urinary proteins. *Nature.* 2001;414:631-634.
43. Stopkova R, Klempt P, Kuntova B, Stopka P. On the tear proteome of the house mouse (*Mus musculus musculus*) in relation to chemical signaling. *PeerJ.* 2017;5:e3541.
44. Liberles SD. Mammalian pheromones. *Ann Rev Physiol.* 2014;76:151-175.
45. Ferrero DM, Moeller LM, Osakada T, et al. A juvenile mouse pheromone inhibits sexual behavior through the vomeronasal system. *Nature.* 2013;502:368-371.
46. Touhara K. Molecular biology of peptide pheromone production and reception in mice. *Adv Genet.* 2007;59:147-71.
47. Kimoto H, Haga S, Sato K, Touhara K. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. *Nature.* 2005;437:898-901.
48. He J, Ma L, Kim S, Nakai J, Yu CR. Encoding gender and individual information in the mouse vomeronasal organ. *Science.* 2008;320:535-538.
49. Kimoto H, Sato K, Nodari F, Haga S, Holy TE, Touhara K. Sex- and strain-specific expression and vomeronasal activity of mouse ESP family peptides. *Curr Biol.* 2007;17:1879-1884.
50. Perricone C, Shoenfeld N, Agmon-Levin N, de Carolis C, Perricone R, Shoenfeld Y. Smell and autoimmunity: a comprehensive review. *Clin Rev Allergy Immunol.* 2013;45:87-96.
51. Strous RD, Shoenfeld Y. To smell the immune system: olfaction, autoimmunity and brain involvement. *Autoimmun Rev.* 2006;6:54-60.
52. Park JH, Ha H. Short-term treatment of daumone improves hepatic inflammation in aged mice. *Korean J Physiol Pharmacol.* 2015;19:269-274.
53. Ferro F, Marcucci E, Orlandi M, Baldini C, Bartoloni-Bocci E. One year in review 2017: primary Sjögren's syndrome. *Clin Exp Rheumatol.* 2017;35:179-191.
54. Maria NI, van Helden-Meeuwesen CG, Brkic Z, et al. Association of increased Treg cell levels with elevated indoleamine 2,3-dioxygenase activity and an imbalanced kynurenine pathway in interferon-positive primary Sjögren's syndrome. *Arthritis Rheumatol.* 2016;68:1688-1699.
55. Davis I, Liu A. What is the tryptophan kynurenine pathway and why is it important to neurotherapeutics? *Expert Rev Neurother.* 2015;15:719-721.
56. Koretzky GA, Abtahian F, Silverman MA. SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat Rev Immunol.* 2006;6:67-78.
57. Pappu R, Cheng AM, Li B, et al. Requirement for B cell linker protein (BLNK) in B cell development. *Science.* 1999;286:1949-1954.
58. Glauzy S, Sng J, Bannock J, et al. Defective early B cell tolerance checkpoints in Sjögren's Syndrome patients. *Arthritis Rheumatol.* 2017;60:2203-2208.
59. Both T, Dalm VASH, van Hagen M, van Daele PLA. Reviewing Sjögren syndrome: beyond the dryness-From pathophysiology to diagnosis and treatment. *Int J Med Sci.* 2017;14:191-200.
60. Luan M, Shang Z, Teng Y, et al. The shared and specific mechanism of four autoimmune diseases. *Oncotarget.* 2017;8:108355-108374.
61. Sharma R, Harris VM, Cavett J, et al. Rare X chromosome abnormalities in systemic lupus erythematosus and Sjögren's syndrome. *Arthritis Rheumatol.* 2017;69:2187-2192.
62. Harris VM, Sharma R, Cavett J, et al. Klinefelter's syndrome (47,XXY) is in excess among men with Sjögren's syndrome. *Clin Immunol.* 2016;168:25-29.
63. Liu K, Kurien BT, Zimmerman SL, et al. X chromosome dose and sex bias in autoimmune diseases: increased prevalence of 47,XXX in systemic lupus erythematosus and Sjögren's syndrome. *Arthritis Rheumatol.* 2016;68:1290-1300.
64. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. *Nat Rev Immunol.* 2010;10:594-604.
65. Invernizzi P, Pasini S, Selmi C, Gershwin ME, Podda M. Female predominance and X chromosome defects in autoimmune diseases. *J Autoimmun.* 2009;33:12-16.
66. Bianchi I, Lleo A, Gershwin ME, Invernizzi P. The X chromosome and immune associated genes. *J Autoimmun.* 2012;38:J187-J192.
67. Ørstavik KH. Why are autoimmune diseases more prevalent in women? *Tidsskr Nor Laegeforen.* 2017;137:866-868.
68. Lagresle-Peyrou C, Luce S, Ouchani F, et al. X-linked primary immunodeficiency associated with hemizygous mutations in the moesin (MSN) gene. *J Allergy Clin Immunol.* 2016;138:1681-1689.
69. Ansa-Addo EA, Zhang Y, Yang Y, et al. Membrane-organizing protein moesin controls Treg differentiation and antitumor

- immunity via TGF- $\beta$  signaling. *J Clin Invest*. 2017;127:1321-1337.
70. Matsui K, Sano H. T helper 17 cells in primary Sjögren's syndrome. *J Clin Med*. 2017;6:E65.
71. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014;13:668-677.
72. Kong N, Lan Q, Chen M, et al. Antigen-specific transforming growth factor  $\beta$ -induced Treg cells, but not natural Treg cells, ameliorate autoimmune arthritis in mice by shifting the Th17/Treg cell balance from Th17 predominance to Treg cell predominance. *Arthritis Rheum*. 2012;64:2548-2558.
73. Nikolov NP, Illei G. Pathogenesis of Sjögren syndrome. *Curr Opin Rheumatol*. 2009;21:465-470.
74. Tsai M-J, Clark JH, Schrader WT, O'Malley BW. Mechanisms of action of hormones that act as transcription-regulatory factors. In: Wilson JD, Kronenberg HM, Larsen PR, eds. *Williams Textbook of Endocrinology*. Philadelphia: WB Saunders; 1998.
75. McPhaul MJ, Young M. Complexities of androgen action. *J Am Acad Dermatol*. 2001;45(suppl 3):S87-S94.
76. Sullivan DA, Wickham LA, Krenzer KL, Rocha EM, Toda I. Aqueous tear deficiency in Sjögren's syndrome: possible causes and potential treatment. In: Pleyer U, Hartmann C, Serry W, eds. *Oculodermal Diseases: Immunology of Bullous Oculo-Muco-Cutaneous Disorders*. Buren, The Netherlands: Aeolus Press; 1997:95-152.
77. Takahashi M, Ishimaru N, Yanagi K, Haneji N, Saito I, Hayashi Y. High incidence of autoimmune dacryoadenitis in male non-obese diabetic (NOD) mice depending on sex steroid. *Clin Exp Immunology*. 1997;109:555-561.
78. Lieberman SM, Kreiger PA, Koretzky GA. Reversible lacrimal gland-protective regulatory T-cell dysfunction underlies male-specific autoimmune dacryoadenitis in the non-obese diabetic mouse model of Sjögren syndrome. *Immunology*. 2015;145:232-241.
79. Hawkins T, Gala RR, Dunbar JC. The effect of neonatal sex hormone manipulation on the incidence of diabetes in nonobese diabetic mice. *Proc Soc Exp Med Biol*. 1993;202:201-205.
80. Rosmalen JG, Pigmans MJ, Kersseboom R, Drexhage HA, Leenen PJ, Homo-Delarche F. Sex steroids influence pancreatic islet hypertrophy and subsequent autoimmune infiltration in nonobese diabetic (NOD) and NODscid mice. *Lab Invest*. 2001;81:231-239.
81. Ono M, Rocha FJ, Sullivan DA. Immunocytochemical location and hormonal control of androgen receptors in lacrimal tissues of the female MRL/Mp-lpr/lpr mouse model of Sjögren's syndrome. *Exp Eye Res*. 1995;61:659-666.
82. Moutsopoulos HM. Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol*. 1994;72:162-165.