American Journal of Pathology, Vol. 151, No. 5, November 1997

Lacrimal Gland Inflammation Is Responsible for Ocular Pathology in TGF-β1 Null Mice

Nancy L. McCartney-Francis,* Diane E. Mizel,* Michelle Frazier-Jessen,* Ashok B. Kulkarni,† James B. McCarthy,‡ and Sharon M. Wahl*

From the Oral Infection and Immunity Branch and the Gene Targeting Research and Core Facility,† National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland, and the Department of Laboratory Medicine and Pathology,‡ University of Minnesota, Minneapolis, Minnesota*

Mice homozygous for a nonfunctional transforming growth factor-β1 gene develop rampant inflammation in vital organs that contributes to a shortened life span. The presence of circulating anti-nuclear antibodies, immune deposits in tissues, leukocyte infiltration, and increased major histocompatibility complex antigen expression resembles an autoimmune-like syndrome. One of the overt symptoms that appears in these mice lacking transforming growth factor-β1 is the development of dry crusty eyes that close persistently as their health declines. Histologically, the eyes appear normal with little or no inflammation. However, inflammatory lesions, predominantly lymphocytic, develop in the lacrimal glands, disrupting their structure and function and severely limiting their ability to generate tears. This histopathology and aberrant function mimic that of Sjögren's syndrome, a human autoimmune disease characterized by dry eyes and dry mouth. Impeding the leukocyte infiltration into the glands with synthetic fibronectin peptides, which block adhesion, not only prevents the inflammatory pathology but also prevents the persistent eye closure characteristic of these mice. ***(Am J Pathol 1997, 151:1281–1288)***

The transforming growth factor (TGF)-β family is involved in fundamental biological processes associated with embryogenesis, development, and immune and inflammatory processes.[1–3] Whereas overlapping functions have been described *in vitro* for the three mammalian isoforms of TGF-β, which share a high degree of amino acid identity and receptor binding, distinctive patterns of expression of TGF-β1, TGF-β2, and TGF-β3 during embryogenesis suggest functional specificity *in vivo*.[4–6] The unique phenotypes of the transgenic mice deficient in each of the isoforms confirm their discrete functional repertoires.[7–10] Beyond their distinct functional patterns, the three isoforms can also be spatially and temporally independent. For example, high levels of TGF-β1 as opposed to TGF-β2 and TGF-β3 are expressed in hematopoietic tissues during murine embryogenesis.[11] In contrast, TGF-β2 is the predominant isoform expressed in ocular tissues,[5,12] suggesting a unique role for this peptide in ocular development. The near absence of TGF-β1 in the eye would suggest that these tissues should be unaffected in the TGF-β1-deficient mice. Surprisingly, the physical appearance of the eyes of the TGF-β1−/− mice suggests that abnormalities exist. TGF-β1−/− mice, which exhibit multifocal inflammation and a wasting disease and die young, develop dry, crusty deposits around the eyes and then the eyes become sealed shut. On the basis of these observations, we investigated the ocular tissues of the TGF-β1−/− mice to determine the pathological basis for the eye disorder. Whereas no abnormalities were apparent in the structure of the eye globes, it became evident that significant lymphocytic infiltration occurred in the lacrimal glands of these mice, consistent with compromised function. The dry eye and recently described dry mouth[13] symptoms in the TGF-β1 null mice resemble the clinical features of the human autoimmune disorder Sjögren's syndrome. Together with serum autoantibodies, immune deposits, leukocyte infiltration, and major histocompatibility (MHC) expression, the pathology that develops in the animals lacking TGF-β1 may also represent an autoimmune-like disorder.[13–16]

Previous studies have demonstrated the therapeutic efficacy of synthetic fibronectin (FN) peptides in chronic inflammation and autoimmune disease.[13,17,18] Consistent with the capacity for soluble FN peptides to block leukocyte adhesion and recruitment into tissues of the TGF-β1−/− mice,[13,18] FN peptide treatment reversed the characteristic inflammatory pathology in the lacrimal glands and prevented the vision impairment in the TGF-β1 knockout mice.

Materials and Methods

Animals

TGF-β1−/− mice were produced by targeted disruption of the TGF-β1 gene in murine embryonic stem (ES) cells derived from 129/SVJ blastocysts.[8] The targeted ES cells

Accepted for publication August 21, 1997.

Address reprint requests to Dr. Nancy McCartney-Francis, Oral Infection and Immunity Branch, National Institute of Dental Research, National Institutes of Health, 30 Convent Drive, Bethesda, MD.

were injected into recipient blastocysts from C57BL/6J mice and transferred into the uteri of pseudopregnant C57BL/6J mice to produce germ-line chimeras. Heterozygous mice were interbred to produce TGF-β1$-/-$ offspring. Mouse genotype was determined by polymerase chain reaction (PCR) analysis of tail biopsies. Mice were maintained on standard mouse chow and supplemented with liquid diet food (Bioserv, Frenchtown, NJ).

Light Microscopy and Immunohistochemistry

Eyes and exorbital lacrimal glands were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) and embedded in paraffin. Five-micron sections were stained with hematoxylin and eosin (H&E) for microscopic examination. Additional sections were stained for T cell (CD3, Dako, Carpinteria, CA), B cell (B220, Pharmingen, San Diego, CA), and monocyte/macrophage antigens (F4/80, Caltag, South San Francisco, CA) using an immunoperoxidase technique (ABC Vectastain rabbit (CD3) or mouse (B220, F4/80) Elite kit, Vector Laboratories, Burlingame, CA) following manufacturer's instructions. Sections were pretreated by microwaving in antigen retrieval solution (Biotek Solutions, Santa Barbara, CA).

Evaluation of Lacrimal Gland Histology

Histological sections were scored from 0 to 4 based on the presence or absence of foci of 50 or more mononuclear inflammatory cells.[19] Grade 0 had no inflammatory cells; grade 1 had inflammation with no foci; grade 2 had at least one focus; grade 3 had two or more foci; and grade 4 had multiple foci and evidence of glandular destruction, glandular tissue replacement by mononuclear cells, and/or fibrosis.

Northern Analysis

Lacrimal glands were homogenized in Trizol reagent (Life Technologies, Gaithersburg, MD), and total RNA was isolated according to manufacturer's instructions. Five micrograms of RNA were electrophoresed in a 1% agarose/formaldehyde gel and transferred to a nylon membrane using the Turboblotter system (Schleicher & Schuell, Keene, NH). The membrane was baked at 80°C for 2 hours, prehybridized at 42°C for at least 4 hours, and hybridized overnight at 42°C with ^{32}P-labeled cDNA. The VCAM-1 (vascular cell adhesion molecule) cDNA probe was generated by PCR from published sequences (bp 1549 to 2585).[20] The VLA-5α (very late antigen) cDNA clone was kindly provided by Dr. V. M. Holers (University of Colorado Health Sciences Center).[21] Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)[22] and ribosomal RNA were used as housekeeping genes. Filters were washed twice at room temperature in 2X SSC, 0.1% SDS for 15 minutes and once at 65°C in 0.1X SSC, 0.1% SDS for 30 minutes. Filters were then exposed to phosphor plates and analyzed by a phosphorimager (Molecular Dynamics, Sunnyvale, CA) using ImageQuaNT software. Images were reproduced using MDImage (National Institutes of Health, Bethesda, MD) and MacDraw (Claris Corp., Santa Clara, CA) software.

Semiquantitative Reverse Transcriptase (RT)-PCR

Total RNA (2 μg) was reverse transcribed using oligo(dT) as a primer and Moloney murine leukemia virus RT (Life Technologies).[23] The cDNA was amplified by PCR using appropriate oligonucleotide primers and predetermined conditions.[13] The amplified products were analyzed by ethidium bromide staining after agarose gel electrophoresis and, in most cases, by Southern analysis. The interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ primer sets were obtained from Clontech (Palo Alto, CA). Sequences for the IL-10 and GAPDH primer sets have been reported previously.[13,23] TGF-β1 (bp 675 to 700 and 1087 to 1110), TGF-β2 (bp 1982 to 2003 and 2355 to 2377), TGF-β3 (bp 1210 to 1227 and 1900 to 1920), IL-4 (bp 56 to 78 and 431 to 454), intercellular adhesion molecule (ICAM)-1 (bp 66 to 93 and 565 to 588), and hypoxanthine phosphoribosyltransferase (HPRT; bp 602 to 626 and 740 to 764) primer sets were synthesized according to published sequences.[24–30] GAPDH and HPRT were used as housekeeping genes to verify efficient cDNA synthesis from each RNA sample and to allow for comparison of RNA species between different samples. Dilutions of a positive cDNA sample (concanavalin-A-stimulated mouse splenocytes) were run simultaneously with the test samples to verify a linear response curve (log concentration *versus* counts per minute or densitometric units) for each primer set.[13]

Fibronectin Treatment

FN peptides were synthesized and purified as described.[31] The peptides included the arginylglycyl aspartic acid (RGD) domain, the alternatively spliced connecting segment (CS-1), two nonoverlapping sequences corresponding to the FN-C/H region of the A chain of FN (FN-C/H-1, Tyr-Glu-Lys-Pro-Gly-Ser-Pro-Pro-Arg-Glu-Val-Val-Pro-Arg-Pro-Arg-Pro-Gly-Val; FN-C/H-V (FN V), Trp-Gln-Pro-Pro-Arg-Ala-Arg-Ile) and a scrambled version of FN V (Scr V, Arg-Pro-Gln-Ile-Pro-Trp-Ala-Arg). All peptides have tyrosine at their carboxyl-terminal end. Mice received daily intraperitoneal injections (400 μg/100 μl) of a mixture of the four peptides, FN V alone, or Scr V peptides for 10 days, starting at day 6.[18]

Results

Ocular Pathology

Mice homozygous for the mutated TGF-β1 gene appear normal for the first 2 weeks of life. Shortly after weaning, the mice begin to exhibit symptoms of a wasting syndrome, appear disheveled with hunched backs, splayed legs, and muscle atrophy and die between 3 and 4 weeks

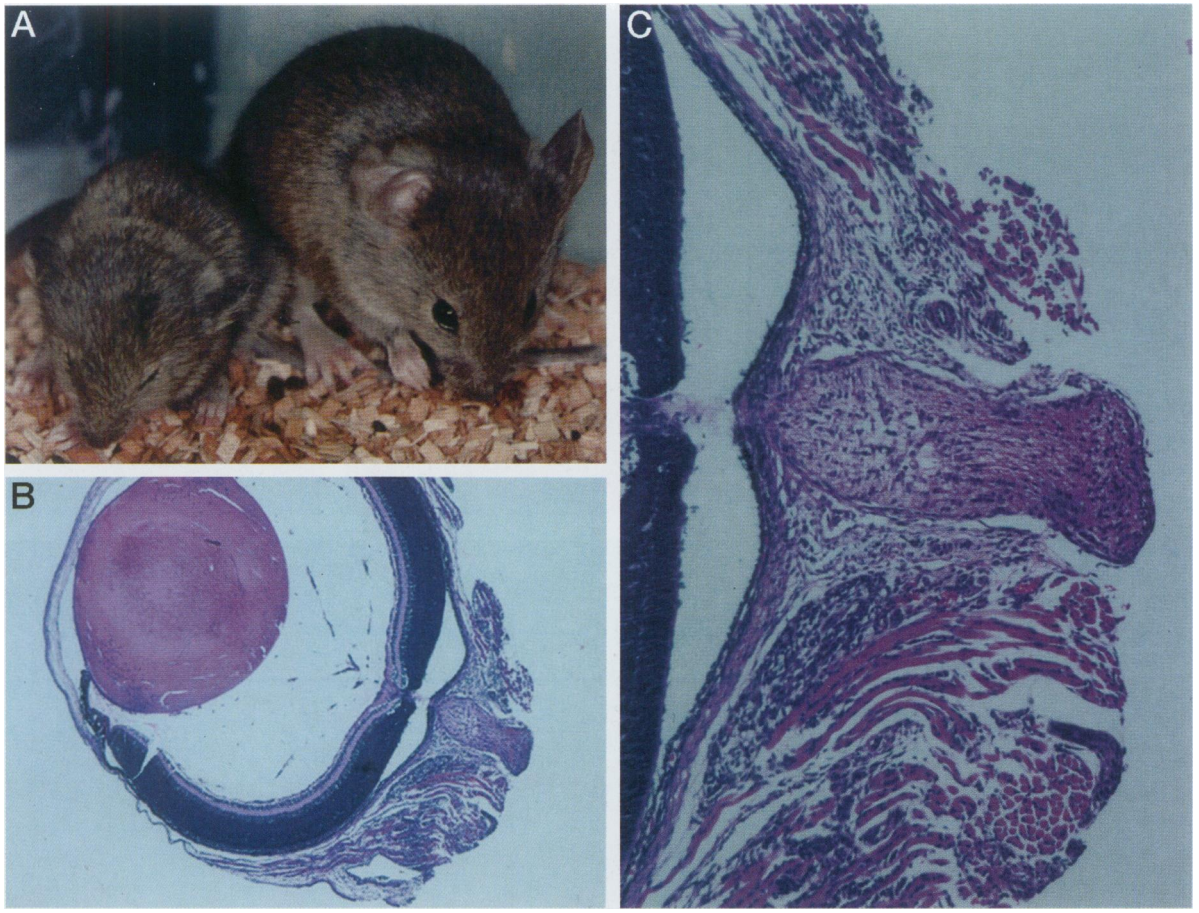

Figure 1. Ocular tissues of the TGF-β1−/− mouse. Physical appearance of TGF-β1−/− eye showing the crusty deposits and eye closure. **A:** Knockout, left; wild-type, right. **B** and **C:** H&E staining of the TGF-β1−/− eye globe (**B**; magnification, ×100) and higher magnification (×250) of optic nerve showing enhanced cellularity (**C**). Cornea, retina, and optic nerve of the TGF-β1−/− mouse appear relatively normal as compared with the TGF-β1+/+ littermate (not shown). Ages of mice ranged from 21 to 29 days.

of age. As symptoms progress, the eyes of the TGF-β1−/− mice develop a dry, crusty appearance, and eventually one or both eye(s) close completely (Figure 1A). However, based on histological examination, the eye globe exhibited a normal structure, including lens, retina, and cornea (Figure 1B), although, in some TGF-β1−/− mice, the optic nerve appeared more cellular, suggestive of inflammatory cell infiltration (Figure 1C).

Lacrimal Gland Histopathology

Because the eye globe exhibited no obvious pathology and ocular dryness is a hallmark of an autoimmune condition known as Sjögren's syndrome in man, we focused on the lacrimal glands as a potential source of pathogenesis in the TGF-β1−/− mice. No inflammatory lesions were evident in glands from asymptomatic (≤12 days) TGF-β1−/− or TGF-β1+/+ mice. However, between the ages of 2 and 4 weeks, inflammatory cell infiltrates were obvious in the lacrimal glands of TGF-β1−/− mice, some as focal lesions and others as large multifocal accumulations of mononuclear cells (Figure 2, A, C, and D). These lesions consisted of perivascular and periductal aggregates of cells that invaded the surrounding parenchyma. As the development of the inflammatory lesions progressed and more leukocytes migrated into the gland, the acini became distorted, shrunken, and atrophic (Figure 2C). In severe lesions, the architecture of the gland was disrupted and focal destruction of the secretory alveoli was evident. The lobular structure of the gland was maintained; however, the space between the lobules increased, indicative of glandular atrophy. In contrast, no inflammatory lesions developed in the lacrimal glands of wild-type mice (TGF-β1+/+; Figure 2, A and B). However, in some of the TGF-β1+/− mice, minimal numbers of mononuclear cells were evident near the ducts and/or vessels but did not develop into inflammatory lesions (graded as 0.5; not shown).

To determine the phenotype of the infiltrating mononuclear cells, lacrimal gland tissue sections were analyzed by immunohistochemical staining (Figure 2, E and F). Significant numbers of CD3-positive mononuclear cells were present within inflammatory sites in the lacrimal glands of TGF-β1−/− mice (Figure 2E), as were a substantial number of B220-positive B cells (Figure 2F). Limited numbers of macrophages were observed throughout the gland (data not shown). The lymphocytic predominance of the inflammatory infiltrate is consistent with the

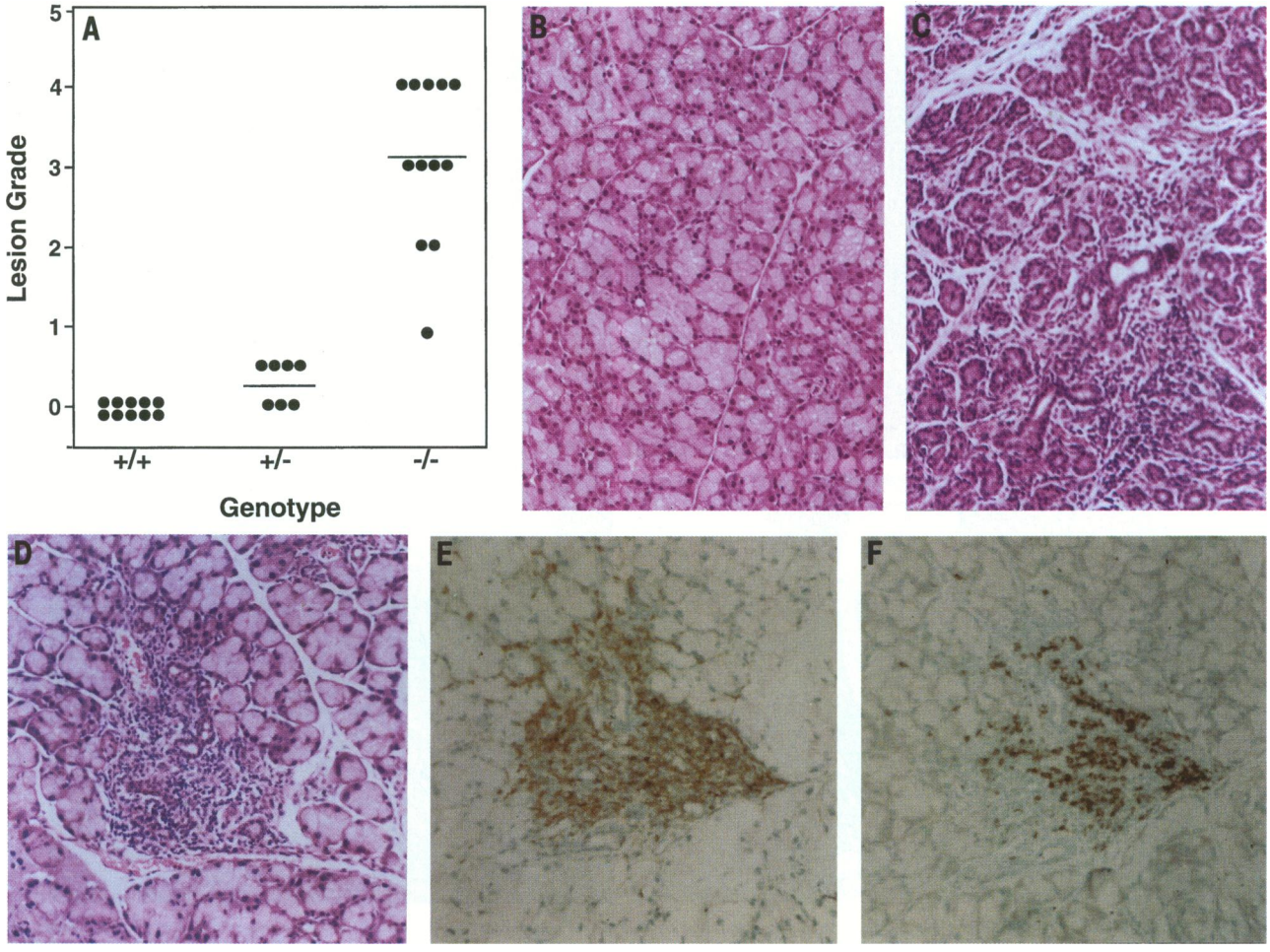

Figure 2. Lacrimal gland histopathology of TGF-β1−/− mouse. **A:** Lacrimal gland sections were stained with H&E and evaluated for inflammatory changes. Cellular infiltration was graded 0 to 4 based on number of inflammatory foci and glandular destruction (see Materials and Methods). No lesions were present in wild-type (+/+, **B**) or heterozygous (+/−) mice. Lacrimal glands from some TGF-β1+/− mice contained a few mononuclear cells and were graded as 0.5 (**A**). Pronounced mononuclear infiltration and tissue disruption were evident in TGF-β1−/− lacrimal glands (**A**, mean lesion grade = 3.08 ± 0.95, n = 12; **C**, grade 3). Ages of mice ranged from 17 to 39 days. Lacrimal gland serial sections from a 23-day-old TGF-β1−/− mouse were stained with H&E (**D**) or by immunohistochemistry with antibodies specific for CD3 (**E**) and B220 (**F**) to demonstrate mononuclear cell infiltration. Dark staining areas represent positive staining. Lacrimal gland tissues from TGF-β1+/+ mice were negative. Control slides without primary antibody were also negative. Magnification, ×250 (**B** and **C**) and ×400 (**D** to **F**).

composition of lacrimal lesions observed in autoimmune mice and in Sjögren's patients.[32,33]

TGF-β Isoforms in Lacrimal Glands

The absence of structural defects in the asymptomatic eye and lacrimal gland suggested that TGF-β1 was not essential for development of these organs. Because the three isoforms of TGF-β have overlapping activities and expression patterns,[3] we measured RNA levels of TGF-β1, -β2, and -β3 to ascertain whether compensatory expression of other isoforms of TGF-β occurred in the absence of TGF-β1 (Figure 3). As anticipated, no TGF-β1 mRNA was detected by RT-PCR in lacrimal gland tissue in the TGF-β1 null mice. However, no TGF-β1 was detected in the lacrimal gland of the wild-type mice either, consistent with reports in human lacrimal glands.[34,35] Immunohistochemical staining for TGF-β1 protein was also negative in the eyes and optic nerves of knockout and wild-type mice (data not shown). Whereas immunohistochemical staining patterns for TGF-β2 and TGF-β3 were similar in TGF-β1−/− and TGF-β1+/+ eyes (data not shown), some increase in TGF-β2, but not TGF-β3 (slight decrease), mRNA was detected in lacrimal gland tissue from TGF-β1−/− mice (Figure 3). The increase in TGF-β2 does not appear to compensate for TGF-β1 in preventing inflammatory events.

Increased Cytokine mRNA Expression in TGF-β1−/− Lacrimal Glands

Several cytokine mRNA species were detected in lacrimal glands of TGF-β1−/− mice (Figure 4A), consistent with studies in other tissues.[7,13,23] In four of four lacrimal gland samples from TGF-β1 knockouts, increased levels of IFN-γ mRNA were detected by RT-PCR as compared with wild-type TGF-β1+/+ mice. Likewise, all knockouts examined expressed TNF-α and IL-4 mRNA, although the level of expression was variable between the knockout

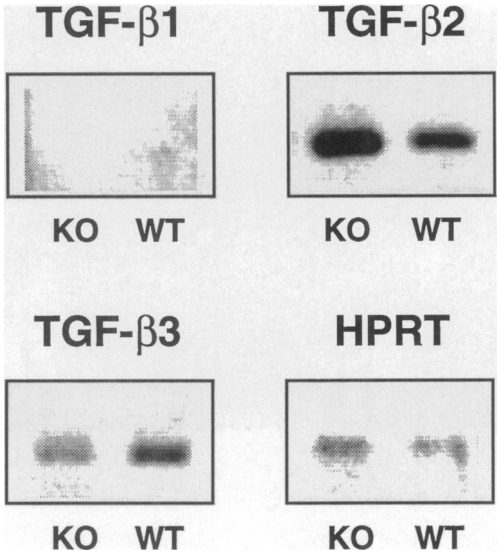

Figure 3. TGF-β isoform expression in lacrimal glands. Total RNA was isolated from lacrimal glands and analyzed for TGF-β isoform and HPRT expression by semiquantitative RT-PCR. PCR products were electrophoresed and visualized by ethidium bromide staining. No TGF-β1 was detected in either knockout or wild-type lacrimal gland samples. (Age of mice, 32 days).

mice. One mouse with elevated TNF-α and IL-4 (TNF-α/GAPDH and IL-4/GAPDH ratios were 2- to 10-fold greater than the other three knockouts) exhibited more severe symptoms, including persistently sealed eyes, emphasizing a pathophysiological link between lacrimal gland and eye pathology and cytokine expression. IL-1β and IL-10 mRNAs, although variable, were higher in all TGF-β1−/− lacrimal glands than in wild-type mice. Wild-type mice did not express detectable amounts of either TNF-α, IL-1β, IL-4, or IL-10 in their lacrimal gland tissue, but some were weakly positive for IFN-γ gene expression.

Adhesion Molecule Expression and Blockade

Because cell-cell interactions and cell adhesion occur early in the development of lacrimal gland inflammation, adhesion molecule expression on endothelial cells and leukocytes was determined (Figure 4B). VCAM-1, present on activated endothelial cells,[36,37] was elevated in lacrimal glands of TGF-β1 knockout mice as compared with wild-type controls. In addition, mRNA for the α5 chain of α5β1 (VLA-5), the FN receptor present mostly on leukocytes and fibroblasts,[21] was also elevated. ICAM-1 mRNA was also increased in lacrimal gland tissue of TGF-β1−/− mice (data not shown).

As adhesion molecule expression likely precipitates leukocyte recruitment into the lacrimal glands of TGF-β1−/− mice, we attempted to block this process with FN peptides, which interfere with integrin-ligand binding.[17] Daily treatment with pooled FN peptides or FN-C/H-V (FN V) peptide alone, previously shown to prevent the infiltration of leukocytes into heart, lung, and salivary gland of the TGF-β1−/− mice and extend the life span of the mice,[13,18] blocked the infiltration of leukocytes into the lacrimal glands. By preventing inflammatory cell accumulation, the FN peptides preserved the structure of the

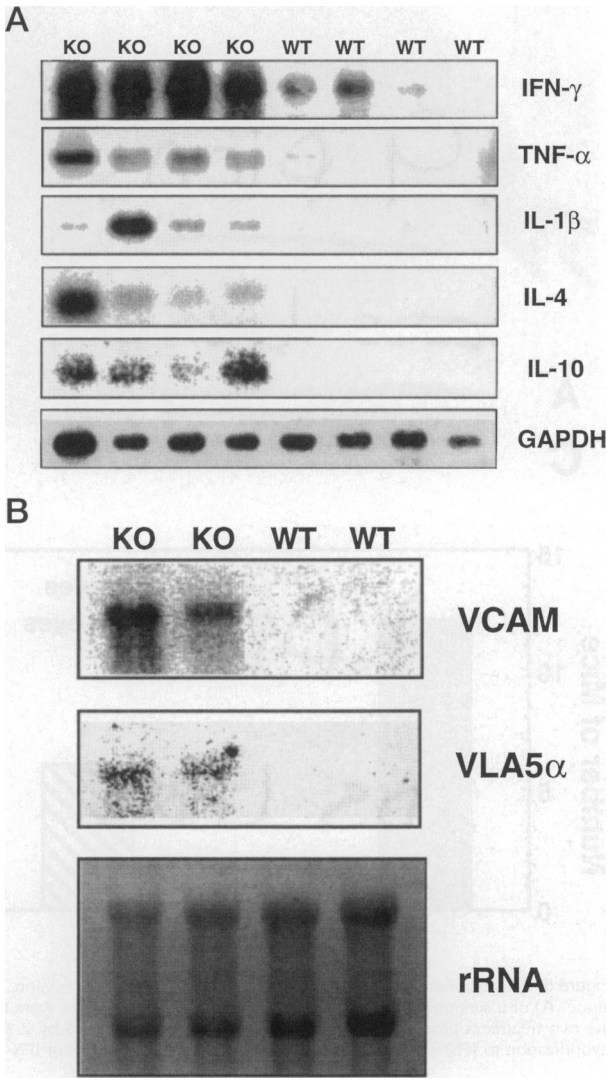

Figure 4. Cytokine and adhesion molecule mRNA expression is increased in lacrimal glands of TGF-β1−/− mice. Total RNA from lacrimal glands was reverse transcribed and amplified by PCR using cytokine-specific primer sets (**A**). PCR products were analyzed by Southern analysis. Adhesion molecule RNA was detected by Northern analysis (**B**). Ages of mice ranged from 22 to 32 days.

gland and, in doing so, maintained its function and tear secretion to keep the eyes open and maintain vision (Figure 5, A and C). By comparison, the eyes of the untreated knockout mice, PBS-treated mice, or mice treated with scrambled FN peptide (Figure 5, B and C) became dry and sealed shut. The inhibition of inflammatory disease in the FN peptide-treated mouse was reflected by a reduction in IFN-γ mRNA (Figure 5D) compared with the scrambled peptide-treated knockout.

Discussion

Mice deficient in TGF-β1 (TGF-β1−/−) develop inflammatory lesions in the lacrimal glands that compromise their function resulting in symptoms of dry eyes and persistent eye closure. Parallel lesions in the salivary

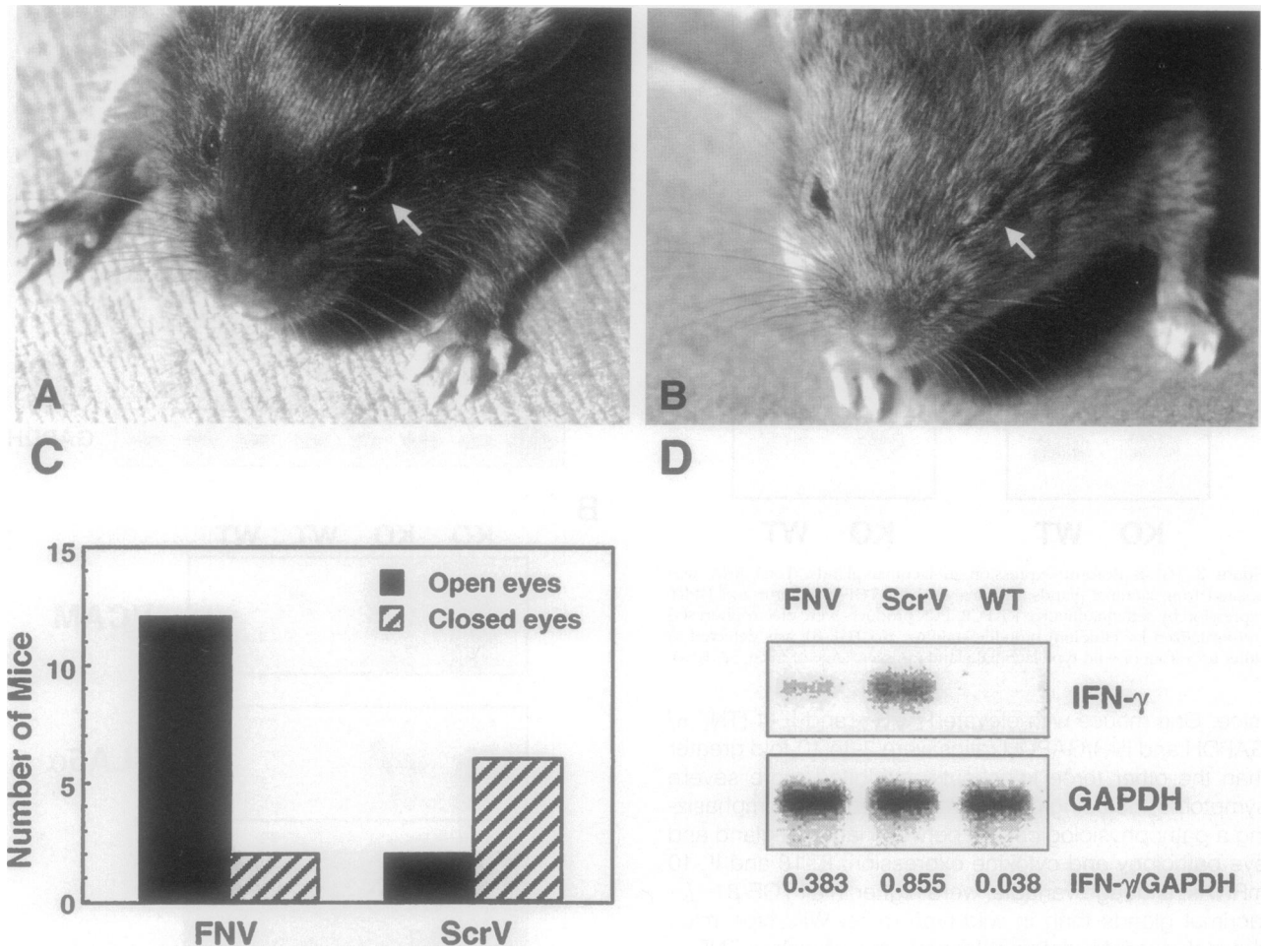

Figure 5. FN treatment prevents eye closure and reduces IFN-γ expression. Mice were treated daily for 10 days with 400 μg of pooled FN peptides, FN V peptide alone (**A**) or a scrambled version of this peptide (Scr V, **B**). Pictures were taken on day 21 and tissues harvested for RNA. The frequency of eye phenotypes in the two treatment groups is shown in **C** ($P < 0.01$ as determined by Z score from odds ratio analysis). After RT-PCR, products were analyzed by Southern hybridization to IFN-γ and GAPDH cDNA (**D**). Densitometric units of IFN-γ mRNA were normalized to GAPDH.

glands of TGF-β1−/− mice result in glandular dysfunction and reduced saliva production.[13] Similar histopathology of the lacrimal and salivary glands to that of the human autoimmune disorder Sjögren's syndrome suggests an autoimmune etiology. Elevations in serum autoantibodies, including antibodies to double-stranded DNA, single-stranded DNA, and Smith (Sm) ribonucleoprotein, and immune deposits in the kidney provide additional evidence for a systemic autoimmune-like response in the TGF-β1−/− mice (M. Frazier-Jessen, M. Christ, N. McCartney-Francis, D. Klinman, S. Bauer, I. Katona, J. Ward, S. Wahl, manuscript submitted).[13–15,38] Furthermore, MHC antigen expression is elevated in TGF-β1−/− mice,[16] and double knockout mice for TGF-β1 and class II (TGF-β1−/−, class II−/−) antigens show partial reduced expression of serum autoantibodies and reduced inflammation.[39]

Although the mechanism of the inflammatory pathology in the TGF-β1−/− mice is not known, the absence of the suppressive milieu of TGF-β1 may contribute to the overexpression of adhesion molecules and cytokines that promote recruitment and activation of inflammatory cells, leading to the development of the autoimmune-like lesions.[40–44] Elevations in local adhesion molecule and cytokine expression as detected by mRNA and immunohistological analyses have been observed in Sjögren's syndrome and in autoimmune mice displaying salivary and lacrimal gland inflammation.[45–49] Interference with leukocyte adhesion through the use of antibodies to integrins or, as in this and previous studies, soluble synthetic FN peptides, prevents inflammatory cell infiltration and facilitates resolution of inflammation.[13,18,50] Furthermore, recent studies suggest that soluble RGD-containing peptides not only interfere with leukocyte adhesion to endothelial cells and matrix but may also block integrin-mediated signal transduction that is critical for the perpetuation of inflammatory processes. In addition to mediating cell adhesion, matrix binding engages a signaling cascade that potentiates cytokine signaling and induction of certain inflammatory and cytokine genes.[37,51] Treatment of TGF-β1−/− mice with adhesion-blocking synthetic FN peptides not only prevented the tissue infil-

tration but also reduced gene expression of cytokines, including IFN-γ.

IFN-γ, in particular, may play an important role in the pathogenesis of these lesions by inducing epithelial cell MHC antigens.[48,49,52] IFN-γ mRNA is elevated in the lacrimal gland and salivary gland of TGF-β1−/− mice,[13] and elevations in IFN-γ protein in the plasma of TGF-β1−/− mice have been observed (Frazier-Jessen et al, manuscript submitted). Furthermore, IFN-γ message levels are increased in the salivary glands of TGF-β1−/− mice before the onset of inflammation, suggesting a role in the early pathogenesis of the inflammatory response.[13] Similarly, IFN-γ mRNA levels are increased at the onset of disease in autoimmune mouse strains[46] and in salivary and lacrimal glands of Sjögren's patients,[48,49] and measurable levels of IFN-γ protein are detectable in saliva of Sjögren's patients.[48] These cytokines may be derived from the infiltrated leukocytes but also may be the products of resident mononuclear cells and salivary and lacrimal epithelium.[48]

Both pro-inflammatory cytokines TNF-α and IL-1β were increased in the lacrimal glands of the knockout mice. The level of cytokine expression correlated with severity of wasting disease and eye closure. The role of TNF-α and IL-1β in the pathogenesis of the inflammatory process in the TGF-β1 knockout mice is not known. Both cytokines may contribute to tissue damage through direct cytotoxic effects or by induction of reactive nitrogen or oxygen pathways. TNF-α and IL-1 can also directly and indirectly up-regulate the expression of other cytokines and adhesion molecules to further amplify the inflammatory process.[43]

In addition to pro-inflammatory cytokine expression, the TH2 cytokines IL-4 and IL-10 were also elevated, suggesting a pandemic activation of the cellular immune response. Expression of TGF-β2 mRNA in the lacrimal glands was also increased as compared with wild-type littermates and may reflect an attempt to compensate for the lack of TGF-β1.

Eye closure generally occurred during the later stages of the inflammatory process and was often used as an indicator of impending death. However, as no changes in the eye globes accompanied the visual impairment, except for occasional inflammation of the optic nerve, the lacrimal glands were considered responsible for the pathogenesis. In autoimmune MRL/MP mice, lacrimal gland inflammation occurs in young (4 to 5 months of age) and old animals, but ophthalmic abnormalities such as choroiditis, scleritis, and orbital vasculitis are primarily limited to older mice.[53] These and other studies suggest that the dynamic ocular changes in a variety of autoimmune mouse strains may be attributed to the effects of lacrimal gland inflammation and decreased tearing.[19,54–56] The extremely short life span of the TGF-β1−/− mice may preclude the development of additional ophthalmic abnormalities. Nonetheless, blockade of lacrimal gland inflammation with systemically delivered adhesion-blocking FN peptides prevented the lacrimal lesions and, consequently, the dry, sealed eyes.

Acknowledgments

The authors thank George McGrady and Paula Worley for their excellent technical assistance and Drs. Robert S. Redman (Veteran Affairs Medical Center, Washington, D.C.), Chi-Chao Chan (National Eye Institute, NIH, Bethesda, MD) and Sue Silverton (University of Pennsylvania School of Dental Medicine) for helpful discussions.

References

1. McCartney-Francis NL, Frazier-Jessen M, Wahl SM: TGF-β: a balancing act. Int Rev Immunol 1997 (in press)
2. McCartney-Francis NL, Wahl SM: Transforming growth factor β: a matter of life and death. J Leukocyte Biol 1994, 55:401–409
3. Letterio JJ, Roberts AB: Transforming growth factor-β1-deficient mice: identification of isoform-specific activities in vivo. J Leukocyte Biol 1996, 59:769–774
4. Roberts AB, Sporn MB: Differential expression of the TGF-β isoforms in embryogenesis suggests specific roles in developing and adult tissues. Mol Reprod Dev 1992, 32:91–98
5. Millan FA, Denhez F, Kondaiah P, Akhurst RJ: Embryonic gene expression patterns of TGF β1, β2 and β3 suggest different developmental functions in vivo. Development 1991, 111:131–144
6. Schmid P, Cox D, Bilbe G, Maier R, McMaster GK: Differential expression of TGF β1, β2 and β3 genes during mouse embryogenesis. Development 1991, 111:117–130
7. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T: Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. Nature 1992, 359:693–699
8. Kulkarni AB, Huh C-G, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S: Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 1993, 90:770–774
9. Proetzel G, Pawlowski SA, Wiles MV, Yin M, Boivin GP, Howles PN, Ding J, Ferguson MWJ, Doetschman T: Transforming growth factor-β3 is required for secondary palate fusion. Nature Genet 1995, 11:409–414
10. Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J: Abnormal lung development and cleft palate in mice lacking TGF-β3 indicates defects of epithelial-mesenchymal interaction. Nature Genet 1995, 11:415–420
11. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ: Defective haematopoiesis and vasculogenesis in transforming growth factor-β1 knock out mice. Development 1995, 121:1845–1854
12. Gatherer D, Ten Dijke P, Baird DT, Akhurst RJ: Expression of TGF-β isoforms during first trimester human embryogenesis. Development 1990, 110:445–460
13. McCartney-Francis N, Mizel DE, Redman RS, Frazier-Jessen M, Panek RB, Kulkarni AB, Ward JM, McCarthy JB, Wahl SM: Autoimmune Sjögren's-like lesions in salivary glands of TGF-β1-deficient mice are inhibited by adhesion-blocking peptides. J Immunol 1996, 157:1306–1312
14. Dang H, Geiser AG, Letterio JJ, Nakabayashi T, Kong L, Fernandes G, Talal N: SLE-like autoantibodies and Sjögren's syndrome-like lymphoproliferation in TGF-β knockout mice. J Immunol 1995, 155:3205–3212
15. Yaswen L, Kulkarni AB, Frederickson T, Mittleman B, Schiffmann R, Payne S, Longenecker G, Mozes E, Karlsson S: Autoimmune manifestations in the TGF-β1 knockout mice. Blood 1996, 87:1439–1445
16. Geiser AG, Letterio JJ, Kulkarni AB, Karlsson S, Roberts AB, Sporn MB: TGF-β1 controls expression of major histocompatibility genes in the postnatal mouse: aberrant histocompatibility antigen expression in the pathogenesis of the TGF-β1 null mouse phenotype. Proc Natl Acad Sci USA 1993, 90:9944–9948
17. Wahl SM, Allen JB, Hines KL, Imamichi T, Wahl AM, Furcht LT, McCarthy JB: Synthetic fibronectin peptides suppress arthritis in rats by interrupting leukocyte adhesion and recruitment. J Clin Invest 1994, 94:655–662
18. Hines KL, Kulkarni AB, McCarthy JB, Tian H, Ward JM, Christ M,

McCartney-Francis N, Furcht LT, Karlsson S, Wahl SM: Synthetic fibronectin peptides interrupt inflammatory cell infiltration in transforming growth factor β1 knockout mice. Proc Natl Acad Sci USA 1994, 91:5187–5191

19. Jabs DA, Enger C, Prendergast RA: Murine models of Sjögren's syndrome: evolution of the lacrimal gland inflammatory lesions. Invest Ophthalmol Vis Sci 1991, 32:371–380
20. Hession C, Moy P, Tizard R, Chishol P, Williams C, Wysk M, Burkly L, Miyake K, Kincade P, Lobb R: Cloning of murine and rat vascular cell adhesion molecule-1. Biochem Biophys Res Commun 1992, 183: 163–169
21. Holers VM, Ruff TG, Parks DL, McDonald JA, Ballard LL, Brown EJ: Molecular cloning of a murine fibronectin receptor and its expression during inflammation: expression of VLA-5 is increased in activated peritoneal macrophages in a manner discordant from major histocompatibility complex class II. J Exp Med 1989, 169:1589–1605
22. Fort P, Marty L, Piechaczyk M, El Sabrouty S, Dani C, Jeanteur P, Blanchard JM: Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res 1985, 13:1431–1442
23. Christ M, McCartney-Francis NL, Kulkarni AB, Ward JM, Mizel DE, Mackall CL, Gress RE, Hines KL, Tian H, Karlsson S, Wahl SM: Immune dysregulation in TGF-β1-deficient mice. J Immunol 1994, 153:1936–1946
24. Derynck R, Jarrett JA, Chen EY, Goeddel DV: The murine transforming growth factor-β precursor. J Biol Chem 1986, 261:4377–4379
25. Silvera MR, Sempowski GD, Phipps RP: Expression of TGF-β isoforms by Thy-1$^+$ and Thy-1$^-$ pulmonary fibroblast subsets: evidence for TGF-β as a regulator of IL-1-dependent stimulation of IL-6. Lymphokine Cytokine Res 1994, 13:277–285
26. Miller DA, Lee A, Pelton RW, Chen EY, Moses HL, Derynck R: Murine transforming growth factor-β2 cDNA sequence and expression in adult tissues and embryos. Mol Endocrinol 1989, 3:1108–1114
27. Miller DA, Lee A, Matsui Y, Chen EY, Moses HL, Derynck R: Complementary DNA cloning of the murine transforming growth factor-β-3 (TGF-β-3) precursor and the comparative expression of TGF-β-3 and TGF-β-1 messenger RNA in murine embryos and adult tissues. Mol Endocrinol 1989, 3:1926–1934
28. Lee F, Yokota T, Otsuka T, Meyerson P, Villaret D, Coffman R, Mosmann T, Rennick D, Roehn N, Smith C, Zlotnik A, Arai K-I: Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell and mast cell-stimulatory activities. Proc Natl Acad Sci USA 1986, 83:2061–2065
29. Siu G, Hedrick SM, Brian AA: Isolation of the murine intercellular adhesion molecule 1 (ICAM-1) gene: ICAM-1 enhances antigen-specific T cell activation. J Immunol 1989, 143:3813–3820
30. Konecki DS, Brennand J, Fuscoe JC, Caskey CT, Chinault AC: Hypoxanthine-guanine phosphoribosyltransferase genes of mouse and Chinese hamster: construction and sequence analysis of cDNA recombinants. Nucleic Acids Res 1982, 10:6763–6775
31. McCarthy JB, Chelberg MK, Mickelson DJ, Furcht LT: Localization and chemical synthesis of fibronectin peptides with melanoma adhesion and heparin binding activities. Biochemistry 1988, 27:1380–1388
32. Jabs D, Prendergast RA: Reactive lymphocytes in lacrimal gland and vasculitic renal lesions of autoimmune MRL.lpr mice express L3T4. J Exp Med 1987, 166:1198–1203
33. Pepose JS, Akata RF, Pflugfelder SC, Voight W: Mononuclear cell phenotypes and immunoglobin gene rearrangements in lacrimal gland biopsies from patients with Sjögren's syndrome. Ophthalmology 1990, 97:1599–1605
34. Wilson SE: Growth factor and receptor messenger RNA production in human lacrimal gland tissue. Lacrimal Gland, Tear Film, and Dry Eyes Syndromes. Edited by Sullivan DA. New York, Plenum Press, 1994, pp 197–204
35. Wilson SE, Lloyd SA: Epidermal growth factor and its receptor, basic fibroblast growth factor, transforming growth factor β-1, and interleukin-1α messenger RNA production in human corneal endothelial cells. Invest Ophthalmol Vis Sci 1991, 32:2747–2756
36. Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994, 8:504–512
37. Wahl SM, Feldman GM, McCarthy JB: Regulation of leukocyte adhesion and signaling in inflammation and disease. J Leukocyte Biol 1996, 59:789–796
38. Frazier-Jessen M, Christ M, Panek RB, McCartney-Francis N, Katona I, Bauer S, Kulkarni A, Ward J, Wahl S: Aberrant B lymphocyte function in TGF-β1 (−/−) null mice. FASEB J 1995, 9:A814
39. Letterio JJ, Geiser AG, Kulkarni AB, Dang H, Kong L, Nakabayashi T, Roberts AB: Autoimmunity associated with TGF-β1-deficiency in mice is dependent on MHC class II antigen expression. J Clin Invest 1996, 98:2109–2119
40. Brennan FM, Feldmann M: Cytokines in autoimmunity. Curr Opin Immunol 1992, 4:754–759
41. Hayashi Y, Haneji N, Hamano H: Pathogenesis of Sjögren's syndrome-like autoimmune lesions in MRL/lpr mice. Pathol Int 1994, 44:559–568
42. Hartwell D, Levine J, Fenton M, Francis C, Leslie C, Beller D: Cytokine dysregulation and the initiation of systemic autoimmunity. Immunol Lett 1994, 43:15–21
43. Ward PA: Cytokines, inflammation, and autoimmune diseases. Hosp Pract 1995, May 15:35–41
44. Nakabayashi T, Letterio JJ, Geiser AG, Kong L, Ogawa N, Zhao W, Koike T, Fernandes G, Dang H, Talal N: Up-regulation of cytokine mRNA, adhesion molecule proteins, and MHC class II proteins in salivary glands of TGF-β1 knockout mice. J Immunol 1997, 158:5527–5535
45. St Clair EW, Angellilo JC, Singer KH: Expression of cell-adhesion molecules in the salivary gland microenvironment of Sjögren's syndrome. Arthritis Rheum 1992, 35:62–66
46. Hamano H, Saito I, Haneji N, Mitsuhashi Y, Miyasaka N, Hayashi Y: Expressions of cytokine genes during development of autoimmune sialadenitis in MRL/lpr mice. Eur J Immunol 1993, 23:2387–2391
47. Saito I, Terauchi K, Shimuta M, Nishiimura S, Yoshino K, Takeuchi T, Tsubota K, Miyasaka N: Expression of cell adhesion molecules in the salivary and lacrimal glands of Sjogren's syndrome. J Clin Lab Anal 1993, 7:180–187
48. Fox RI, Kang H-I, Ando D, Abrams J, Pisa E: Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. J Immunol 1994, 152:5532–5539
49. Boumba D, Skopouli FN, Moutsopoulos HM: Cytokine mRNA expression in the labial salivary gland tissues from patients with primary Sjögren's syndrome. Br J Rheumatol 1995, 34:326–333
50. Diebold RJ, Eis MJ, Yin M, Ormsby I, Boivin GP, Darrow BJ, Saffitz JE, Doetschman T: Early-onset multifocal inflammation in the transforming growth factor β1-null mouse is lymphocyte mediated. Proc Natl Acad Sci USA 1995, 92:12215–12219
51. McCarthy JB, Vachhani BV, Wahl SM, Finbloom DS, Feldman G: Human monocyte binding to fibronectin enhances IFN-γ-induced early signaling events. J Immunol 1997, 159:2424–2430
52. Fox RI, Bumol T, Fantozzi R, Bone R, Schreiber R: Expression of histocompatibility antigen HLA-DR by salivary gland epithelial cells in Sjögren's syndrome. Arthritis Rheum 1986, 29:1105–1111
53. Jabs DA, Alexander EL, Green WR: Ocular inflammation in autoimmune MRL/MP mice. Invest Ophthalmol Vis Sci 1985, 26:1223–1229
54. Kessler HS, Cubberly M, Manski W: Eye changes in autoimmune NZB and NZB × NZW mice: comparisons with Sjögren's syndrome. Arch Ophthalmol 1971, 85:211–219
55. Hoffman RW, Alspaugh MA, Waggie KS, Durham JB, Walker SE: Sjögren's syndrome in MRL/l and MRL/n mice. Arthritis Rheum 1984, 27:157–165
56. Jabs DA, Prendergast RA: Ocular inflammation in MRL/MP-lpr/lpr mice. Invest Ophthalmol Vis Sci 1991, 32:1944–1947