STAT6-Mediated Keratitis and Blepharitis: A Novel Murine Model of Ocular Atopic Dermatitis

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PURPOSE. Atopic dermatitis (AD) is a common inflammatory disease that can affect the eye, resulting in ocular pathologies, including blepharitis, keratitis, and uveitis; however, the pathogenic mechanisms underlying the ocular manifestations of AD are not well understood.

METHODS. In the present study, we characterized the ocular pathologies that develop in the Stat6VT mouse model of AD. We examined the cytokine profile of the eyelid lesions, measured the behavioral response, and documented the treatment response to topical steroids.

RESULTS. Our results show that Stat6VT mice spontaneously developed blepharitis, keratitis, and uveitis similar to that observed in patients with AD. Histologic findings of allergic inflammation in affected eyelids in this model include the presence of a lymphocyte-predominant infiltrate and tissue eosinophilia in the dermis. Gene expression analysis of affected eyelid tissue by quantitative PCR revealed increased amounts of mRNAs for the Th2 cytokines IL-4, IL-5, and IL-13. In addition, increased eyelid scratching was seen in Stat6VT mice with blepharitis. Topical treatment with the corticosteroid clobetasol reduced eyelid inflammation, tissue eosinophilia, and Th2 cytokine expression.

CONCLUSIONS. The development of AD-like ocular pathologies in this model supports the idea that in humans, AD-associated disease of the eye may be driven by Th2-mediated inflammation and demonstrates that the Stat6VT mouse may be a useful system in which to further investigate pathogenesis of and treatment strategies for blepharitis and other ocular diseases that develop in association with AD.

Keywords: STAT6, ocular atopic dermatitis, atopic blepharitis

Atopic dermatitis (AD) is a chronic inflammatory disease that can involve periocular skin and the cornea.¹ Ocular manifestations of AD include blepharitis, keratitis with neovascular formation, uveitis, subcapsular cataracts, and, rarely, retinal detachment.^{2–5} Blepharitis involves chronic inflammation of the eyelid and is characterized histologically by a mixed inflammatory cellular infiltrate in the dermis and clinically by dry eye and visual impairment.^{6–8} Infectious and immune-mediated mechanisms have been proposed to play a significant role in the pathogenesis of blepharitis.^{7,8} Another AD-associated ocular disease is keratitis, which involves neovascularization of the peripheral corneal stroma and subsequent infiltration of inflammatory cells.² A better understanding of the immunopathogenic mechanisms of AD-associated ocular disease is needed to better manage these conditions, which can result in significant vision loss.^{6,9}

Interleukin 4 (IL-4) is a Th2 cytokine implicated in AD pathogenesis.¹⁰ Binding of IL-4 to its receptor on target cells activates STAT6 via the Janus kinase (Jak)/STAT signaling pathway, promoting transcription of a number of immunomodulatory genes.¹¹ Expression of skin barrier function genes, including filaggrin, also can be attenuated by IL-4.¹² Recently

Stat6VT mice on the C57BL/6 background have been shown to develop AD-like skin lesions and blepharitis as the result of transgenic expression of a constitutively active form of human Stat6 (Stat6VT) in the lymphocyte compartment.^{13,14} To more easily monitor lesion development, these mice were crossed into the outbred SKH1 hairless background.¹⁵

In this study, we used Stat6VT mice on the SKH1 hairless background to define novel aspects of AD-like blepharitis and discovered that additional manifestations of AD-like ocular pathologies, including keratitis and uveitis, develop in this model. Moreover, we identified clinical, histologic, and molecular markers of blepharitis that improve in response to treatment with topical steroids, thus providing a new model system in which to investigate pathogenesis and test therapeutics at the preclinical stage.

METHODS

Reagents

Anti-IL-4 antibody for immunoblotting was obtained from Santa Cruz Biotech (Dallas, TX, USA). Anti-IL-5, IL-10, and IL-13 were

from Biolegend (San Diego, CA, USA). All other reagents were obtained from Sigma (St. Louis, MO, USA).

Animals

All animal experiments followed the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care Committee of Indiana University. The Stat6VT transgenic mouse has been previously described; mice used in this report were on the SKH1 background.^{10,11} The transgene used to generate these mice includes the human CD2 locus control region upstream of a human Stat6 cDNA with V547 and T548 codons mutated to encode alanine.^{10,11} All mice were maintained in standard microisolator cages under a simulated 12-hour day/night cycle and were fed and watered ad libitum. All mice were maintained by the Laboratory Animal Resource Center. After weaning at 3 to 4 weeks of age, wild-type (WT) and Stat6VT mice were monitored biweekly up to 30 weeks of age to calculate the cumulative incidence of blepharitis.

Histology of the Eye and the Orbit

Mice were euthanized and the eyelids of age-matched mice (Stat6VT and WT) were harvested, sectioned, and fixed in 10% neutral buffered formalin for 1.5 hours and then transferred to 70% EtOH for storage before further processing and paraffin-embedding. Orbital tissues were embedded in paraffin and vertical sections were obtained at 10- μ m thickness through the papillary-optic nerve plane. Hematoxylin and eosin (H&E), periodic acid-Schiff, and anti-CD31 staining were performed.

Quantification of Eosinophils by IHC

Images of H&E-stained slides were scored by a trained pathologist (JB) in a blinded manner with a Nikon Eclipse E400 microscope (Nikon Corp., Melville, NY, USA) at $\times 200$ to $\times 400$ magnification.

Immunoblot Analysis

Cell lysates were subjected to SDS-PAGE followed by immunoblot analysis with the indicated antibodies. Equal amounts of protein were run on 10% to 12% gel and transferred to nitrocellulose membrane BioRad (Hercules, CA, USA). Membranes were blocked in 5% nonfat dried milk in PBS. Primary and secondary antibodies were diluted in concentrations as described.¹³ An Odyssey Imaging system (Li-cor Biosciences, Lincoln, NE, USA) was used to analyze immunoblots.

Total RNA Extraction and cDNA Synthesis

For quantitative real-time PCR, eyelid tissues were homogenized using a tissue lyser (Qiagen, Valencia, CA, USA) followed by RNA isolation using the RNeasy fibrous tissue kit (Qiagen), after which cDNA was prepared with the high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA, USA). Transcripts were quantified using TaqMan chemistry and a 7500 Fast Real-Time PCR System (Applied Biosystems). Primer and probe mixtures for IL-4, IL-5, IL-10, IL-13, IL-17A, IFN γ , TNF α , TSLP, and GAPDH were purchased from Applied Biosystems. The relative expression of selected cytokine transcripts was determined using the comparative C_T method (i.e., $2^{-\Delta\Delta C_T}$ method) with GAPDH as the endogenous reference transcript. The fold changes in expression of cytokine transcripts in samples from Stat6VT mice relative to

samples from WT mice are equal to $2^{-\Delta\Delta C_T}$, where C_T is the threshold cycle, and $\Delta\Delta C_T$ is calculated using the following equation: $\Delta\Delta C_T = ([C_T^{\text{Cytokine transcript Stat6VT}} - C_T^{\text{GAPDH Stat6VT}}] - [C_T^{\text{Cytokine transcript WT}} - C_T^{\text{GAPDH WT}}])$.

Quantification of Animal Behavior

Age- and sex-matched littermate progeny of Stat6VT and WT mice at 4 to 8 months of age were monitored behaviorally in two groups. In brief, cohorts of Stat6VT and WT mice (4 mice total per observation) were deprived of food and water during 10 minutes of habituation to a new cage, followed immediately thereafter by 30 minutes of videotaping to monitor grooming and eyelid-scratching behaviors of the mice. All investigators were kept out of the room during videotaping. Two experimenters blinded with respect to genotypes of the mice then reviewed the videos and independently quantified scratching and grooming behaviors of individual mice. Data were analyzed using a two-way ANOVA.

Treatment of Blepharitis With Topical Corticosteroids

Eyelids of Stat6VT mice with blepharitis and WT control mice were treated daily for 7 days with either clobetasol dipropionate ointment (0.05%, 50 mg) or white petrolatum as the vehicle control. Upper eyelid thickness was determined with calipers (Mediline 0793211; Mundelein, IL, USA) based on the average of triplicate midline upper eyelid thickness determinations per mouse, per time point on days 0, 4, and 7 of treatment. Mice were then euthanized and tissues were collected for histology and isolation of total RNA.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Shapiro-Wilk was used to test normality and equal variance of the data. Mann-Whitney tests were used to compare Stat6VT versus WT when groups were non-normal or had unequal variance. Student's *t*-test was used when normality and equal variance assumptions were met.

RESULTS

Development of Blepharitis and Keratitis in Stat6VT Mice

To determine the cumulative incidence of blepharitis in Stat6VT mice on the SKH1 background, SKH1-*Hr^{br}* WT mice and mice that express a constitutively active STAT6 (Stat6VT), V547A/T548A, were monitored biweekly up to 30 weeks of age. In Stat6VT mice, periocular edema and eyelid erythema with associated excoriations and madarosis (loss of hair) were evident as early as 7 weeks of age and developed in most of these mice (85%) by 20 weeks of age ($n = 20$ and 24 for Stat6VT and WT, respectively; Figs. 1A, 1B). No such clinical abnormalities were noted in the age- and sex-matched WT controls.

In Stat6VT mice, findings of keratitis also were present. Slit-lamp examination of the eye revealed peripheral neovascularization of the cornea, which extended centrally in many of the mice (Fig. 1C). Findings of keratitis on slit-lamp examination were present in 35% of Stat6VT mice by 20 weeks of age, compared with only 4% of age-matched WT mice ($n = 20$ Stat6VT; $n = 24$ WT).

FIGURE 1. Clinical features of ocular inflammation in Stat6VT mice. **(A)** Photographs showing blepharitis in Stat6VT mouse (*right*: arrow denotes lid edema) compared with unaffected WT control (*left*). Scale: 5 mm. **(B)** Cumulative incidence of blepharitis in Stat6VT mice ($n = 20, 24$ respectively for Stat6VT and WT). **(C)** Slit-lamp photographs of WT and Stat6VT mice showing diffuse corneal neovascularization in Stat6VT mouse (*arrow*). Scale: 500 μ m. **(D)** Behavioral assay with video recording determined the rate of grooming versus lid scratching over a 30-minute period in both WT and Stat6VT transgenic mice ($n = 5, 7$ respectively of WT and Stat6VT). Counts were obtained by two independent observers; * $P < 0.05$).

Increased Scratching in Stat6VT Mice

To further evaluate the clinical features of the blepharitis in Stat6VT mice, scratching and grooming behaviors of the mice were quantified using a method previously reported to study allergic inflammation in the skin of mice.¹² In brief, eyelid-scratching and grooming behaviors of mice in an undisturbed environment (i.e., no investigators in the room) were recorded on video for 30 minutes. Videos were then reviewed by two investigators blinded to the genotype of the mice. Behaviors of the mice that were quantified were categorized as “lid (eyelid) scratching” and nonspecific facial cleaning was referred to as “grooming.” The rate of scratching in Stat6VT mice was elevated as compared with WT controls (Fig. 1D, 14/min versus 8/min, $P < 0.05$). In contrast, there were no differences in “grooming” behavior between the two genotypes.

Histopathologic Findings of AD-Like Ocular Pathologies in Stat6VT Mice

Histologic analysis of Stat6VT mice showed marked infiltration of a mixed inflammatory cell infiltrate in the dermis of both upper and lower lids. A lymphocyte-predominant infiltrate containing variable numbers of eosinophils, macrophages, and neutrophils was identified in the substantia propria of the eyelids as well (Fig. 2A). A prominent inflammatory infiltrate was also present in the Meibomian glands of Stat6VT, but not WT mice (Fig. 2B). Further analysis of the subepithelial inflammatory cell infiltrate revealed a nearly 4-fold increase in total cells (Figs. 2C, 2D; $n = 15$ mice, 203 ± 20 vs. 50 ± 21 cells/mm²; $P < 0.05$) and an approximately 40-fold increase in eosinophils in Stat6VT compared with WT mice (Fig. 2E; $n =$

15 mice, 179.7 ± 65.0 cell/mm² vs. 4.4 ± 4 cell/mm²; $P < 0.05$).

Corneal disease (neovascular pannus formation) also was seen in Stat6VT but not WT mice (Figs. 1C, 2F). Fluorescein staining did not show any corneal abrasions associated with the stromal neovascular changes (Supplementary Fig. S1). Histologic analysis revealed corneal stromal inflammatory infiltrates in Stat6VT mice but not in WT controls (Fig. 2F). The cornea from affected Stat6VT mice also exhibited keratinization of the epithelial layer, increased stromal inflammatory cells, neovascularization, and corneal edema. Periodic acid-Schiff staining demonstrated the basement membrane was not significantly affected by the cellular infiltrate (Fig. 2F, bottom panels). Finally, although Descemet's membrane appeared to be thinner in Stat6VT compared with WT mice, the difference was not statistically significant (data not shown).

Several findings of uveitis were present in Stat6VT mice. First, the anterior chamber of the eye revealed inflammatory exudates. In addition, peripheral anterior synchia formation was noted (Fig. 2G). Finally, proteinous exudates with cellular infiltrates were seen along with erosion of the Descemet's membrane (Fig. 2G).

Th2 Cytokine Expression Is Increased in Eyelids of Stat6VT Mice

Histologic features of a lymphocyte-predominant inflammatory cell infiltrate and tissue eosinophilia were consistent with allergic inflammation causing the blepharitis in Stat6VT mice. To further evaluate for evidence of allergic inflammation, transcripts for Th2 cytokines were measured by quantitative real-time PCR. As shown in Figure 3A, these studies revealed increased levels of IL-4, IL-5, IL-10, and IL-13 transcripts in

FIGURE 2. Histological analysis of eyelid and corneal tissue from WT and Stat6VT mice. (A) Samples were fixed stained with H&E, original magnification $\times 400$ (Inset: arrow indicates eosinophil). (B) Meibomian glands of WT and Stat6VT transgenic mice. Scale bar: 250 μ m. (C) Subepithelial cellular infiltration in Stat6VT mice. Scale bar: 250 μ m. (D) Bar graph represents quantification of cellular infiltrate in WT and Stat6VT mice ($*P < 0.05$, paired Student's *t*-test). Representative of the average for 10 high-power fields examined and three independent experiments by two observers. (E) Quantitation of eosinophils in WT and Stat6VT mice ($*P < 0.05$, paired Student's *t*-test). Representative of average of 10 high power fields from three to five mice examined and three independent experiments. (F) Histologic staining of corneal tissue in WT and Stat6VT mice (arrow, intrastromal neovascularization). Scale bar: 30 μ m. (G) Anterior chamber inflammation with loss of Descemet's membrane (arrowhead). Scale: 50 μ m.

eyelids from Stat6VT mice compared with WT controls. No differences in expression were noted for thymic stromal lymphopoietin, an epithelial/dendritic cell-derived cytokine implicated in some forms of allergic inflammation in the skin.¹⁶ Mild elevations in IL-17A, IFN- γ , and TNF α were also noted (Supplementary Fig. S2).

To further investigate the cytokine profile of the blepharitis in Stat6VT mice, immunoblotting was performed on eyelids from Stat6VT and WT mice. Consistent with findings at the mRNA level, the total amount of IL-4 was increased in eyelids of Stat6VT mice with blepharitis compared with WT control eyelids (Fig. 3B). Similar findings were seen for IL-5, IL-10, and IL-13 (Supplementary Fig. S3).

Response of Blepharitis in Stat6VT Mice to Topical Application of Clobetasol

Topical corticosteroids are part of the therapeutic treatment for blepharitis.⁶ To further validate the Stat6VT mouse as a model of AD-like blepharitis, we investigated the responsive-

ness of this phenotype to topical corticosteroids, hypothesizing that treatment should improve clinical and histologic features of blepharitis and reduce the level of IL-4 mRNA.

To establish an appropriate system, eyelid thickness was assessed in Stat6VT mice with blepharitis and found to be significantly increased compared with WT mice (Fig. 4A). Cohorts of Stat6VT mice with blepharitis and WT mice were then treated daily for 7 days with clobetasol ointment 0.05% (50 mg) or vehicle control (white petrolatum). In contrast to those treated with vehicle, eyelid thickness decreased in Stat6VT mice treated with the topical corticosteroid, whereas eyelid thickness was not affected by this treatment in WT controls (Fig. 4A). Treatment with topical corticosteroid also reduced eyelid edema and erythema in Stat6VT mice compared with vehicle-treated Stat6VT mice (Fig. 4A). Treatment of blepharitis in Stat6VT mice with topical corticosteroids also led to a marked reduction in tissue eosinophilia (Fig. 4B; 89 cells/mm² for vehicle treated versus 14 cells/mm² for clobetasol-treated, $P < 0.05$). Finally, the quantity of IL-4 mRNA was significantly reduced in affected eyelids of Stat6VT mice

FIGURE 3. Increased Th2 cytokine levels in eyelids of Stat6VT mice with blepharitis. **(A)** RNA was isolated from eyelids of WT and Stat6VT mice with active lesions and cytokine mRNA (IL-4, IL-5, IL-10, IL-13, TSLP) was analyzed using quantitative PCR. Results are the average of three independent experiments performed in triplicate, from five to seven mice per group. **(B)** Total protein from eyelids of WT and Stat6VT transgenic mice were subjected to SDS-PAGE; immunoblot analysis was performed using anti-IL4 and β -actin. β -actin was used as a loading control. *Bar graph* represents the average densitometry of three samples normalized to β -actin (* $P < 0.05$, Student's *t*-test; representative of three independent experiments).

treated with topical corticosteroid compared to vehicle-treated Stat6VT mice (Fig. 4C).

DISCUSSION

Here we demonstrated that the Stat6VT mouse model of AD exhibits three of the ocular pathologies associated with AD in patients, including blepharitis, keratitis, and uveitis. Further investigation of blepharitis in Stat6VT mice revealed clinical and histologic features of allergic inflammation in the eyelid. Additional evidence of allergic inflammation in affected eyelids from Stat6VT mice included increased levels of mRNAs for several Th2 cytokines, including IL-4 and increased quantities of IL-4 protein in whole eyelid tissue. With respect to other ocular manifestations of the AD-like phenotype in Stat6VT mice, findings of keratitis were identified on slit-lamp examination and findings of both keratitis and uveitis were identified on histologic analysis of affected tissues.

Further investigations of blepharitis in Stat6VT mice, including behavioral and interventional studies, faithfully recapitulated what would be expected of a model of AD-associated blepharitis. For example, eyelid scratching, which is a common behavior in AD patients with blepharitis, was increased in Stat6VT mice with blepharitis. Moreover, topical

FIGURE 4. Treatment of eyelids with a topical corticosteroid reverses blepharitis and restores Th2 imbalance in Stat6VT mice. **(A)** Clobetasol (50 mg) was applied topically daily for 7 consecutive days on both WT and Stat6VT transgenic mice. Upper eyelid thickness at midline was measured on days 0, 4, and 7 (* and ** $P < 0.05$, two-tailed Student's *t*-test). *Inset photographs* shown for appearance of blepharitis phenotype in the mouse before (left, D0) and 7 days after (right, D7) initiating treatment with clobetasol ointment. Scale: 25 μ m. (Representative of the average for three independent experiments by two observers.) **(B)** Eosinophil counts in the subdermal tissue were performed for both WT and Stat6VT mice treated with either vehicle or clobetasol. Representative of the average for 10 high-power fields examined and three independent experiments. **(C)** RNA isolated from eyelids of WT and Stat6VT mice treated with vehicle or clobetasol was used to quantify IL-4 transcripts (normalized to GAPDH). Representative of the average from three independent experiments (* $P < 0.05$, two-tailed Student's *t*-test).

corticosteroids, a treatment for severe blepharitis in patients, also improved blepharitis in Stat6VT mice. Given the high frequency of blepharitis (>80%) in Stat6VT mice and the identification of several markers of disease that are reduced with therapy (e.g., eyelid thickness, tissue eosinophilia, and IL-4 mRNA levels), Stat6VT mice may be a valuable tool for studying pathogenesis and as a preclinical model in which to develop and test novel therapeutics.

Several models of experimentally induced eyelid inflammation have been described, including those triggered by mechanical (laser or cryoablation), infectious (HSV-1), or pharmacologic (repeated epinephrine injections) modalities.^{17–19} Immunologic models of experimental blepharitis include a mouse model of experimental systemic lupus erythematosus induced by administration of anti-DNA antibody, NC/Nga mice, and IL-4 transgenic mice.^{3,8,11,20} Tepper et al.¹¹ demonstrated development of blepharitis in transgenic mice that overexpress IL-4 in lymphocytes. Binding of IL-4 to its receptor leads to phosphorylation and homodimerization of STAT6, which then translocates to the nucleus to regulate target genes.¹⁰ The development of a similar phenotype in Stat6VT mice highlights the importance of the STAT6 arm of the IL-4 signaling pathway in allergic inflammation and demonstrates that activation of STAT6 in the lymphocyte compartment is sufficient to trigger the development of allergic blepharitis in mice. Future studies of the molecular and cellular pathways downstream of STAT6-mediated transcription in lymphocytes will be needed to better understand the pathogenesis of blepharitis and other AD-associated ocular pathologies in this model. In addition, patients with anterior eyelid inflammation frequently develop corneal complications; therefore, it will be important to further examine the corneal pathology in Stat6VT mice and investigate the mechanisms underlying this process.

In conclusion, Stat6VT mice represent a unique model of chronic anterior blepharitis resulting from allergic inflammation and exhibit additional AD-associated ocular pathologies. This model may serve as a useful system to further investigate disease mechanisms and develop novel therapies for AD-associated ocular diseases.

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References

- Leung DY, Bieber T. Atopic dermatitis. *Lancet*. 2003;361:151–160.
- Carmi E, Defossez-Tribout C, Ganry O, et al. Ocular complications of atopic dermatitis in children. *Acta Derm Venereol*. 2006;86:515–517.
- Ebihara N, Funaki T, Matsuda H, Okumura K, Murakami A, Ra C. Corneal abnormalities in the NC/Nga mouse: an atopic dermatitis model. *Cornea*. 2008;27:923–929.
- Matsuo T, Saito H, Matsuo N. Cataract and aqueous flare levels in patients with atopic dermatitis. *Am J Ophthalmol*. 1997;124:36–39.
- Iijima Y, Wagai K, Matsuura Y, Ueda M, Miyazaki I. Retinal detachment with breaks in the pars plicata of the ciliary body. *Am J Ophthalmol*. 1989;108:349–355.
- Jackson WB. Blepharitis: current strategies for diagnosis and management. *Can J Ophthalmol*. 2008;43:170–179.
- Giese MJ, Adamu SA, Pitchikian-Halabi H, Ravindranath RM, Mondino BJ. The effect of *Staphylococcus aureus* phage lysate vaccine on a rabbit model of staphylococcal blepharitis, phlyctenulosis, and catarrhal infiltrates. *Am J Ophthalmol*. 1996;122:245–254.
- Chan CC, Gery I, Kohn LD, Nussenblatt RB, Mozes E, Singer DS. Periocular inflammation in mice with experimental systemic lupus erythematosus. A new experimental blepharitis and its modulation. *J Immunol*. 1995;154:4830–4835.
- Nagaki Y, Hayasaka S, Kadoi C. Cataract progression in patients with atopic dermatitis. *J Cataract Refract Surg*. 1999;25:96–99.
- Zeng WP. 'All things considered': transcriptional regulation of T helper type 2 cell differentiation from precursor to effector activation. *Immunology*. 2013;140:31–38.
- Tepper RI, Levinson DA, Stanger BZ, Campos-Torres J, Abbas AK, Leder P. IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. *Cell*. 1990;62:457–467.
- DaSilva SC, Sahu RP, Konger RL, Perkins SM, Kaplan MH, Travers JB. Increased skin barrier disruption by sodium lauryl sulfate in mice expressing a constitutively active STAT6 in T cells. *Arch Dermatol Res*. 2012;304:65–71.
- Sehra S, Yao Y, Howell MD, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol*. 2010;184:3186–3190.
- Bruns HA, Schindler U, Kaplan MH. Expression of a constitutively active Stat6 in vivo alters lymphocyte homeostasis with distinct effects in T and B cells. *J Immunol*. 2003;170:3478–3487.
- Turner MJ, Dasilva-Arnold SC, Yi Q, Mehrotra P, Kaplan MH, Travers JB. Topical application of a vitamin D analogue exacerbates atopic dermatitis and induces the atopic dermatitis-like phenotype in Stat6VT mice. *Pediatr Dermatol*. 2013;30:574–578.
- Zhang Y, Zhou B. Functions of thymic stromal lymphopoietin in immunity and disease. *Immunol Res*. 2012;52:211–223.
- Gossman DM, Brightwell JR, Huntington AC, Newton C, Yung R, Egger S. Experimental comparison of laser and cryosurgical cilia destruction. *Ophthalmic Surg*. 1992;23:179–182.
- Jester JV, Nicolaidis N, Kiss-Palvolgyi I, Smith RE. Meibomian gland dysfunction. II. The role of keratinization in a rabbit model of MGD. *Invest Ophthalmol Vis Sci*. 1989;30:936–945.
- Allen SJ, Mott KR, Ghiasi H. Involvement of STAT4 in IgG subtype switching and ocular HSV-1 replication in mice. *Mol Vis*. 2010;16:98–104.
- Matsuda H, Watanabe N, Geba GP, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol*. 1997;9:461–466.