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^{lpr} (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.[1,2] This lymphocyte infiltration may severely damage acinar and ductal epithelial cell function, resulting in a significantly diminished output of aqueous tears.[1] In consequence, Sjögren syndrome is a leading cause of aqueous-deficient dry eye disease.[1]

One of the most compelling features of Sjögren syndrome is that it affects predominantly females.[3–5] In fact, female sex is a significant risk factor for the development of Sjögren syndrome, given that 93% of the patient population is female.[3–5] This sexual dichotomy is frequently linked to fundamental sex-related differences in the immune system.[4,6,7] 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.[3,4,6,7] Indeed, women constitute almost 80% of the 20 million people in the United States with autoimmune disease.[8]

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.[9]

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^{lpr} (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.[10] 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).[10,11] 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_2 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°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.[12] cDNA was synthesized from RNA (2 μg) 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°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).[13]

The second method to examine differential gene expression involved the hybridization of each cRNA (20 μg) 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[10] from MRL/lpr ($n =$ 18 mice/sex; age = 19.8 ± 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,[14] and is more highly expressed in lacrimal

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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,[15–17] as well as those of others,[18–21] which discovered little agreement between CodeLink and Affymetrix microarrays in the detection of differential gene expression. Although these platforms seem to measure different things,[20] most gene expression changes revealed by each of the platforms are thought to be biologically correct.[19,20]

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.

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

Accession No.	Gene	Ratio	*P*	Ontology
F>M, CodeLink				
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
F>M, Affymetrix				
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
M>F, CodeLink				
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
M>F, Affymetrix				
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 pathwayal 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
MRL/lpr			
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		
NOD			
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.

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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
Biological process				
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	
Molecular function				
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	
Cellular components				
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.[22] 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

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

Ontology	CodeLink Genes ↑	Affymetrix Genes ↑	CodeLink *z*-score	Affymetrix *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

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Table 6. Continued

Ontology	CodeLink Genes ↑	Affymetrix Genes ↑	CodeLink z-score	Affymetrix 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 (≥50 genes/ontology, ≥6.0 CodeLink z-score), molecular functions (≥5 genes/ontology, ≥2.0 z-score) and cellular component (≥4 genes/ontology, ≥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 Fcγ 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 ↑, z-score = 2.43; NOD male, 25 genes ↑, 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 ↑, z-score = 9.64; Affymetrix, 17 genes ↑, z-score = 3.03) and NOD (CodeLink, 53 genes ↑, z-score = 10.78; Affymetrix, 59 genes ↑, z-score = 17.5) mice. Similarly, more that 81% of the proteasome KEGG pathways were significantly higher in lacrimal tissues of female MRL/lpr (Codelink, 22 genes ↑, z-score = 5.87; Affymetrix, 10 genes ↑, z-score = 2.77) and NOD (CodeLink, 21 genes ↑, z-score = 5.09; Affymetrix, 20 genes ↑, 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.[5]

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),[23] thereby facilitating viral infection and increasing glandular inflammation.[23–25] Chronic HCV infection, in turn, is linked to an enhanced prevalence of keratoconjunctivitis sicca[26] and mimics the clinical manifestations of Sjögren syndrome.[24,25,27,28] In addition, ASGPR is an autoantigenic target of both T and B cells.[29] 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.[30] Anti-TRIM21/Ro52 autoantibodies have also been detected in MRL/lpr and NOD mice.[31,32] TRIM21/Ro52 is a ubiquitin E3 ligase that may be induced by interferons (type I or type II) and has immunomodulatory

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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	
Chemokine (C-X-C motif) ligand 13	*7.29*	*5.5*	*0.0021*	*0.0060*
Chitinase 3-like 3	6.43		0.0015	
Serine (or cysteine) peptidase inhibitor, clade A, member 1B	*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
Complement component 3	*4.38*	*3*	*0.0050*	*0.0255*
Interleukin 4 receptor, α	*4.35*		*0.0039*	
Amine oxidase, copper containing 3		4.26		0.0377
Chemokine (C-X-C motif) ligand 9	*4.21*	*3.45*	*0.0053*	*0.0243*
Elastase 2, neutrophil	3.96		0.0088	
Tryptase β2	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
Chemokine (C-C motif) receptor 1	*3.4*		*0.0025*	
Toll-like receptor 2	*3.31*	*2.54*	*0.0033*	*0.0044*
Integrin β2	3.24	2.2	0.0334	0.0167
Acid phosphatase 5	*3.14*		*0.0004*	
C-type lectin domain family 7, member a	*3.11*	*3.63*	*0.0025*	*0.0092*
Mediterranean fever	*3.05*		*0.0483*	
Phospholipase A2, group VII	*3.02*		*0.0120*	
Interleukin 23 receptor	3		0.0001	
Lymphocyte antigen 86	*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 γ	2.87		0.0080	
Fc receptor, IgG, high affinity I	*2.85*		*0.0299*	
Yamaguchi sarcoma viral oncogene homolog		2.83		0.0082
Fc receptor, IgG, low affinity IIb	*2.79*	*2.63*	*0.0101*	*0.0207*
Fatty acid binding protein 4, adipocyte	2.73	3.16	0.0055	0.0182
Fcγ receptor III	*2.73*	*3.03*	*0.0174*	*0.0080*
Complement component factor h		2.6		0.0008
Orosomucoid 1	2.56		0.0237	
Chemokine (C-C motif) ligand 8	*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
CD47 antigen	*2.35*		*0.0432*	
Complement component 4B		*2.35*		*0.0021*
Toll-like receptor 1	*2.34*		*0.0083*	
Chemokine (C-C motif) receptor 2	*2.31*	*2.02*	*0.0063*	*0.0419*
Arachidonate 5-lipoxygenase activating protein	2.3		0.0019	
CD55 antigen	*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	
Cytochrome b-245, polypeptide	*2.21*		*0.0057*	
Annexin A1	*2.18*		*0.0209*	
Janus kinase 2	*2.13*		*0.0062*	
CD44 antigen		2.12		0.0196
INAP for IL-1 inducible nuclear ankyrin-repeat protein		2.11		0.0034
Heme oxygenase 1	*2.09*		*0.0027*	
Chemokine (C-X-C motif) ligand 11	2.07		0.0112	
Chemokine (C-X-C motif) ligand 10	*2.04*		*0.0324*	
Chemokine (C-C motif) ligand 5		2.05		0.0286
UDP-Gal:β.GlcNAc β 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 ≥2-fold. Italicized genes were also found to be upregulated in lacrimal glands of male NOD mice (Table 8).

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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 γ	24.92		0.0008	
Chemokine (C-X-C motif) ligand 9	*15.74*	*81.01*	*0.0000*	*0.0001*
Chemokine (C-C motif) ligand 20	13.04	44.52	0.0001	0.0001
Chemokine (C-X-C motif) ligand 10	10.13	6.79	0.0001	0.0055
CD28 antigen		8.44		0.0009
Serine (or cysteine) peptidase inhibitor, clade A, member 1B	*6.9*	*17.57*	*0.0004*	*0.0013*
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
Forkhead box P3	6.56		0.0007	
Lymphocyte antigen 86	*6.45*	*9.55*	*0.0001*	*0.0003*
Chemokine (C-C motif) ligand 8	*5.52*	*7.81*	*0.0001*	*0.0000*
C-type lectin domain family 7, member a	*5.45*	*6.08*	*0.0024*	*0.0000*
Complement component 4B	*5.38*	*6.94*	*0.0053*	*0.0002*
Cytochrome b-245, α polypeptide	*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, α	4.92		0.0016	
Fc receptor, IgG, low affinity IIb	*4.83*	*6.17*	*0.0005*	*0.0003*
Adenosine A2b receptor	4.73		0.0023	
Toll-like receptor 1	*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 β2	4.07	7.53	0.0002	0.0011
Transforming growth factor, β1	3.94		0.0008	
Interleukin 4 receptor, α	*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, γ polypeptide	3.6	4.94	0.0038	0.0014
Nucleotide-binding oligomerization domain containing 2	3.54		0.0014	
Chemokine (C-C motif) receptor 2	*3.38*		*0.0028*	
CD55 antigen	*3.36*	*2.96*	*0.0052*	*0.0264*
Tumor necrosis factor receptor superfamily, member 1b		3.31		0.0246
Fc receptor, IgG, high affinity I	*3.21*	*4.57*	*0.0006*	*0.0082*
Phospholipase A2, group VII	*3.2*	*4.45*	*0.0044*	*0.0003*
Complement component 3	*3.14*	*3.52*	*0.0050*	*0.0035*
Toll-like receptor 2	*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 1β	2.72	2.78	0.0001	0.0027
Neutrophil cytosolic factor 1	2.71		0.0468	
Acid phosphatase 5		*2.66*		*0.0007*
Serine (or cysteine) peptidase inhibitor, clade B, member 9		2.53		0.0054
Mitogen-activated protein kinase 8	2.48		0.0198	
Fcγ receptor III	*2.46*	*2.48*	*0.0001*	*0.0060*
Janus kinase 2		*2.4*		*0.0003*
Mediterranean fever	*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	
Heme oxygenase 1		*2.26*		*0.0257*
CD47 antigen		*2.24*		*0.0015*
Unc-13 homolog D	2.17		0.0033	
Solute carrier family 11		2.12		0.0160
Annexin A1		*2.11*		*0.0003*
Phosphatidylinositol 3-kinase γ 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 ≥2-fold. Italicized genes were also found to be upregulated in lacrimal glands of female MRL/lpr mice (Table 7).

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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.[33–37] 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.[38,39] 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[40–43] and implied effects include sexual attraction, aggression, hormone modulation, individual recognition, and spatial learning.[41,44,45] 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.[43,46–48] ESP1 is male-specific, and its expression increases in response to androgen administration.[46] In contrast, ESP36 is female-specific and is negatively regulated by androgen.[46] Further, it has been suggested that the reception of ESPs in the vomeronasal system differs according to sex.[49] The vomeronasal system is an accessory olfactory system, and pheromones also can be detected by the anatomically distinct main olfactory system.[46] 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.[50]

Research has also provided evidence that the olfactory system may be inextricably linked to immunological function.[51] 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 γ 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.

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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-γ	3.46		0.0071	
Histocompatibility 2, O region β 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, α	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, β1		2.33		0.0209
Bactrianus MHC class II antigen H-2E α precursor	2.32		0.0382	
Histocompatibility 2, class II antigen E β	2.30	1.98	0.0429	0.0195
CD8 antigen, α 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, α, class A member 1	2.10		0.0008	
Preprolegumain	2.00	2.25	0.0209	0.0144
Interferon γ 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 ≥ 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 α locus 8	6.20	6.52	0.0003	0.0004
Histocompatibility 2, class II antigen A, β1	5.87	5.45	0.0002	0.0001
Histocompatibility 2, O region β locus	5.83		0.0000	
Histocompatibility 2, class II, locus DMa		5.42		0.0002
Histocompatibility 2, O region α 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 β	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	
β2 microglobulin, segment 1, clones PBRCB-(1–3)	4.53	5.43	0.0002	0.0004
Histocompatibility 2, class II antigen A, α	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, β	3.51	3.09	0.0148	0.0017
CD8 antigen, β 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, α 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 γ inducible protein 30	2.00	1.80	0.0035	0.0002
Proteasome 28 subunit, α	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 ≥2.0-fold in either the CodeLink or Affymetrix microarray.

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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 γ	2.78		0.0379	
Vav 1 oncogene	2.78		0.0008	
Arrestin, β2	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 γ	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 ≥2.0-fold in either the CodeLink or Affymetrix platform.

treatment suppresses hepatic inflammation in mice.[52] 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.[50] 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.[50]

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-κB; 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.[53–57]

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.[4,58,59] The increased expression of KEGG pathways related to lysosomes and Fcγ 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.[60]

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).[5,53] 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.[61–63] In fact, attention has been drawn to X-chromosome vulnerability as a possible explanation for the high female prevalence of autoimmune diseases in general.[64–67] 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.[68,69] 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, β	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 γ	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, α 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, γ 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, β2	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 γ 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, γ 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 ≥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.[70–73] The reasons for this shift are not completely known, but may be due, at least in part, to moesin activity and other microenvironmental stimuli.[52]

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.[74,75] 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.[5,76] These effects are analogous to those found

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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.[5,76] Indeed, androgen deficiency seems to be a risk factor for the development of lacrimal gland inflammation in women with Sjögren syndrome.[5,76] In contrast, androgens induce lymphocyte infiltration into the lacrimal glands of NOD mice.[5,77,78] This anomalous effect appears to be mediated through the lacrimal gland microenvironment,[11] as well as male-specific factors that cause CD4(+) CD25(+) Foxp3(+) regulatory T-cell dysfunction.[78] Further, this androgen response differs markedly from the androgen-induced decrease of inflammation in NOD salivary and pancreatic tissues.[11,79,80]

It is noteworthy that acinar and ductal epithelial cells contain the androgen receptors that are the target for androgen activity in lacrimal tissue.[81] In addition, these cells are thought to be the primary cells involved in the initiation and perpetuation of glandular autoimmune reactivity in Sjögren syndrome.[82] 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

1. Bron AJ, de Paiva CS, Chauhan S, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15:438–510.
2. Psianou K, Panagoulias 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.
3. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol*. 2014;35:347–369.
4. 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.
5. Sullivan DA, Rocha EM, Aragona P, et al. TFOS DEWS II sex, gender, and hormones report. *Ocul Surf*. 2017;15:284–333.
6. Whitacre CC. Sex differences in autoimmune diseases. *Nat Immunol*. 2001;2:777–780.
7. Klein SL, Flanagan KL. Sex differences in immune response. *Nat Rev Immunol*. 2016;16:626–638.
8. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. *Am J Pathol*. 2008;173:600–609.
9. 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.
10. Toda I, Sullivan BD, Rocha EM, Da Silveira LA, Wickham LA, Sullivan DA. Impact of gender on exocrine gland inflammation in mouse models in Sjogren syndrome. *Exp Eye Res*. 1999;69:355–366.
11. 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.
12. 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.
13. 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
14. 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.
15. 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.
16. 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
17. 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.
18. 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.
19. Yauk CL, Berndt ML, Williams A, Douglas GR. Comprehensive comparison of six microarray technologies. *Nucleic Acids Res*. 2004;32:e124.
20. Hollingshead D, Lewis DA, Mirnics K. Platform influence on DNA microarray data in postmortem brain research. *Neurobiol Dis*. 2005;18:649–655.
21. MAQC Consortium. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol*. 2006;24:1151–1161.
22. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol*. 2003; 4:R.
23. 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.
24. 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.
25. Toussirot E, Le Huede G, Mougin 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.
26. Siagris D, Pharmakakis N, Christofidou M, et al. Keratoconjunctivitis sicca and chronic HCV infection. *Infection*. 2002;30:229–233.
27. 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.
28. 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.
29. 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. Aqrawi 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-Meeuwsen 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-β 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 β-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, Sterry 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 nonobese 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.