Stat6-Independent Tissue Inflammation Occurs Selectively on the Ocular Surface and Perioral Skin of IκBζ−/− Mice

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PURPOSE. 1κBζ−/− mice have been reported to be affected by allergic dermatitis. This study was conducted to analyze the pathophysiological role of 1κBζ and to address the functional relevance of Th2-mediated immune responses in the development of ocular surface inflammation and dermatitis by 1κBζ−/− mice.

METHODS. BALB/c background 1κBζ−/− mice were established without individual differences; 1κBζ/Stat6 double-knockout (WKO) mice unable to produce Th2 cytokine were created; and microscopic-, histologic-, and immunochemical studies were performed. In 1κBζ−/− mice the serum IgE levels were examined by ELISA, and quantitative PCR was used to study the gene expression of IFN-γ, IL4, IL10, TNFa, IL6, IL17a, and CCL11 in eyelid tissue.

RESULTS. 1κBζ−/− mice exhibited a severe inflammatory phenotype on the ocular surface and perioral skin. The inflammatory infiltrates in the perioral skin consisted primarily of CD4+ and CD8+ cells; CD4+ and CD45R/B220+ cells were mainly detected in the conjunctiva. In eyelid and perioral skin tissue, the expression of IL-17a and of Th1 and Th2 cytokines, but not of CCL11, was augmented. 1κBζ−/− and 1κBζ+/− mice did not differ significantly in their serum total IgE levels before, 0 to 4 weeks, and 5 to 9 weeks after disease onset. 1κBζ/Stat6 WKO mice showed the same or slightly more severe inflammation than did 1κBζ+/− mice.

CONCLUSIONS. IgE and Stat6 are not responsible for the immune pathologic response leading to the development of ocular surface and perioral skin inflammation in 1κBζ−/− mice. 1κBζ−/− mice may be a suitable model for Stevens-Johnson syndrome, but not for atopic dermatitis. (Invest Ophthalmol Vis Sci. 2008;49:3387–3394) DOI: 10.1167/iovs.08-1691

1κBζ (also known as MAIL and INAP) is an ankyrin-repeat containing nuclear protein that is highly homologous to the IκB family member Bcl3.1–3 1κBζ was originally reported to be a regulator of transcription factor NF-κB, which is strongly induced by interleukin (IL)-1 and lipopolysaccharide (LPS).1–4 1κBζ, induced by diverse PAMPs (pathogen-associated microbial products), such as peptidoglycan (PGN), bacterial lipoprotein, flagellin, MALP-2, R-848, and CpG DNA,5 regulates NF-κB activity, possibly to prevent the excessive inflammation caused by bacterial components.3,6

We have reported that 1κBζ−/− mice with a 129/Ola×C57BL/6 background expressly exhibit severe, spontaneous ocular surface inflammation accompanied by the eventual loss of almost all goblet cells and suggested that 1κBζ participates in the negative regulation of ocular surface inflammation.6 We also proposed 1κBζ−/− mice as a suitable model for Stevens-Johnson syndrome (SJS), an ocular surface inflammatory disease, because they manifest the loss of goblet cells that occurs in human SJS.6

Another group reported that MAIL (molecular-possessing ankyrin repeats induced by LPS, equal to 1κBζ−/−) mice, also from a 129/Ola×C57BL/6 background, represent a valuable new animal model for research on atopic dermatitis, because these animals were affected by allergic dermatitis.7

The inflammatory phenotypes of previously reported 129/Ola×C57BL/6 background 1κBζ−/− mice were not uniform, and there were individual variations. For example, although all 1κBζ−/− mice manifested ocular surface and perioral skin inflammation, only some developed dermatitis in the perioral area, neck, or ventral trunk.7,7 To analyze the pathophysiological role of 1κBζ, we established BALB/c background 1κBζ−/− mice.

STAT6 is a critical transcriptional factor that regulates IL-4-mediated Th2 immune responses.8,9 It is phosphorylated and activated through an IL-4R-mediated signal. It translocates as a phosphorylated homodimer and subsequently regulates IL-4-mediated transcriptional events, including Th2 differentiation and Ig class switching to IgE. IL-4-mediated STAT6 activation is an efficient cascade for the generation of Th2 cells during primary T-cell activation. The disruption of the STAT6 gene in mice has revealed its requirement for the development of Th2 cells and Th2-specific immune responses, such as IgE hyperproduction and atopic bronchial asthma.10,11

To address the functional relevance of Th2-mediated immune responses in the development of ocular surface inflammation and dermatitis in 1κBζ−/− mice, we created mice lacking both 1κBζ and Stat6 (1κBζ/Stat6 WKO) that are not able to produce Th2 cytokines, such as IL-4.9
**MATERIALS AND METHODS**

**BALB/c Background IκBζ KO Mice and IκBζ/Stat6 WKO Mice**

BALB/c background IκBζ knockout (KO) mice were produced by back-crossing 129/Ola×C57BL/6 IκBζ KO mice with BALB/c mice for six generations. For genotyping we used genomic DNA isolated from the tail of 2- to 3-week-old heterozygous parents (DNase1 kit; Qiagen, Valencia, CA). PCR amplification was as previously reported.6 Briefly, PCR amplification on a thermal cycler (GeneAmp: Applied Biosystems, Foster City, CA), with the IκBζ gene primer pair for wild IκBζ, IκBζ-wild (GTCTCATCCAGCTTACACTGAACAGTGT), and IκBζ-ex03 (GTTTAAAGTGGGCTTCTGGCTTGGT) resulted in an 1200-bp fragment from wild-type (IκBζ+/−) mice. The gene primer pair for the inserted neomycin gene, IκBζ-ex03, and PKG-rc2 (CTAAAGCGGATGTCTGCA-GACTGGCTTG) yielded an 1200-bp fragment from the homozygotes (IκBζ−/−). Both fragments were obtained from the heterozygotes (IκBζ+/-).

BALB/c background IκBζ/Stat6 WKO mice were produced by mating BALB/c background IκBζ−/− Stat6 KO mice obtained from BALB/c background IκBζ−/− mice and BALB/c background Stat6 KO mice. To genotype the Stat6 gene, we performed PCR amplification with the Stat6 gene primer pair for wild Stat6, as previously reported.9 The use of primer A, specific for the targeted Stat6 gene (TCACTGGGGAGCGGATACGGATCCTG), and primer B, specific for the Stat6 gene downstream of the targeting construct (GGCTGACTGCTGGCTCATACACATTA), resulted in an 1300-bp fragment from wild-type (Stat6+/+) mice. The gene primer pair for the inserted neomycin gene, primer B and primer C, which is specific for the neo resistance gene (ATGCCTCTCATTGCTGCTGACAGG), yielded an 1300-bp fragment from homozygotes (Stat6−/−). Both fragments were obtained from heterozygotes (Stat6+/-). All studies were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Histologic Analysis**

The whole eyeball, together with the eyelids, conjunctiva, and perioral skin of the mice, were fixed in 10% neutral buffered formalin. Fixed tissues were then embedded in paraffin, 6-μm sections were cut, and representative sections from each sample were stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) reagent.

**Immunohistologic Analysis**

The whole eyeball, together with the eyelids, conjunctiva, and perioral skin of the mice, was embedded in OCT compound (Sakura Finetek, Torrance, CA) and then flash frozen in liquid nitrogen. Sections (6 μm thick) were cut and fixed with 100% acetone at 4°C for 10 minutes and blocked (30 minutes) with 10% normal donkey serum in phosphate-buffered saline (PBS). The rat monoclonal antibody was reactive with mouse CD45R/B220 (BD Biosciences, San Diego, CA); mouse CD4 (BD Biosciences), and mouse CD8 (eBioscience, San Diego, CA). The rat IgG2a isotype (BD Biosciences) was used for the negative control. The secondary antibody (biotin-SP-conjugated AffiniPure F(ab')2 fragment donkey anti-rat IgG(H+L), 1:500 dilution; Jackson ImmunoResearch, West Grove, PA) was applied for 30 minutes. The ABC reagent (Vectorstain; Vector Laboratories, Inc., Burlingame, CA), for increased sensitivity, and peroxidase substrate solution (DAB substrate kit; Vector Laboratories) were used as chromogranin substrates.

**Measurement of Total IgE in Serum**

Serum from collected blood was separated by centrifugation at 1000g for 10 minutes. Serum total IgE levels were assessed by ELISA using a mouse IgE ELISA (OptEIA; BD Bioscience) according to the manufacturer’s recommendation.

**Quantitative RT-PCR**

The upper and lower eyelids were collected and homogenized in liquid nitrogen. Total RNA was extracted (RNasy mini kit; Qiagen, Tokyo, Japan) and treated with DNase1 (DNase1 kit; Qiagen) to remove any residual genomic DNA. Reverse transcription (SuperScript Preamplification System; Invitrogen) followed by quantitative PCR was performed on a StepOne Plus (Prism 7700; Applied Biosystems [ABI], Foster City, CA), according to published procedures.9 The primers and probes for mouse IκBζ, CCL11, TNF-α, IFN-γ, IL-4, IL-10, IL-6, IL-17α, and mouse GAPDH were from ABI. The quantification data were normalized to the expression of the housekeeping gene GAPDH.

**RESULTS**

**Macroscopic Observations on IκBζ−/− Mice**

To analyze the pathophysiological role of IκBζ we established BALB/c background IκBζ−/− mice. The phenotype of these mice is uniform, and there are no individual differences, although the phenotype of 129/Ola×C57BL/6 background IκBζ−/− mice varies, and there are individual differences. The ratio of wild-type (+/+) to heterozygous (+/−) to homozygous (−/−) mutant mice born from heterozygous intercrosses was 107:220:33 (1.2:0.3:1), indicating that 70% of IκBζ−/− embryos died in utero. The birth ratio of IκBζ−/− mice is higher than that of 129/Ola×C57BL/6 background mice (1:1.7:0.1).4 None of the IκBζ−/− or IκBζ+/− mice exhibited symptoms of ocular surface- or skin inflammation until the age of 32 weeks (data not shown).

IκBζ−/− mice manifested a severe inflammatory phenotype on the ocular surface, especially along the eyelids, and on the perioral skin. Kinetic monitoring of the inflammatory phenotype in the eyes and perioral skin revealed that the phenotype was absent at the time of birth. When these mice were between 4 and 6 weeks of age, the inflammatory phenotype of the eyelids became evident. Its appearance was followed by inflammatory symptoms in the perioral skin and became more severe as the animals grew older (Fig. 1A). Their severe eyelid inflammation was characterized by eyelid swelling, alopecia, and abnormal hair growth. No inflammation appeared on the abdominal and dorsal skin (data not shown). Severe perioral skin inflammation in these mice was characterized by erythema with excoriations and partial hair loss. Their dermatitis gradually progressed during the observation period until 32 weeks and resulted in lichenified chronic dermatitis. No morphologic or behavioral abnormalities were evident.

**Histologic Analysis of IκBζ KO Mice**

Histologic analysis of the perioral skin of IκBζ−/− mice at 6 weeks of age, 2 weeks after symptom onset, revealed hyperplasia and spongiosis in the epidermis, including the hair follicles, inter- and intracellular edema in the epidermis, and heavy infiltration of the dermis by inflammatory cells (Fig. 1B). Histologically, the abdominal and dorsal skin of IκBζ−/− mice did not manifest this inflammatory phenotype (data not shown). In the perioral skin of IκBζ−/− mice of the same age, we detected neither obvious pathologic changes nor infiltrated inflammatory cells (Fig. 1B). Before the manifestation of inflammation, the perioral skin of IκBζ−/− mice exhibited no distinct histologic changes (data not shown).

Histologic analysis of the eyes of the same IκBζ−/− mice, performed 2 weeks after symptom onset, showed heavy infiltration by inflammatory cells into the submucosal area of the conjunctiva. Moreover, there was a severe decrease in the number of goblet cells in conjunctival epithelia (Fig. 1C). Similar to the perioral skin, histologic analysis of the perioral skin (eyelids) of the same IκBζ−/− mice revealed hyperplasia.
and spongiosis in the epidermis, inter- and intracellular edema in the epidermis, and infiltration of the dermis by inflammatory cells (data not shown). Neither obvious pathologic changes nor infiltrated inflammatory cells were detected in the eyes of IκBζ−/− mice of the same age (Fig. 1C). Before the manifestation of inflammation, the periocular skin and conjunctiva of IκBζ−/− mice exhibited no distinct histologic changes (data not shown). However, within 1 week after symptom onset, the infiltration of inflammatory cells into the conjunctival epithelia was evident, and we observed a moderate loss of goblet cells (Fig. 1C), possibly as a consequence of inflammatory cell infiltration. No pathologic changes were evident in other eye compartments such as the lens, retina, uvea, and sclera of the IκBζ−/− mice (data not shown), or in other tissues such as the thymus, spleen, liver, kidney, lung, small intestine, large intestine, and brain (data not shown).

**Immunohistologic Analysis of the Ocular Surface and Perioral Skin of IκBζ KO Mice**

The inflammatory infiltrates in the perioral skin of 10-week-old IκBζ−/− mice (6 weeks after symptom onset) consisted primarily of CD4+ and CD8+ cells (Fig. 2). A few CD45R/B220+ cells were present (Fig. 2). CD8+ cells infiltrated the outer sheet of
hair follicles, and CD4\(^+\) cells infiltrated between the hair follicles (Fig. 2). Moreover, CD4\(^+\) and CD8\(^+\) cells infiltrated not only the dermis but also the epidermis. These cells were not detected in the perioral skin of I\(/H9260\)B\(/H9256\)/H11001/H11002 mice.

On the other hand, the inflammatory infiltrates in the subconjunctival tissue of the eyelids of the same mouse were mainly composed of CD4\(^+\) and CD45R/B220\(^+\) cells. CD8\(^+\) cells were detected in conjunctival epithelium, but not in subconjunctival tissue (Fig. 3). Moreover, like their perioral skin, the dermis of the eyelids of I\(/H9260\)B\(/H9256\)/H11002 mice was infiltrated by many CD4\(^+\), some CD8\(^+\), and a few CD45R/B220\(^+\) cells (Fig. 3). No CD4\(^+\), CD45R/B220\(^+\), or CD8\(^+\) cells were detected in the subconjunctival tissue and eyelids of I\(/H9260\)B\(/H9256\)/H11001 mice.

**Figure 2.** Immunohistologic analysis of the perioral skin of an I\(/B\) KO mouse. Perioral skin tissue sections from 10-week-old I\(/B\) and I\(/B\) mice (6 weeks after symptom onset) were immunohistologically stained for the isotype control (IgG2a) and for CD4, CD8, and CD45R/B220. Boxed areas in (b), (e), (h), and (k) are enlarged in (c), (f), (i), and (l), respectively. Scale bar, 100 µm.

**Figure 3.** Immunohistologic analysis of eye of I\(/B\) KO mice. Eye tissue sections from 10-week-old I\(/B\) and I\(/B\) mice (6 weeks after symptom onset) were immunohistologically stained for the isotype control (IgG2a) and for CD4, CD45R/B220, and CD8. Boxed areas in (a, b) are enlarged in the columns to the right and are in the same position in all of the I\(/B\) images. Scale bar, 500 µm.
Serum Total IgE

The serum total IgE levels of Ixβκ−/− mice were reportedly higher than those of Ixβκ+/− and Ixβκ+/+ mice.7 To confirm this finding, we examined the serum total IgE levels of Ixβκ−/− mice at various time points before and after symptom onset. To our surprise, we detected no significant difference between Ixβκ−/− and Ixβκ+/− mice before, 0 to 4 weeks, and 5 to 9 weeks after disease onset (Fig. 4A). However, at 10 weeks after onset, the serum total IgE level of Ixβκ−/− mice became significantly higher than that of Ixβκ+/− mice (data not shown). This result suggests that the high serum total IgE levels in Ixβκ−/− mice may be secondary to the observed tissue inflammation.

Quantitative RT-PCR of Eyelid Tissue

To elucidate the cytokine milieu of the observed inflammation, we studied the gene expression profiles. We first confirmed the Ixβκ mRNA expression level in eyelid tissues. Although Ixβκ−/− mice did not express Ixβκ mRNA, in Ixβκ+/− mice, the Ixβκ mRNA expression was ~50% of that in Ixβκ+/+ mice (Fig. 4B). The CCL11 mRNA level did not differ significantly among Ixβκ−/−, Ixβκ+/−, and Ixβκ+/+ mice (Fig. 4C). The expression of IFN-γ, IL4, IL10, TNFa, IL6, and IL17α mRNA was upregulated in the eyelids of Ixβκ−/− mice (Fig. 4D). It should be noted that IL6 gene expression was upregulated in the eyelid tissues. Our analysis of perioral skin tissue returned the same results as the eyelid tissue (data not shown). These findings suggest that the eyelid- and perioral skin inflammation of Ixβκ−/− mice is the result of the interplay among the helper T-cell subsets Th17, -1, and -2.

Severe Inflammatory Symptoms in Ixβκ/Stat6 KO Mice

To address the functional relevance of IgE and Th2-mediated immune responses in the development of ocular surface and perioral skin inflammation in Ixβκ−/− mice, we created Ixβκ/ Stat6 WKO mice that were unable to mount the Th2-polarized IL-4-mediated immune response required for IgE class switching. IL4-specific gene expression was actually ablated in Ixβκ/ Stat6 WKO mice, directly indicating the absence of Th2-mediated immune responses (data not shown). To our surprise, in the Ixβκ/Stat6 WKO mice, severe inflammatory symptoms were elicited (Fig. 5A). Moreover, these mice not only presented with severe dermatitis of the facial skin but also of the abdominal skin (Fig. 5A). No obvious dermatitis was seen in Stat6 single-KO mice (Fig. 5A).

Histologic analysis of the perioral skin of 23-week-old Ixβκ/ Stat6 WKO mice, 19 weeks after symptom onset, revealed epidermal hyperplasia, hyperkeratosis, inter- and intracellular edema in the epidermis, and heavy infiltration of the dermis by inflammatory cells. In the perioral skin of Ixβκ−/− Stat6−/− and Ixβκ+/−/Stat6−/− mice we observed slight acanthosis and mild dermal infiltration by inflammatory cells (Fig. 5B). On the other hand, the abdominal skin of Ixβκ/Stat6 WKO, but not of Ixβκ−/− Stat6−/− and Ixβκ+/−/Stat6−/− mice, showed moderate infiltration of the dermis by inflammatory cells (data not shown).

Our analysis of the eyes of the same 23-week-old Ixβκ/Stat6 WKO mice showed heavy inflammatory cell infiltration into the submucosal area of the conjunctiva. Moreover, as in Ixβκ−/− mice, PAS staining revealed a severe decrease in the number of goblet cells in conjunctival epithelia (Fig. 5C). No obvious pathologic change and no inflammatory cell infiltration was detected in the eyes of Ixβκ−/− Stat6−/− and Ixβκ+/−/Stat6−/− mice of the same age (Fig. 5C). The other eye compartments such as the lens, retina, uvea, and sclera of Ixβκ/ Stat6 WKO mice exhibited no pathologic changes, and there was no distinctive difference from Ixβκ−/− Stat6−/− and Ixβκ+/−/Stat6−/− mice (data not shown).

The inflammatory infiltrates in the perioral skin of the 23-week-old Ixβκ/Stat6 WKO mice were mostly CD4+ cells; a few CD8+ and a few CD45R/B220+ cells were present (Fig. 5D). In the perioral skin of Ixβκ/Stat6 WKO mice, CD4+ and CD8+ cells infiltrated not only the dermis but also the epidermis (Fig. 5D). On the other hand, in the perioral skin of Ixβκ−/− Stat6−/− and Ixβκ+/−/Stat6−/− mice, only a few CD4+ cells infiltrated the dermis and epidermis (Fig. 5D). In Ixβκ/Stat6 WKO mice, as in Ixβκ−/− mice, the inflammatory infiltrates in the subconjunctival tissue of the eyelids were CD4+ and CD45R/B220+ cells (data not shown). As their perioral skin, many CD4+, a few CD8+, and few CD45R/B220+ cells infiltrated the dermis of the eyelids of Ixβκ/Stat6 WKO mice (data not shown).

![Figure 4](https://iovs.arvojournals.org/)
DISCUSSION

Unlike 129/Ola×C57BL/6 background mice that exhibited individually varying disease symptoms, BALB/c background IκBζ−/− mice manifested a uniform disease phenotype. This renders them useful for the pathologic investigation of chronic inflammatory symptoms on the human ocular surface. One of the typical pathologic phenotypes confirmed in this study is a concurrent loss in the conjunctival epithelia of goblet cells and the presence of intensive inflammatory infiltrates the submucosa of the conjunctival epithelia, and the dermis and epidermis of the perioral skin. Our findings suggest that IκBζ−/− mice may be a suitable model for Stevens-Johnson syndrome and that these mice may be useful for mimicking the secondary conjunctival inflammation that often occurs in patients with SJS.6

SJS is an acute-onset mucocutaneous disease induced by infectious agents and/or inciting drugs.12-16 The pathobiologic mechanisms underlying the onset of SJS/TEN have not been fully established. Patients with SJS manifest vesiculobullous skin lesions and severe conjunctivitis in the acute stage. Ocular surface complications such as dry eye due to loss of goblet cells persist in the chronic stage.17 Thus, loss of goblet cells is an important ocular surface feature of SJS in the chronic stage.18,19 Moreover, in a patient with conjunctival inflammation due to SJS, CD4+ T-cells were identified in the cell population infiltrating the conjunctivalized tissues over the cor-

**FIGURE 5.** (A) Inflammatory phenotype of IκBζ/Stat6 WKO. The face and ventral trunk of an IκBζ/Stat6 WKO mouse and a Stat6 single-KO mouse. (B) Histologic analysis of the perioral skin of IκBζ/Stat6 WKO mice at 19 weeks after symptom onset. The perioral skin of IκBζ+/− /Stat6−/− (Ba, Bc) and IκBζ/Stat6 WKO (Bb, Bd) mice. Boxed areas in (Ba) and (Bb) are enlarged in (Bc) and (Bd). H&E stains. (C) Histologic analysis of the eyelid of IκBζ+/− /Stat6−/− (Cc, Cg) and IκBζ/Stat6 WKO ( Cf, Ch) mice. H&E (Cc, Cf) and PAS (Cg, Ch) stains. (D) Immunohistologic analysis of the perioral skin of IκBζ KO mice. Perioral skin tissue sections from a 23-week-old IκBζ/Stat6 WKO mouse (19 weeks after symptom onset) were immunohistologically stained for the isotype control (IgG2a) and for CD4 and CD8. Scale bar: (B) 200 μm; (C) 100 μm.
nea.\(^2\) CD4\(^+\) T-cells were also recognized in the ocular surface inflammation of Ix\(_B^\beta\) KO mice.

The almost complete loss of goblet cells in the conjunctival epithelia of Ix\(_B^\beta\)\(^{-/-}\) mice made the ocular surface inflammation in these mice distinct from that previously reported in mice with allergic conjunctivitis, other rodent models of allergic conjunctivitis, and NC/Nga mice with spontaneous atopic dermatitis. Rodent models of allergic conjunctivitis display no change in these cells\(^2\)\(^1\),\(^2\) and NC/Nga mice with spontaneous atopic dermatitis manifest an increase in goblet cell density.\(^2\)\(^4\)

There have been no rodent models showing the spontaneous loss of goblet cells in their conjunctiva as observed in Ix\(_B^\beta\)\(^{-/-}\) mice. Of note, the reduction of goblet cells followed the infiltration of inflammatory cells. Thus, we posit that the observed loss of goblet cells may be a consequence of inflammatory cell infiltration into the conjunctival epithelia of Ix\(_B^\beta\)\(^{-/-}\) mice.\(^6\) On the ocular surface in SJS, inflammation distinct from allergic inflammation may be involved in the loss of goblet cells in the conjunctiva.

Ix\(_B^\beta\) was expressed, not only on the ocular surface, but also in mucosal tissues such as the trachea and small intestine.\(^6\) The inflammatory disorders were recognized in limited tissues of Ix\(_B^\beta\)\(^{-/-}\) mice. In these animals, spontaneous chronic inflammation was selectively elicited on the ocular surface and in perioral skin but not in other tissues. Canker sores, oral mucositis, and ocular surface inflammation may actually be essential to SJS with ocular surface complications in the acute stage.

To the best of our knowledge, there are no reports describing the association between the Ix\(_B^\beta\) gene and SJS. However, in our investigation of the disordered innate immune response in SJS, the gene expression of Ix\(_B^\beta\) by peripheral CD14\(^+\) mononuclear cells of SJS patients was found to be reduced compared with that in healthy control subjects (our unpublished data, 2007). Thus, we speculate that, in part, Ix\(_B^\beta\) gene expression may participate in the onset of SJS.

The members of the NF-\(\kappa B\) family are evolutionarily conserved pleiotropic transcription factors that play a crucial role in many biological processes. A variety of stimuli lead to the phosphorylation of IxBs.\(^2\)\(^4\) Unlike typical IxB proteins, Ix\(_B^\beta\) rather stably accumulates in the nucleus. In line with its function as a negative regulator of NF-\(\kappa B\), a proapoptotic effect of Ix\(_B^\beta\) has been reported; it antagonizes the antiapoptotic function of NF-\(\kappa B\).\(^7\)\(^9\) The Toll-like receptor-mediated production of IL-6 was inhibited in Ix\(_B^\beta\)-deficient macrophages.\(^5\) We found that compared with Ix\(_B^\beta\)\(^{-/-}\) and Ix\(_B^\beta\)\(^{+/+}\) mice, in the eyelids of Ix\(_B^\beta\)\(^{-/-}\) mice, the mRNA expression of IL-6 mRNA was dramatically increased, as was the expression of TNF-\(\alpha\), IL-10, IL-4, IFN-\(\gamma\), and IL-17\(\alpha\). This finding suggests that Ix\(_B^\beta\) exerts regulatory effects selectively not only on cytokines through NF-\(\kappa B\), but also in a tissue- or cell-type-specific manner (spatially orchestrated regulation). The regulation of NF-\(\kappa B\) varies among distinct mucous tissues.\(^8\)

Th1 T-cells can mediate proinflammatory or cell-mediated immune responses, whereas Th2 T-cells promote immediate-type hypersensitivity reactions. Th1 and Th2 responses are often considered to be mutually exclusive. However, there is a growing body of evidence suggesting that interactions between Th1 and Th2 immune elements are not solely antagonistic, but may in fact modulate the immune response in a much more exquisite way.\(^7\)\(^9\)\(^2\)\(^0\) These new findings and considerations should be integrated to explain the observed elevation of Th1/Th2 cytokine genes in the eyelid tissues of Ix\(_B^\beta\)\(^{-/-}\) mice and to gain a better understanding of the inflammatory disease observed in these animals.

Our finding that in Ix\(_B^\beta\)/Stat6 WKO mice, severe inflammation comparable to that observed in Ix\(_B^\beta\)\(^{-/-}\) mice was elicited, indicates that Stat6 and Th2 immunity is not causally related to the development of ocular surface- and perioral skin inflammation in Ix\(_B^\beta\)\(^{-/-}\) mice. Allergic reactions acting through IL-4 receptors involve a group of signal transducers and activators of transcription (Stat) proteins.\(^8\) Stat6 appears to have the most prominent role during IL-4-mediated responses, including Th2 differentiation and immunoglobulin-class switching to IgE.\(^8\)

With respect to the severe inflammation observed in Ix\(_B^\beta\)/Stat6 WKO mice, it is notable that Stat6, despite its marginal additive effect, may also operate as a negative regulator of proinflammatory mediator production.

In the eyelids and perioral skin of Ix\(_B^\beta\)\(^{-/-}\) mice, we observed the augmented gene expression of IL-17\(\alpha\), but not of CCL11. The immune pathologic response on the ocular surface and perioral skin of Ix\(_B^\beta\)\(^{-/-}\) mice may not be solely dependent on the Th2 response. There may be a possibility that the Th17 response, which is elicited by IL-17\(\alpha\), is involved in the immune pathologic response on the ocular surface and perioral skin of Ix\(_B^\beta\)\(^{-/-}\) mice. We have begun detailed studies on spatial and temporal regulations, to identify the mechanism(s) that underlies the inflammation on ocular surface- and perioral skin tissues.

Intestinal epithelial cell-specific inhibition of NF-\(\kappa B\) spontaneously causes severe chronic intestinal inflammation in mice.\(^2\)\(^9\)\(^3\)\(^0\) Thus, the transcription factor NF-\(\kappa B\), a master regulator of proinflammatory responses, functions in gut epithelial cells to control epithelial integrity and the interaction between the mucosal immune system and gut microflora. Of interest, Ix\(_B^\beta\) transcript is predominantly distributed in the epithelia of the ocular surface.\(^6\) Considering the growing body of evidence suggesting the interactions among the Th1,-2, and -17 immune elements, the sole participation of Th1,-2, or -17 may not be plausible, but the interaction among them may be probable in collaboration with TNF-\(\alpha\) and the newly emerging cytokine IL-17\(\alpha\). Deregulated NF-\(\kappa B\)-activity in Ix\(_B^\beta\)-gene-targeted mice may lead to deregulated homeostasis of conjunctival epithelial cells. The defect may trigger a chronic inflammatory response, initially by innate immune cells but also later by acquired immune responses. Studies are under way in our laboratory to identify the precise molecular mechanisms of Ix\(_B^\beta\)-mediated transcriptional regulation in efforts to gain a better understanding of the immune/inflammatory axis in Ix\(_B^\beta\) gene-disrupted mice.

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References


