Original Article

Blockade of IL-27 signaling ameliorates herpes stromal keratitis with upregulated CD4$^+$ Foxp3$^+$ regulatory T cells influx in mice

Likun Xia, Tianchang Tan, Yang Li, Qiuyue Zhong, Mei Shi

Purpose: The purpose of this study was to investigate the production of IL-27 p28 and EBI3 in the ocular inflammatory sites, and the role of IL-27 signaling in a model of HSV-1 induced herpetic stromal keratitis (HSK). **Methods:** The BALB/c mice were injected intraperitoneally (24 h before infection) with anti-IL-27 antibody or IgG antibody as control, infected with HSV-1 via corneal scarification, and then injected intraperitoneally with anti-IL-27 antibody or IgG antibody at 1, 3, and 5 days postinfection. Slit lamp and histopathology were used to assess disease outcome. The levels of IL-27 p28 and EBI3 in corneas were determined by western blotting and immunofluorescence. Furthermore, viral titers were determined, and immune cell infiltrates were collected and analyzed by flow cytometry. **Results:** We found that the levels of IL-27 p28 and EBI3 in corneas were elevated significantly at the peak of HSK, and both of them were expressed simultaneously in the epithelium, stroma, and endothelium of corneas. In the group of anti-IL-27 treatment, the severity of the corneal lesion and CD4$^+$ T cells infiltration were significantly decreased, and the percentage of CD4$^+$ Foxp3$^+$ Tregs was upregulated markedly in the spleen, DLNs and cornea of HSK mice compared to IgG treatment. **Conclusion:** These results provided evidence that IL-27 as a pathogenic pro-inflammatory cytokine controlled CD4$^+$ Foxp3$^+$ Tregs production in HSK, which ultimately resulted in promoting the progression of HSK and poor prognosis.

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Herpes stromal keratitis (HSK), a chronic immuno-inflammatory reaction in the cornea, caused by herpes simplex virus type 1 (HSV1) infection, is one of the major causes of infectious blindness in developed countries.[1,2] Outstanding clinical manifestations of HSK include stroma opacity and neovascularization, leading to scarring and permanent loss of vision.[3] In animal models, studies have indicated that the corneal stromal lesions are orchestrated by CD4$^+$ T cells principally.[4-6] Lesions severity are affected by the balance of different CD4 T cell subsets. Th1 cells which lead to delayed-type hypersensitivity (DTH) are seen to be more predominant in HSK than Th2 cells.[7,8] As our previous study, Th17 cells also infiltrate to the HSV-infected cornea and possibly are involved in HSK pathogenesis.[9-11] Moreover, recent studies have demonstrated that CD4$^+$ regulatory T cells (Tregs) are present in the ocular inflammatory sites and play a critical role in controlling HSK severity in mouse models.

Interleukin-27 (IL-27), an IL-12 superfamily cytokine composed of the p28 and Epstein–Barr-virus-induced gene 3 (EBI3) subunits, is recently appreciated to be a multifaceted heterodimeric cytokine with pronounced pro- and anti-inflammatory as well as immunoregulatory functions.[12,13] Early studies have demonstrated that IL-27 acts as a pro-inflammatory cytokine, inducing proliferation of human and mouse naïve CD4$^+$ T cells, and driving naïve T cells into Th1 cells.[12,14,15] Nevertheless, subsequent reports have indicated that IL-27 also has profound anti-inflammatory effects and well-characterization effects in controlling infection due to its ability to inhibit Th1 and Th17 responses.[16-19] Of note, more recent studies have shown that the dual role of IL-27 in Tregs is due to the suppression of the formation of Tregs by IL-27[20,21] and the inhibitory effect of Tregs by IL-27 under certain circumstances.[22-24]

However, the effect of IL-27 on HSK diseases remains unclear. In this study, we demonstrated that blocking IL-27 signaling during the clinical period of the disease with an anti-IL-27 antibody made the severity of HSK ameliorated. Furthermore, we explored the underlying mechanisms of IL-27 as a pro-inflammatory factor in an ongoing inflammatory condition.

Methods

Mice

Specific-pathogen-free female BALB/c Mice, 5 to 7 weeks old, weighing 20–24 g each, were purchased from Liaoning Changsheng Biotechnology Company (Benxi, China). They were housed under standard conditions with food and

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water available *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of China Medical University.

Corneal HSV-1 infection and viral titration

HSV-1 KOS strain was propagated and titrated on VERO cells using standard protocols. To establish an HSK model, mice were anesthetized, and the corneal epithelium of the right eye was abraded in a cross-shaped pattern with a sterile 27-gauge needle using a dissecting microscope according to the previous description. A 5 µl volume containing 1 × 105 PFU of HSV-1 KOS was then dropped onto the corneal surface.[25] Mock-infected control mice were abraded and dropped with VERO cells culture medium. To detect HSV-1 load in infected corneas, swabs were collected from the infected corneal surface, put it into sterile tubes, and then kept frozen at -80°C on day 1, 3, and 5 post-infection. Finally, the swabs were plated onto the monolayer of VERO cells, which were then monitored for cytopathic effects 48 and 96 h later. Then viral titers were calculated through plaque counting and expressed as PFU/ml according to previously described.[25-27]

Western blotting

The HSV-1 uninfected mice and infected mice were terminated on day 14 postinfection, and their corneas were aseptically removed. The total corneal proteins were extracted, and protein concentration was determined as previously described.[11] Equal amounts of protein (50 µg) were separated by 10% SDS-PAGE and then transferred electrophoretically onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, United States). After blocking, the membranes were incubated with the following primary antibodies overnight at 4°C: IL-27 p28 (1:3000, R and D System), or against EBI3 (1:10000, Abcam) or against GAPDH (1:15000; Abcam). Blots were probed with a horseradish peroxidase-conjugated secondary antibody, and an enhanced chemiluminescence (ECL) kit (Amersham) was utilized to visualize the membrane. Densitometry analysis was performed using ImageJ 6.0 software.

Immunofluorescence assay

On the 14th day after corneal HSV-1 infection, corneal slides were prepared as previously described.[11] For immunofluorescence staining of IL-27 p28 and EBI3 in the cornea, cryostat slides were permeabilized with 0.5% (v/v) Triton X-100 for 20 min at room temperature and blocked with 5% bovine serum albumin (Sigma) for 30 min. And then the slides were incubated with primary antibodies (goat anti-mice IL-27 p28 antibody, rabbit anti-mice EBI3 antibody, and PBS) at 4°C for 24 h. Subsequently, slides were incubated with secondary antibody (donkey anti-goat IgG, donkey anti-rabbit IgG) at 37°C for 3 h. The slides were stained with DAPI (Invitrogen) to visualize the nuclei. Finally, using a fluorescent microscope to view these immunolabeled slides. Above antibodies were purchased from R and D System or Abcam.

Anti-IL-27 antibody administration and clinical observations

Mice were administrated intraperitoneally with 100 µg of anti-IL-27 antibodies (R and D Systems) in 200 µl PBS. The first injection was performed 24 h before ocular infection, followed by three additional injections on days +1, +3, and +5 relative to corneal HSV-1 infection. Control mice were injected with 100 µg of isotype control rat IgG in 200 µl PBS. Each group included 12 animals. The clinical severity of keratitis for individually scored mice was recorded by slit-lamp examination on different days postinfection.[25,28]

Flow cytometry analysis

Corneas, cervical draining lymph nodes (DLNs), and spleens from anti-IL-27- or IgG-treated mice were harvested on day 14 postinfection. Single-cell suspensions from individual mouse corneas, DLNs, and spleens were prepared as described previously.[25] Antibodies used for flow cytometry were purchased from BD Pharmingen. Antibodies included an unconjugated anti-CD32/CD16, anti–CD3e-FITC, anti–CD4-PerCP-Cy5.5, anti-IL-17A-PE, anti-IL-10-PE, anti-IFN-γ-PE, anti-FoxP3-APC, and isotype control antibodies. Briefly, mononuclear cells and splenocytes surface and intracellular staining were accomplished according to the manufacturer's instructions. After staining, the samples were washed in staining buffer and analyzed on a FACS-Calibur flow cytometer (BD Bioscience).

Histopathology

IgG-treated and anti-IL-27 treated mice were terminated on the 14th day after corneal HSV-1 infection. The eyes were taken off and put them in 4% formaldehyde solution fixed. The pathological changes of corneal tissue were observed by H and E staining following paraffin embedding and sectioning.

Statistical analysis

All experiments were performed twice. Data were expressed as mean ± standard deviation (SD). Differences between experimental groups were analyzed using the Student's *t*-test. $P < 0.05$ was considered statistically significant. GraphPad Prism software (GraphPad Software, Inc, La Jolla, CA) was used for statistical analysis.

Results

Expression levels of IL-27 p28 and EBI3 protein are elevated in the cornea of HSK mice

In the model of HSK, ocular infection with HSV-1 initiated in epithelia. The epithelial defects were extremely evident at 3 days. Repair occurred rapidly, and the epithelial lesion was no longer evident on day 6 after infection. However, beginning at 7–8 days after infection, the stromal opacity and edema of the cornea (called HSK) became evident and persisted. Severe stromal keratitis peaked on day 14 after infection. To understand the possible functional relevance of IL-27 in HSK, the first step was to determine whether IL-27 p28 and EBI3 were expressed in the cornea of HSK mice when corneal stromal keratitis peaked. As the western blotting results presented in Fig. 1a, both IL-27 p28 and EBI3 levels were significantly elevated in the cornea of HSK mice compared to control mice. Accordingly, immunofluorescence staining showed that neither IL-27 p28 nor EBI3 subunit was found in the uninfected cornea. However, both IL-27 p28 and EBI3 subunit were expressed simultaneously in the corneal epithelium, stroma, and endothelium of HSK mice [Fig. 1b]. These results indicate that the expression levels of IL-27 protein are significantly increased at the peak of corneal inflammation.

Administration of anti-IL-27 antibody decreases the severity of HSK and inhibits CD4$^+$ T Cells infiltration in infected corneas

To evaluate whether IL-27 has a role in mediating HSK immunopathology, the anti-IL-27 or IgG control antibodies were used to treat BALB/c mice in an HSK model. The severity of HSK lesions was determined by slit-lamp biomicroscopy,

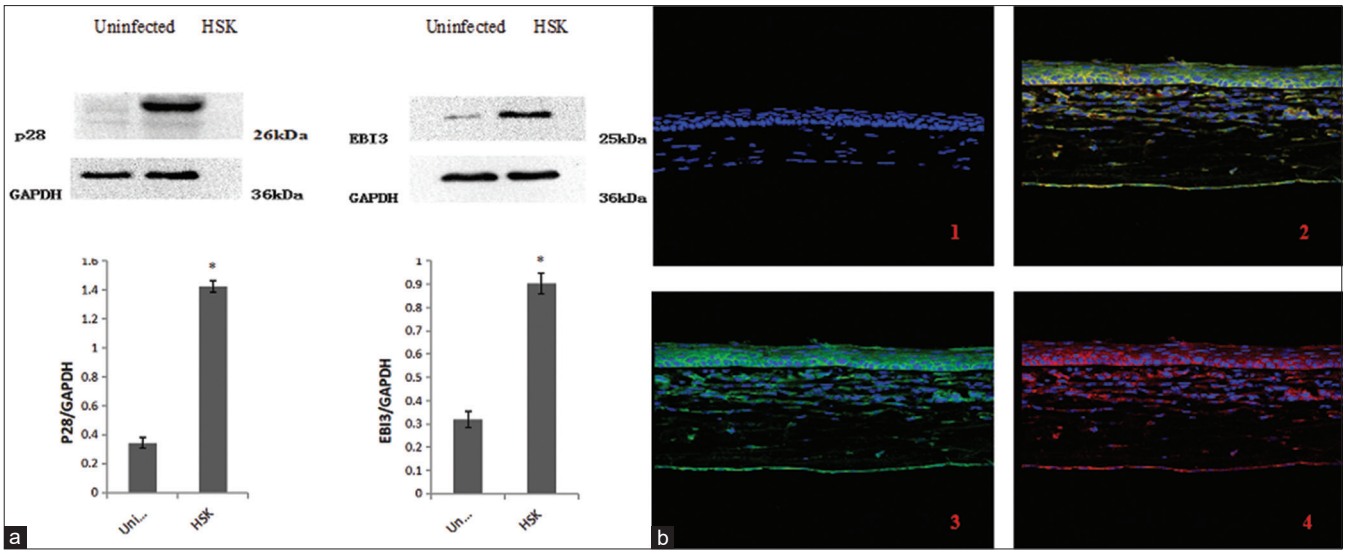

Figure 1: Expression of IL-27 p28 and EBI3 protein in the cornea of HSK mice following corneal HSV-1 infection. (a) Expression of IL-27 p28 and EBI3 protein in the corneas was assessed by western blot analysis. **P* < 0.01 indicates differences between HSK mice and uninfected mice (*n* = 6 in each group). (b) The corneal cryostat sections were immunostained with goat anti-mice IL-27 p28 antibody and donkey anti-goat IgG (green), and/or with rabbit anti-mice EBI3 antibody and donkey anti-rabbit IgG (red). Nuclei were counterstained with DAPI (blue fluorescence). (1) Neither IL-27 p28 nor EBI3 protein was expressed in the cornea of uninfected mice, only nuclei are shown in blue, (2) Both IL-27 p28 and EBI3 subunit were expressed simultaneously in the corneal epithelium, stroma, and endothelium of HSK mice (yellow-green fluorescence), (3) IL-27 p28 protein was shown in green, and (4) EBI3 protein was shown in red. Original magnification, ×400

and the clinical severity score of stromal keratitis mice was recorded individually over 14 days after corneal HSV-1 infection. As shown in Fig. 2a, anti-IL-27 treated group showed the decreased corneal lesion compared to IgG control group, with apparent differences on days 8, 10, 12, and 14 ($P < 0.01$). Fig. 2b depicts the corneal opacity score of per individual BALB/c mice eye each group on the 14th-day post infection. 11 of 12 IgG-treated eyes developed lesion severity scores 3 or 4, with a mean corneal opacity score of 3.25. In contrast, 10 of 12 anti-IL-27-treated eyes had mild opacity scores 1 or 2, with a mean corneal opacity score of 1.17. The typical eye pictures of the two groups] showed the severity of corneal opacities on day 14 post-infection [Fig. 2c]. Histological examination] showed that the reduced inflammatory reactions in the infected corneas treated by anti-IL-27 antibody compared to those treated by IgG control antibody [Fig. 2d]. In other experiments, corneal samples were collected from six randomly selected eyes taken from different treatment groups at 14 days post-infection to prepare single cell suspensions following collagen digestion for phenotypic analysis by flow cytometry. To determine whether the reduction of inflammatory reactions in the corneas of anti-IL-27 treated mice had functional consequences in terms of impacting the migration of infiltrating cells, the frequency and cell numbers of CD4$^+$ T cells in the cornea was analyzed. As is shown in Fig. 2e-h, the total number of viable cells and CD4$^+$ T cells were significantly lower in the anti-IL-27 group compared to isotype control. These results indicated that along with the decreased severity of virus-induced corneal disease, the CD4$^+$ T cell immune response in the cornea of anti-IL-27 treated mice was also reduced.

Anti-IL-27 treatment does not affect the expression of IL-17, IFN-γ, and IL-10 in the cervical DLNs of HSK mice

In previous studies including infectious disease and autoimmune disease have confirmed that IL-27 played the pro- or anti-inflammatory role during immune response mediated by Th1, Th2, and Th17. To determine the mechanism of anti-IL-27 protection in HSK, we first checked the effects of anti-IL-27 treatment on the expression of major inflammatory factors such as IL-17, IFN-γ, and IL-10. Lymphocytes were isolated from the cervical DLNs of IgG-treated or anti-IL-27-treated mice at the peak of HSK (on the 14th-day post-infection), and the expression of IL-17, IFN-γ, IL-10 was analyzed on lymphocytes from each group by flow cytometry. The results showed that there was no difference in the percentage of lymphocytes expressing IFN-γ-, IL-10-, IL-17 between the anti-IL-27-treated group and the IgG-treated group [Fig. 3].

Anti-IL-27 treatment upregulate the frequencies of CD4$^+$ Foxp3$^+$ Tregs in the spleen, DLNs, and cornea of HSK mice

Recent studies by others have shown that CD4$^+$ Foxp3$^+$ Tregs are present in the ocular inflammation site which may help limit the severity of HSV-induced immunopathological lesions in the cornea and more recent work has described the dual influence of IL-27 on Tregs populations. As a consequence, we investigated the function of anti-IL-27 treatment on CD4$^+$ Foxp3$^+$ Tregs in the BALB/c mouse model of HSK. On day 14 after HSV-1 infection, IgG-treated and anti-IL-27-treated mice were terminated, and single-cell suspensions of spleens, cervical DLNs and corneas were prepared for flow cytometry. As shown in Fig. 4, flow cytometric analysis demonstrated higher frequencies of Foxp3$^+$ Tregs in the spleen (18.69%), DLNs (35.86%) and cornea (73.12%) of anti-IL-27-treated mice compared to those of IgG-treated mice (10.32%, 11.65%, and 40.23%, respectively). Anti-IL-27-treatment significantly increased the percentage of CD4$^+$ Foxp3$^+$ Tregs on an absolute basis compared to those of IgG-treated mice. These findings

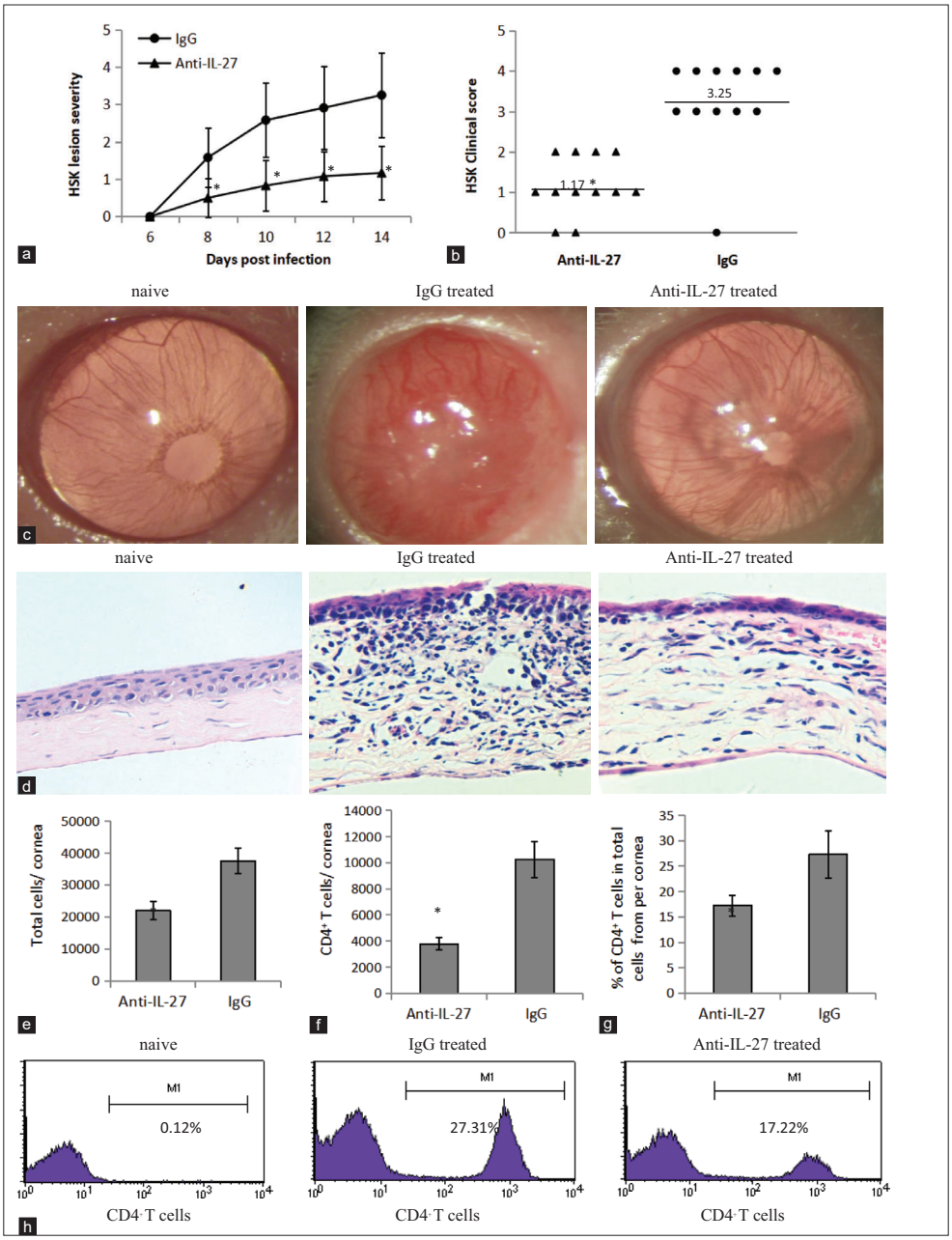

Figure 2: Anti-IL-27 treatment reduces the severity of HSK and CD4⁺ T cell infiltrations in cornea. BALB/c mice were infected with 105 PFU HSV-1 (KOS). BALB/c mice received an intraperitoneal injection of 100 μg anti-IL-27 antibodies or IgG isotype control on days -1, +1, +3, and +5 relative to the viral infection. HSK lesion severity was determined by slit-lamp biomicroscopy, and the clinical severity of stromal keratitis of individually scored mice was recorded. Stromal keratitis was graded from 0 to 4+, depending on the corneal opacity with neovascularization, edema, and infiltration. Each group of mice consisted of twelve animals, and the results shown are the representative of two similar experiments. (a) The average scores of twelve mice per group over a period of 14 days post infection. Data are recorded as mean±SEM. Anti-IL-27 treated group showed the decreased corneal lesion compared to IgG treated group, with significant differences occurring on days 8, 10, 12 and 14 (*P<0.01). (b) Each dot represents corneal opacity of an individual BALB/c eye on the 14th day following HSV-1 infection. Crossbar indicates the mean. *P<0.01 vs IgG treated group. (c) Pictures representative for naïve eye, IgG treated eye, and anti-IL-27 treated eye were taken at day 14 post infection. (d) The typical histological findings of cornea stained with hematoxylin and eosin by day 14 after infection were shown. The naïve mouse showed normal corneal tissue. The cornea of the IgG treated group exhibited severely swollen, heavily infiltrated with inflammatory cells, and numerous neovascular tissues in the stroma. The cornea of anti-IL-27 treated group showed decreased stromal swelling, few inflammatory cell infiltration, and less neovasculars in the stroma. Original magnification, 400× (e) Six corneal samples from each group of treated mice were liberase digested at day 14 postinfection. The bars represent the total number of viable cells present per cornea of two groups. *P<0.01 compared with IgG-treated group. (f) The cells isolated from infected corneas were stained for CD4 marker and the bars represent the total number of CD4⁺ T cells present per cornea from two groups of mice. Decreased number of CD4⁺ T cells in the cornea of anti-IL-27 treated mice (*P<0.01 compared with IgG-treated group). (g) The percentage of CD4⁺ T cells in total cells of per cornea from two groups. Reduced percentage of CD4⁺ T cells in the cornea of anti-IL-27 treated mice (*P<0.01 compared with IgG treated group). (h) Representative plot denotes the percentage of inflammatory cells expressing CD4⁺ T cells markers on cornea of different group mice

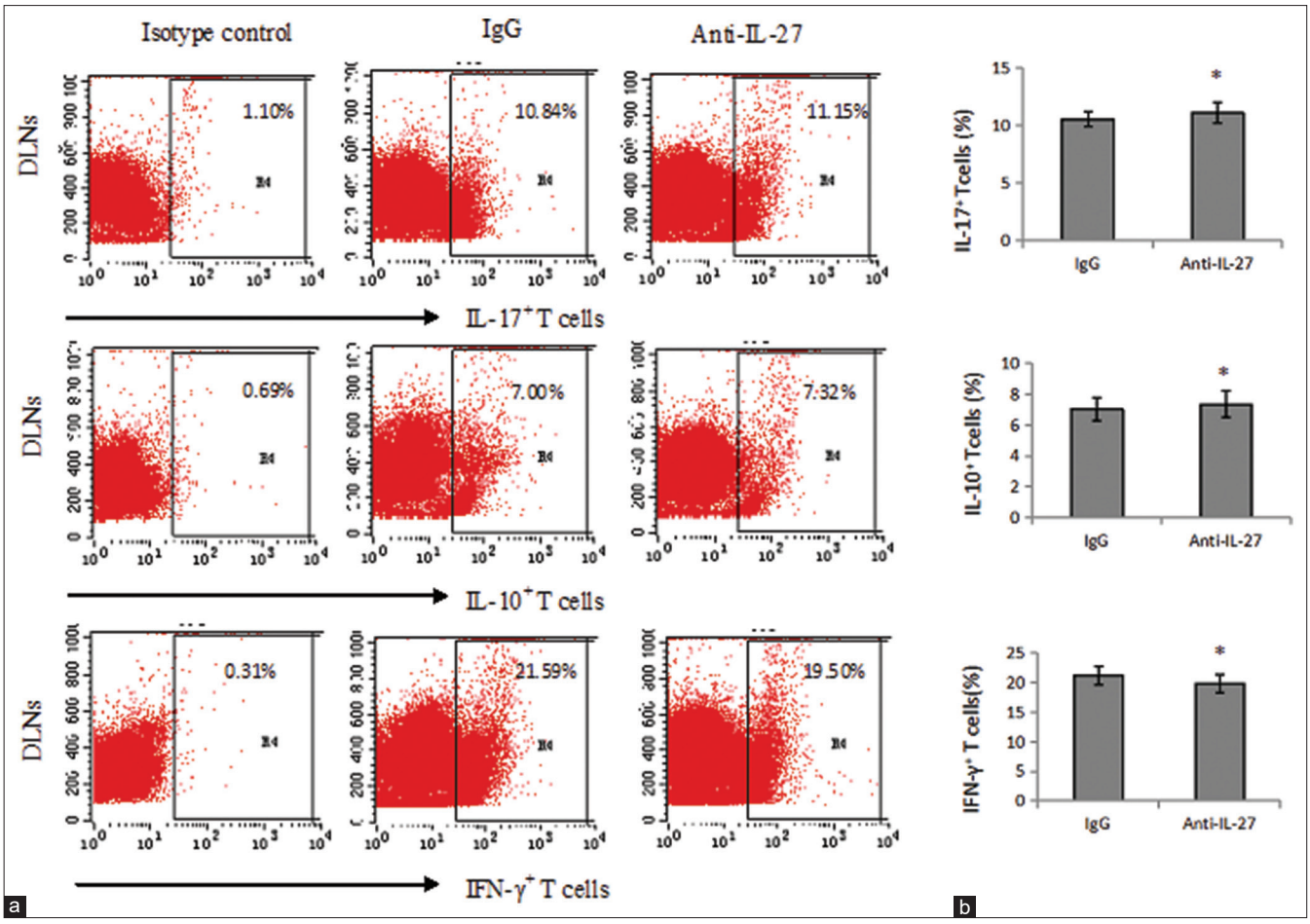

Figure 3: Anti-IL-27 treatment does not regulate the expression of IL-17, IFN-γ, and IL-10 in the cervical draining lymph nodes (DLNs) of HSK mice. On day 14 after HSV-1 infection, single-cell suspensions of cervical DLNs were stimulated with PMA and ionomycin (4 h) before intracellular cytokine staining. The expression of IL-17, IL-10, and IFN-γ was analyzed on the lymphocytes from each group using flow cytometry. (a) The representative flow cytometric dot plots for each group are shown. (b) The bar diagram demonstrates the percentage of IL-17$^+$, IL-10$^+$, and IFN-γ$^+$ T cells in the DLNs in two groups (n = 6 in each group). *P > 0.05 indicates no differences between anti-IL-27-treated mice and IgG-treated mice

manifested the anti-IL-27 treatment significantly upregulated the CD4$^+$ Foxp3$^+$ Tregs influx during the progression of HSK.

Anti-IL-27 treatment does not alter viral load in the cornea

To determine whether anti-IL-27 treatment is better able to control the virus and reduce viral load, we detected tear film virus titers at days 1, 3, and 5 following infection in two groups. Although the mean viral titers in tear film of anti-IL-27-treated mice were lower than those of IgG-treated mice at any time points monitored, the difference did not reach statistical significance [Fig. 5]. The results indicated that anti-IL-27 treatment does not alter viral load in the corneas.

Discussion

It is well accepted that HSV-1-induced HSK is the result of immunopathology mediated by CD4$^+$ T cells. The balance between effective viral control and limited immunopathology is especially critical during HSV-1 corneal infections to prevent corneal opacification, scarring, and permanent loss of vision. IL-27 is a critical immunomodulatory cytokine with important roles in the balance of inflammatory immune responses and CD4$^+$ T cell development.[29] IL-27 has been proved by independent groups to play various roles in the immunity or pathogenesis in several viral infections including human immunodeficiency virus-1, hepatitis C virus, hepatitis B virus, Respiratory syncytial virus, cytomegalovirus, influenza virus infection.[30-33] We are the first to report the pro-inflammatory effects of IL-27 during HSV-1-induced HSK in mice.

In this research project, we first analyzed whether the ocular inflammatory sites induced IL-27 production after HSV-1 infection. Our studies demonstrated that the levels of IL-27 p28 and EBI3 protein were evidently elevated in the cornea of HSK mice at the peak of corneal stromal keratitis, and both IL-27 p28 and EBI3 subunit were expressed simultaneously in the corneal epithelium, stroma, and endothelium of HSK mice rather than in the healthy control group. These findings indicated that IL-27 plays a role in HSV-1 induced corneal immunopathology. In an animal model of experimental autoimmune encephalomyelitis, an inhibitory antibody directed against p28 has been described to be advantageous. In our research, we directly examined the importance of IL-27 in the pathogenesis of HSK by using a specific inhibitory antibody that neutralizes IL-27 p28. The clinical scores of corneal stromal keratitis revealed a clear-cut difference in the stromal lesion between anti-IL-27 treated and IgG-treated groups of mice. Down-regulation of IL-27

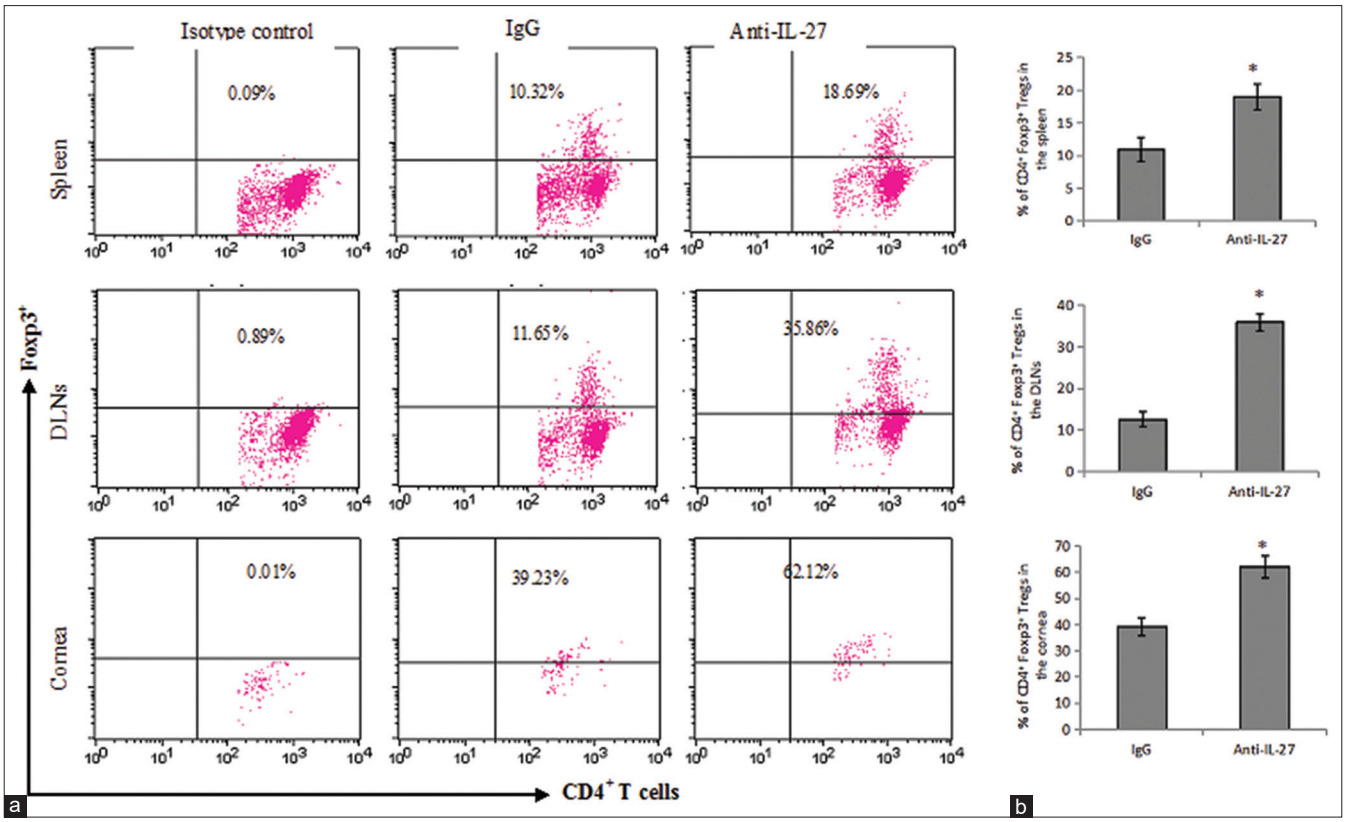

Figure 4: Upregulated CD4+ Foxp3+ Treg cells influx in the anti-IL-27-treated mice. (a) Dot plots represent the frequencies of CD4+ Foxp3+ Tregs from the spleens, DLNs, and corneas of different groups when gated on the lymphocytes. The percentage of CD4+ T cells expressing Foxp3 is shown in the upper right quadrants. (b) The bars represent the percentage of CD4+ Foxp3+ Tregs present in the spleen, DLNs or cornea from different groups ($n = 6$ in each group). *$P < 0.01$ indicates statistically significant differences between anti-IL-27-treated mice and IgG-treated mice

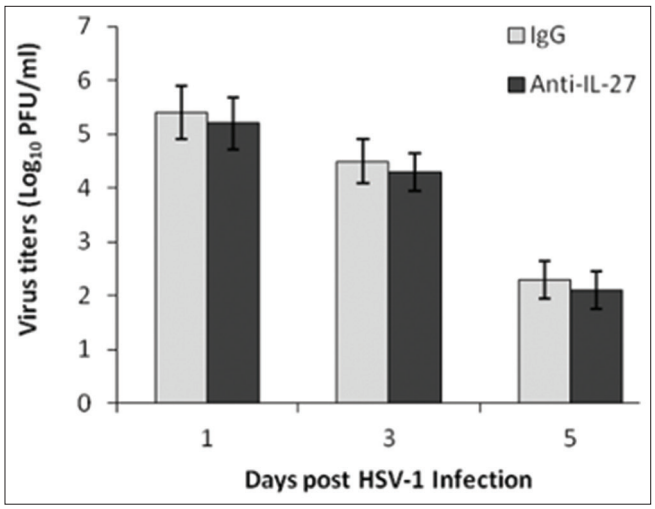

Figure 5: Anti-IL-27 treatment does not alter viral loads in the cornea. On days 1, 3, and 5, swabs of the corneal surface were collected following HSV-1 attacked. The virus titers were calculated as $\log_{10}$PFU/ml. Results are expressed as means ± SD for individual eye swabs ($n = 6$ in each group). The mean viral titers in tear film of anti-IL-27-treated mice were lower than those of IgG-treated mice at any time points monitored, but the difference did not reach statistical significance (*$P > 0.05$)

significantly alleviated HSV-1 induced herpetic stromal keratitis (HSK) in mice. Additionally, histological examination showed that fewer inflammatory cells infiltration and flow cytometry measurement revealed a decreased CD4+ T cells influx in the corneas of anti-IL-27 treatment group. All above these data showed that the blockade of IL-27 signaling inhibited the infiltration of CD4+ T cells into the inflamed cornea, led to significant suppression of corneal stromal inflammation and reduced the severity of ocular diseases. These results strongly suggested that IL-27 played a pro-inflammatory role in CD4+ T cell-mediated HSK immunopathological responses.

Early studies with IL-27 and T cell differentiation demonstrated that IL-27 induces Th1 and Tr1, but inhibits Th2, Th17 and Treg differentiation and function.[14,15,20,21] However, under certain conditions, opposite effects on certain T cell subsets have been observed, for example, inhibition of Th1 and enhancement of Th17 or Treg generation and activity.[16-19,22-24] Tregs are suppressive CD4+ T cells that express the transcription factor Foxp3, maintain self-tolerance during homeostasis, and limit immune-pathology following viral infection. Recently, a series of studies have confirmed that Tregs play a protective role in the control of immune-mediated inflammatory process induced by HSV-1.[26,34-37] To try and understand the mechanism(s) whereby IL-27 signaling play pro-inflammatory role during HSK, we first checked the function of anti-IL-27 antibody in the secretion of IL-17, IFN-γ, IL-10, which involved in the immunoinflammatory reaction to HSV-1 infection.[7-11] We did not find, by flow cytometry, that anti-IL-27 treatment affected the expression of IL-17, IFN-γ, and IL-10 in the cervical DLNs of HSK mice. These verified that the amelioration in

inflammatory response observed in anti-IL-27-treated mice was not associated with the altered Th1, Th2, Th17 responses. However, our further studies showed that anti-IL-27-treated mice had markedly higher percentage of $CD4^+$ $Foxp3^+$ Tregs in the spleen (18.69%), DLNs (35.86%) and cornea (73.12%) than those of IgG-treated mice (10.32%, 11.65%, and 40.23%, respectively), accompanied by the decreased $CD4^+$ T cells response and disease severity of HSK. This finding suggested that IL-27 appeared to exert its pro-inflammatory effect on HSK by inhibiting the frequencies of $CD4^+$ $Foxp3^+$ Tregs in the spleen, DLNs and cornea of HSV-1 infected mice. While our current study focused on the role of anti-IL-27 treatment on virus-specific CD4 T-cells and $CD4^+$ $Foxp3^+$ Tregs during HSV-1 infection, the changes of $CD8^+$ T-cells and $CD8^+$ $Foxp3^+$ Tregs were also tested. However, no difference in either CD8 T cells population or $CD8^+$ $Foxp3^+$ Tregs expression was revealed between two groups (data not shown).

Converging evidence have shown that corneal pathological lesions in HSK mice are not the direct aftermath of viral replication in the cornea, but rather due largely to the host's exuberant immune-inflammatory response to the virus. To ascertain whether the decreased corneal lesions by anti-IL-27 treatment correlates with better virus control, two groups of mice were compared in terms of tear film virus titers. Although the mean viral titers in tear film of anti-IL-27-treated mice were lower than those of IgG-treated mice at any time points monitored, the difference did not reach statistical significance. The results indicated the reduced corneal lesion by anti-IL-27 treatment is not related to limiting viral replication in the cornea.

Conclusion

Collectively, the overall outcome of the present study demonstrates that IL-27 is significantly up-regulated in the ocular inflammatory sites of HSK mice and functions as a pathogenic pro-inflammatory cytokine during the $CD4^+$ T cell-mediated immunity against HSV-1 primarily by controlling $CD4^+$ $Foxp3^+$ Tregs production. Anti-IL-27 treatment evidently decreases the severity of HSK, while does not alter viral load in the corneas. These results suggest that IL-27 can be used as an adjuvant to antiviral to control inflammation during HSV-1 induced keratitis.

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Conflicts of interest

There are no conflicts of interest.

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