Cornea

Interleukin-11 Suppresses Ocular Surface Inflammation and Accelerates Wound Healing

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Received: June 17, 2023
Accepted: September 26, 2023
Published: November 1, 2023

Citation: Singh A, Cho WJ, Pulimamidi VK, Mittal SK, Chauhan SK. Interleukin-11 suppresses ocular surface inflammation and accelerates wound healing. *Invest Ophthalmol Vis Sci.* 2023;64(14):1. https://doi.org/10.1167/iovs.64.14.1

Purpose. Regulation of inflammation is critical for achieving favorable outcomes in wound healing. In this study, we determine the functional role and mechanism of action of IL-11, an immunomodulatory cytokine, in regulating inflammatory response at the ocular surface.

Methods. Corneal injury was induced by mechanical removal of the epithelium and anterior stroma using an AlgerBrush II. Transcript and protein levels of IL-11 in injured cornea were quantified using real-time PCR and ELISA analysis. Corneal inflammation was assessed by measuring frequencies of total $CD45^+$ inflammatory cells, $CD11b^+Ly6G^+$ polymorphonuclear cells (neutrophils), and $CD11b^+Ly6G^-$ mononuclear cells (macrophages, monocytes) at the ocular surface using flow cytometry. To assess the effect of IL-11 on innate immune cell function, cell activation marker and inflammatory cytokines including major histocompatibility complex (MHC) class II, myeloperoxidase (MPO), TNFα, and inducible nitric oxide synthase (iNOS) were measured following recombinant IL-11 treatment (1 µg/mL). Injured corneas were topically treated with IL-11 (1 µg/mL), and wound healing was evaluated using corneal fluorescein staining.

Results. Corneal injury resulted in increased levels of IL-11 in the cornea, particularly in the stroma. Neutrophils and $CD11b^+$ mononuclear cells (macrophages, monocytes) substantially expressed IL-11 receptor. Interestingly, IL-11 significantly downregulated the activation of immune cells, as evidenced by the lower expression of MHC II and TNFα by $CD11b^+$ mononuclear cells and lower levels of MPO by neutrophils. Topical administration of IL-11 to injured corneas led to faster wound healing and better retention of tissue architecture.

Conclusions. Our findings demonstrate IL-11 is a key modulator of ocular surface inflammation and provide novel evidence of IL-11 as a potential therapeutic to control inflammatory damage and accelerate wound repair following injury.

Keywords: corneal wound healing, IL-11, inflammation

A transparent and compact cornea is critical for the transmission of light to form a clear image of an object on the retina. Corneal injury induced by trauma or infection triggers a cascade of inflammatory response, a physiologic healing response. During this repair process, immune cells clear tissue debris and epithelial cells proliferate and migrate to reepithelize the lesion.[1,2] However, if this process is uncontrolled or prolonged, proper reepithelization does not occur and can lead to significant tissue damage, which manifests as corneal thinning, perforation, and scarring.[3] Corneal injury is characterized by epithelial disruption and an influx of immune cells from the limbus and activation of resident immune cells of the cornea, leading to a state of inflammation and loss of corneal homeostasis.[4] These activated immune cells release myeloperoxidase (MPO) and TNFα, which can cause nonspecific tissue damage to delay corneal wound healing.[5]

IL-11 is a pleiotropic cytokine belonging to the IL-6 superfamily.[6] It has a double helical structure and acts via binding to its transmembrane receptor, IL-11Rα, and gp130.[7,8] IL-11 has first been identified to promote megakaryocyte maturation and currently is approved for the prevention of thrombocytopenia in patients receiving chemotherapy.[6,9] Beyond their role in megakaryocyte maturation, IL-11 has been implicated in the pathogenesis of fibrotic diseases and various autoimmune disorders, including ulcerative colitis, rheumatoid arthritis, and graft-versus-host disease.[10–12] In the eye, expression of IL-11 has been reported to be elevated in human retinal pigment cells and corneal fibroblasts when stimulated with inflammatory cytokines in vitro.[13] However, no study has investigated the functional role of IL-11 in an inflamed cornea.

The purpose of the study was to identify the function of IL-11 during corneal injury and delineate its underlying mechanism. We evaluated the levels of IL-11 in naive and injured corneas and showed that high levels of IL-11 are present in the corneal stroma following injury. Having observed increased levels of IL-11 in the inflamed cornea,

Investigative Ophthalmology & Visual Science

we used a gain-of-function approach to determine the function of IL-11 on the varied infiltrating immune cells using in vivo and in vitro models. Using our well-established model of corneal injury and topical administration of IL-11, we demonstrate exogenous supplementation of IL-11 results in faster wound closure and dampened inflammation.

Materials and Methods

Mice

Six- to 8-week-old male BALB/c mice (Charles River Laboratories, Wilmington, MA, USA) were used for indicated experiments. The study (2020N000010) was approved by the Schepens Eye Research Institute (SERI) Animal Care and Use Committee. Mice were housed in a pathogen-free environment at the animal facility of SERI and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Corneal Injury

Mice were anesthetized via intraperitoneal injection of ketamine (120 mg/kg) and xylazine (20 mg/kg). The central cornea of the right eye was marked with a 2-mm trephine, and the corneal epithelium and anterior stroma (approximately one-third of total corneal thickness) were removed mechanically using a handheld motor brush (AlgerBrush II; Alger Company, Lago Vista, TX, USA), as described previously.[14,15] Triple antibiotic ointment (Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment USP; Bausch + Lomb, Wilmington, MA, USA) was applied to the eye after injury. Subcutaneous injection of buprenorphine extended-release suspension (3.25 mg/kg; Ethiqa XR, Fidelis Animal Health, NJ, USA) was administered to minimize postprocedure pain. Mouse recombinant IL-11 (0.3 mg/mL, cat. #78026; STEMCELL Technologies, Cambridge, MA, USA) was administered topically three times a day for 5 days after corneal injury. Mouse serum albumin (MSA; 0.3 mg/mL) served as the control treatment.

Corneal Fluorescein Staining and Slit-Lamp Biomicroscopy

Corneal epithelial healing was assessed using corneal fluorescein staining (CFS). One microliter of 1% sodium fluorescein dye (Sigma-Aldrich, St. Louis, MO, USA) was applied to the injured ocular surface, and positive staining was evaluated under cobalt blue light on the slit-lamp biomicroscope, as described previously.[16] CFS assessment was performed immediately following injury (day 0) and on alternate days thereafter until day 6 postinjury. Captured slit-lamp micrographs were analyzed to calculate the area of epithelial defect using ImageJ, version 1.5.3 (National Institutes of Health, Bethesda, MD, USA).

Corneal Tissue Digestion

Single-cell suspensions were prepared from corneas for flow cytometry analysis.[17,18] Harvested corneas were digested in RPMI media (Lonza, Walkersville, MD, USA) containing 2 mg/mL collagenase type IV (Sigma-Aldrich) and 2 mg/mL DNase I (Roche, Basel, Switzerland) for 45 minutes at 37°C followed by filtration through a 70-micron cell strainer.

Real-Time PCR

Total RNA was isolated from harvested corneas using the RNeasy Micro kit (cat. #74004; Qiagen, Hilden, Germany). Isolated RNA was reverse transcribed to synthesize cDNA using the SuperScript III First-Strand Synthesis System (ThermoFisher Scientific, USA). Real-time PCR was performed using TaqMan Universal PCR Mastermix (Life Technologies, ThermoFisher Scientific, Waltham, MA, USA) in the Mastercycler RealPlex platform (Eppendorf, Hamburg, Germany). Preformulated TaqMan primers for *Il11* (Mm00457645_m1), *Tnfα* (Mm99999068_m1), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; Mm99999915_g1) were used. The comparative threshold cycle method was used to analyze the results, which were normalized to *Gapdh* as an internal control.

ELISA

Levels of IL-11 in the murine cornea and culture supernatants were analyzed using commercially available ELISA kits (murine: cat. #DY218, human: Cat. #DY418, R&D Systems, Minneapolis, MN, USA). To separate the corneal epithelium from the stroma, harvested corneas were first incubated in EDTA (1:25 in PBS) for 30 minutes at 37°C. Thereafter, the epithelium was peeled off from the underlying stroma using nontoothed forceps. Lysates were prepared by subjecting the corneal tissue to three freeze-thaw cycles at −180°C/37°C, followed by homogenization with a motorized pestle and centrifugation at 10,000 rpm for 10 minutes.[19] The results were read using a SpectraMax Plus 384 microplate reader (Molecular Devices, San Jose, CA, USA).

Isolation of Bone Marrow–Derived Immune Cells

Bone marrow (BM) was harvested from femurs of euthanized BALB/c mice. The bone marrow was flushed out of the femurs with PBS in a petri dish followed by gentle pipetting and centrifugation at 1300 rpm for 5 minutes. Depletion of red blood cells was carried out using red blood cell lysis buffer (1 mL per 100 million cells) (cat. #R7757; Sigma-Aldrich).

Cell Culture

Primary human corneal fibroblasts (HCFs) were isolated from multiple donor human corneas and cultured as described previously.[20,21] Human macrophages were differentiated from the human monocytic cell line, THP-1 (ATCC, Manassas, VA, USA), using phorbol 12-myristate 13-acetate (PMA), as per the standardized protocol.[22] In detail, THP-1 cells were seeded in a 24-well culture plate (density: 2.5×10^5 cells/mL) and incubated with PMA (0.2 µM, cat. #P8139; Sigma-Aldrich) for 72 hours. Thereafter, PMA-containing media were replaced with fresh supplemented RPMI. Cells were visualized under an inverted microscope (Leica DMi1; Leica Microsystems, Wetzlar, Germany) to confirm macrophage-like morphology characterized by cell spreading, adhesion, and increased cytoplasmic volume.[22] Monocytes and HCFs were analyzed for IL-11 expression in the resting state as well as after incubation with IL-1β (100 ng/mL) for 24 hours.

In Vitro Culture Assays

BM-isolated cells (density: 1.0×10^6 cells/mL) were incubated with IFNγ (10 ng/mL) in the presence or absence of

recombinant murine IL-11(1 µg/mL, cat. 756102; BioLegend, San Diego, CA, USA) for 24 hours. Brefeldin A (cat. #420601, BioLegend, San Diego, CA, USA) was added at 12 hours to capture intracellular cytokines and TNFα. To evaluate the effect of IL-11 on neutrophil activation, BM-isolated cells were stimulated with formyl-methionyl-leucyl-phenylalanine (fMLP, 1 µM; Sigma-Aldrich) with or without recombinant murine IL-11 (1 µg/mL) for 1 hour.

Human macrophages (THP-1 derived) were incubated with IFNγ (10 ng/mL, cat. #570204; BioLegend) in the presence or absence of recombinant human IL-11 (1 µg/mL, cat. #585902; BioLegend) for 24 hours. Brefeldin A (1×, cat. #420601, BioLegend) was added at 12 hours to capture intracellular cytokines. Macrophages were harvested by gently rinsing the attached cells twice with cold PBS (Corning, Tewksbury, MA, USA) for flow cytometry analysis.

MPO Assay

Neutrophil activation was additionally studied by evaluating MPO enzyme activity in culture supernatants and corneal lysates. MPO activity was measured using a commercial colorimetric assay kit (cat. #ab105136; Abcam, Waltham, MA, USA).

Flow Cytometry

Single-cell suspensions were stained with fluorochrome-conjugated antibodies to study the expression of specific markers. Anti-CD45 (clone: 30-F11), anti-CD11b (clone: M1/70), anti-Ly6G (clone:1A8), anti-TNFα (clone: MP6-XT22), anti–iNos-2 (clone: W16030C), anti–major histocompatibility complex (MHC) class II (clone: M5/114.15.2), anti-HLA-DR (clone: L243), and their respective isotypes were procured from BioLegend. Anti–IL-11RA (cat. #10264-1- AP) and goat anti-rabbit IgG (heavy chain) secondary antibody (cat. #A27039), as well as their respective isotypes, were obtained from ThermoFisher Scientific. The stained cells were read on a LSR-II flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed using the Summit software (Dako Colorado, Fort Collins, CO, USA).

Histology

Whole eyeballs were harvested 5 days postinjury. Formalin-fixed, paraffin-embedded cross sections were stained with hematoxylin and eosin (H&E) and examined using a bright-field microscope (Leica DM750; Leica Microsystems) at 20× magnification. Number of epithelial cell layers, stromal infiltration of immune cells, and tissue architecture were visualized.[23]

Statistical Analysis

To compare the means between the two groups, unpaired two-tailed *t*-test was used. For the comparison of more than two groups, one-way ANOVA was used. The significance level was set at $P < 0.05$. Results are presented as the mean ± SD (in vitro) experiments and mean ± SEM (in vivo) of at least three independent experiments. CFS evaluation was performed by two observers in a masked fashion. Sample sizes were estimated based on previous experimental studies on corneal injury and inflammation.[16,24,25]

Results

High Levels of IL-11 in the Corneal Stroma Following Injury

IL-11 cytokine has been reported to be expressed in different inflammatory diseases; however, its role in the eye has largely been unexplored.[26,27] Corneas were harvested 24 hours after injury to assess levels of IL-11 in naive and injured corneas. Real-time PCR analysis showed a 10-fold increase in the expression of IL-11 in the injured cornea, as compared to naive cornea (Fig. 1A). Similar findings were observed at the protein level showing a 6-fold increase in IL-11 levels in the injured cornea as compared to the naive cornea (Fig. 1B). To delineate the source of IL-11 expression among the corneal layers, we compared its expression profile in the different corneal layers, namely, the epithelium and stroma. IL-11 was detected primarily in the stroma, with the epithelium showing no detectable levels of IL-11 ($P < 0.001$) (Fig. 1C). As resident macrophages and fibroblasts are major components of the corneal stroma, levels of IL-11 were quantified in HCFs and human monocytic cells. Interestingly, monocytic cells did not express detectable levels of IL-11, while HCFs constitutively expressed high levels of IL-11, both at resting and under inflammatory conditions (Fig. 1E).

IL-11 Receptor Is Expressed by Neutrophils and Monocytic Cells in the Wounded Cornea

IL-11 binds to its receptor IL-11Rα and gp130 to form a hexameric complex that initiates downstream signaling.[28] As innate immune cells quickly infiltrate the cornea in large numbers following injury, we investigated whether infiltrating $CD45^+$ total immune cells, $Ly6G^+CD11b^+$ polymorphonuclear cells (neutrophils), and $Ly6G^-CD11b^+$ mononuclear cells (macrophages, monocytes) express IL-11R. At 24 hours postinjury, corneas were harvested and single-cell suspension for flow cytometry analysis was prepared. Infiltrating $CD45^+$ immune cells ($P < 0.0001$), neutrophils ($P < 0.001$), and $CD11b^+$ mononuclear cells ($P < 0.01$) expressed significant levels of IL-11R (Fig. 2A). As most immune cells at the ocular surface following injury infiltrate from the bone marrow, immune cells were isolated from the bone marrow and characterized for their IL-11R expression using flow cytometry. Consistent with our analysis on infiltrating immune cells in the cornea, significant expression of IL-11R on neutrophils and $CD11b^+$ mononuclear cells was observed ($P < 0.001$ and $P < 0.05$, respectively) (Fig. 2B). Similar to our findings on the murine setting, human monocytic immune cells expressed significant levels of IL-11R ($P < 0.01$) (Fig. 2C).

IL-11 Suppresses the Effector Function of Innate Immune Cells

Given the high expression of IL-11R by infiltrating immune cells, we next sought to investigate the effect of IL-11 on the key innate immune cells: neutrophils and mononuclear cells. BM cells were stimulated with fMLP (1 µM), a specific neutrophil stimulant, in the presence or absence of IL-11 (1 µg/mL). Levels of MPO activity, which was substantially upregulated following fMLP ($P < 0.05$), were dampened by 50% in the presence of IL-11 ($P < 0.01$; Fig. 3A). Expression of CD11b, a functional marker for neutrophil

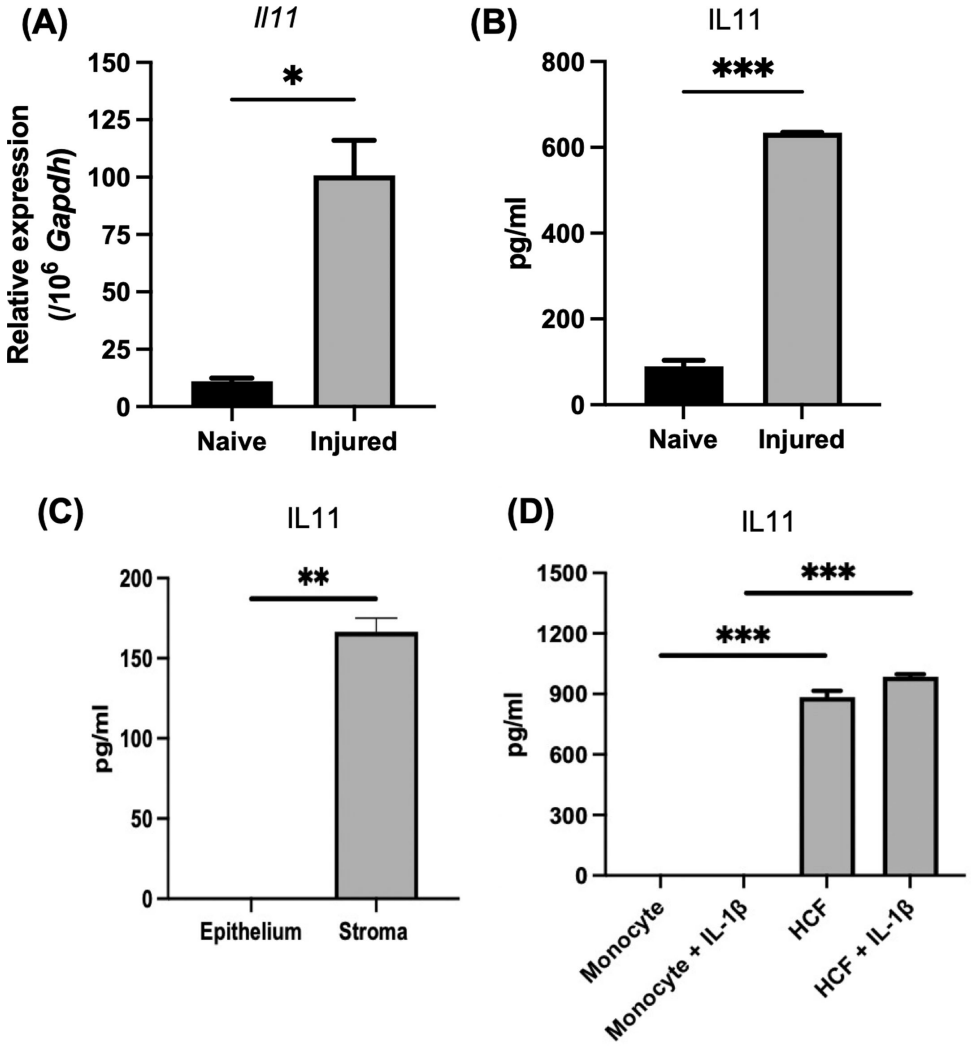

Figure 1. IL-11 expression in the cornea following injury. Corneal injury was induced by mechanical removal of the corneal epithelium and anterior stroma in BALB/c mice. At 24 hours postinjury, corneas were harvested. Corneal tissue harvested from naive mice served as control. (**A**) mRNA expression of IL-11 (normalized to *Gapdh*) by naive and injured corneas. (**B**) Protein levels of IL-11 in lysates of whole corneas collected from naive and injured mice. (**C**) Epithelial and stromal layers were separated from harvested corneas, and protein levels of IL-11 were quantified using ELISA. (**D**) HCFs and human monocytes were stimulated with IL-1β (100 ng/mL) for 24 hours. HCFs and monocytes cultured in media alone served as controls. Bar chart depicting protein levels of IL-11 in the culture supernatants of indicated groups. Data from three independent experiments are shown, and each experiment consisted of 4 to 6 animals/group. The values are shown as mean ± SEM (*error bar*). Student *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

activation, was assessed on these neutrophils using flow cytometry. Compared to the untreated group, the IL-11–treated cells showed a significant reduction in CD11b expression ($P < 0.05$; Fig. 3B), paralleling the MPO finding.

To analyze the effect of IL-11 on mononuclear cell function, we studied expression of its activation marker, MHC II, and its functional cytokines TNFα and inducible nitric oxide synthase (iNOS) by CD11b^{+}Ly6G^{-} mononuclear cells (macrophages, monocytes) in the presence or absence of IL-11. A 3-fold increase in MHC II expression was observed following IFNγ (10 ng/mL) stimulation, which was significantly reduced when cultured with IL-11 ($P < 0.001$; Fig. 3C). Similarly, TNFα and iNOS expression by CD11b^{+} mononuclear cells were significantly downregulated when cultured with IL-11, compared to untreated controls (Figs. 3D, 3E).

To assess whether our findings can be translated in the human setting, THP-1–derived human macrophages were stimulated with IFNγ (10 ng/mL), and expression of HLA-DR, TNFα, and iNOS in the presence or absence of IL-11 was evaluated. Flow cytometry analysis demonstrated significant downregulation in the expression of HLA-DR and both effector molecules in human macrophages cultured in the presence of IL-11, compared to those cultured in its absence ($P < 0.0001$; Figs. 4A–C). Taken together, our data demonstrate that IL-11 exerts anti-inflammatory effects on neutrophils and macrophages, key modulators of acute corneal inflammation.

Exogenous Supplementation of IL-11 Suppresses Injury-Induced Inflammation

Using our in vivo injury model, we assessed whether IL-11 supplementation can regulate ocular inflammation. Following corneal injury, IL-11 (0.3 mg/mL) was topically applied

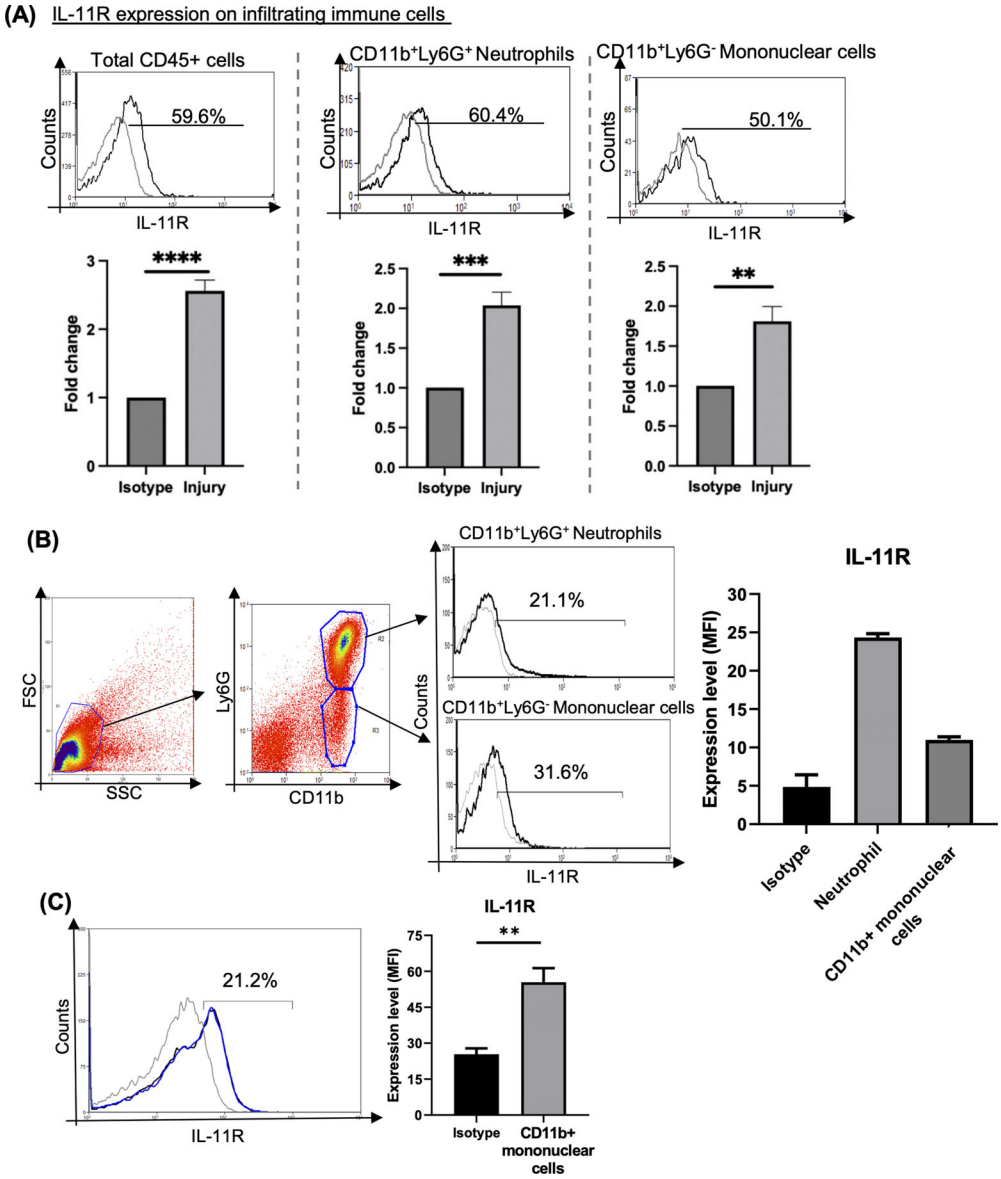

Figure 2. IL-11 receptor expression by infiltrating immune cells. Corneas were harvested 24 hours following injury and single-cell suspensions were prepared for flow cytometry analysis. Corneas harvested from naive mice served as controls. (**A**) Representative histograms (*upper panel*) showing frequencies of IL-11R expressing $CD45^+$ total immune cells, $CD11b^+Ly6G^+$ neutrophils, and $CD11b^+Ly6G^-$ monocytes in the cornea. Bar charts (*lower panel*) quantifying fold change in IL-11R expression (MFI, mean fluorescence intensity) by the indicated cells. (**B**) Representative dot plots (left) showing the gating strategies of bone marrow–isolated $CD11b^+Ly6G^+$ neutrophils and $CD11b^+Ly6G^-$ mononuclear cells (macrophages, monocytes). Bar chart (*right*) quantifying expression (MFI) of IL-11R by $CD11b^+Ly6G^+$ neutrophils and $CD11b^+Ly6G^-$ mononuclear cells (macrophages, monocytes). (**C**) Representative histogram (*left*) and bar chart (*right*) quantifying expression of IL-11R by human monocytes. Data from three independent experiments are shown, and each experiment consisted of 4 to 6 animals. Data are presented as mean ± SD (*error bar*). Student *t*-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

to the ocular surface thrice daily (Fig. 5A). MSA-treated mice served as controls. Twenty-four hours following corneal injury, corneas were harvested, and frequencies of $CD45^+$ total immune cells, $Ly6G^+CD11b^+$ neutrophils, and Ly6G-$CD11b^+$ mononuclear cells (macrophages, monocytes) were analyzed using flow cytometry. IL-11 treatment resulted in a 58% decline in total $CD45^+$ leukocyte frequencies, compared to MSA treatment ($P < 0.05$; Fig. 5B). Specifically, topical treatment with IL-11 showed a 54% reduction in neutrophils ($P < 0.01$) and a 55% reduction in $CD11b^+$ mononuclear cells ($P < 0.05$), relative to MSA treatment (Fig. 5C). We further evaluated IL-11–mediated suppression of immune cell function, via measuring MPO activity and TNFα expression at the ocular surface. A 3.5-fold reduction in MPO activity was observed in IL-11–treated corneas, compared to MSA-treated corneas ($P < 0.0001$; Fig. 5D). In addition, transcript analysis showed significant downregulation of TNFα expression in corneas treated with IL-11 compared to those treated with MSA ($P < 0.05$; Fig. 5E).

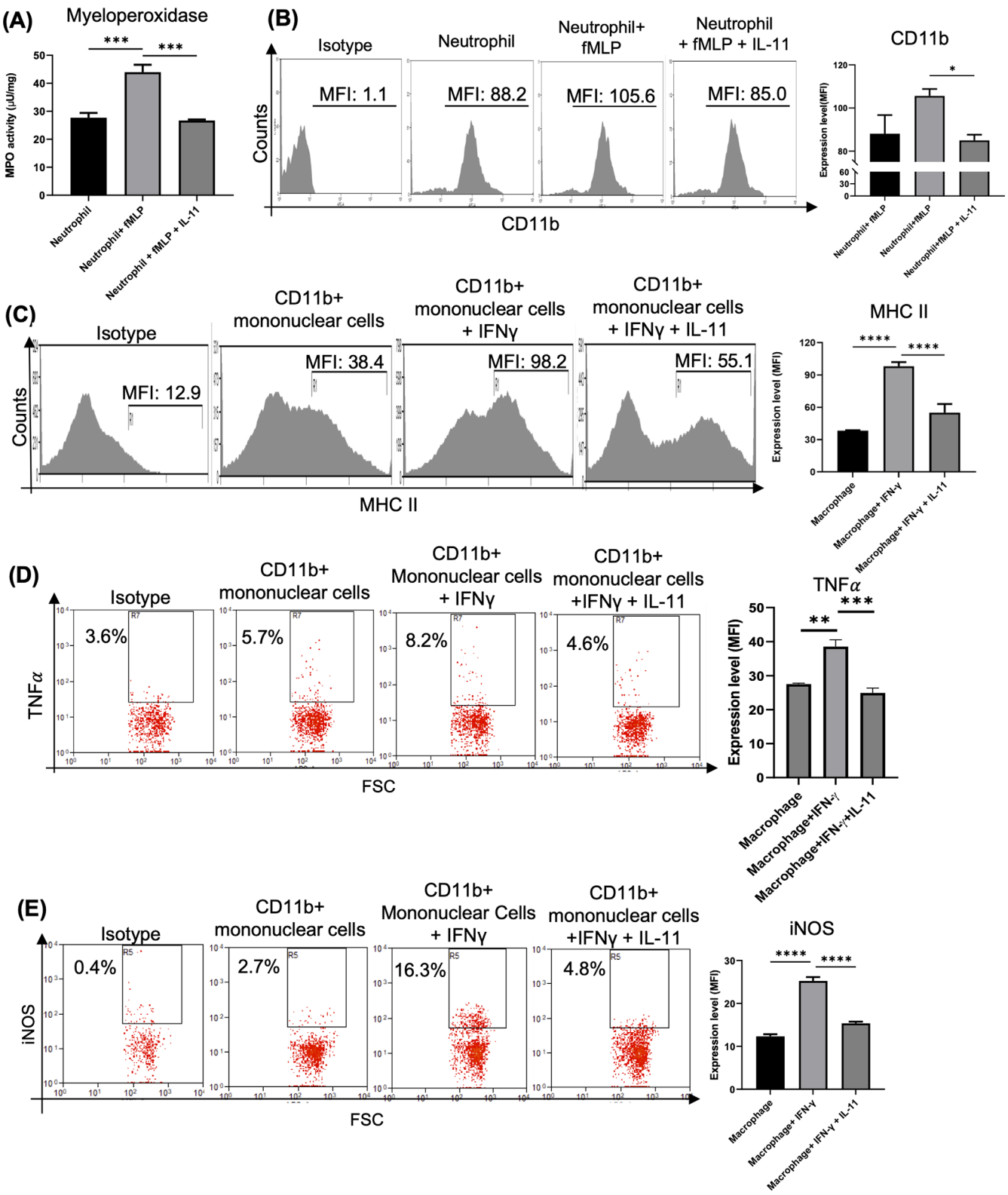

Figure 3. IL-11 suppresses activation of neutrophils and monocytic cells. Bone marrow was harvested from BALB/c mice and single-cell suspensions were made. (**A**) Cells were stimulated with a neutrophil activator, fMLP (1 μM), in the presence or absence of IL-11 (1 μg/mL). Cells cultured in media alone served as control. Bar chart depicting MPO levels in supernatants of indicated cell cultures. (**B**) Representative histogram (*left*) and bar chart (*right*) depicting expression of neutrophil activation marker CD11b (MFI). (**C–E**) Bone marrow cells were stimulated with IFNγ (10 ng/mL) and cultured in the presence or absence of IL-11 (1 μg/mL). (**C**) Representative histogram (*left*) and bar chart (*right*) depicting MHC II expression (MFI) by mononuclear cells (gated on $CD11b^{+}Ly6G^{-}$). (**D**) Representative dot plots (*left panel*) and bar chart (*right panel*) quantifying mononuclear cells (gated on $CD11b^{+}Ly6G^{-}$) expression (MFI) of TNFα, a proinflammatory cytokine. (**E**) Representative flow cytometry dot plots (*left*) and bar chart (*right*) depicting differential expression (MFI) of iNOS by mononuclear cells (gated on $CD11b^{+}Ly6G^{-}$). Data from three independent experiments are shown, expressed as mean ± SD (*error bar*). Data in each group are from triplicate wells. One-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

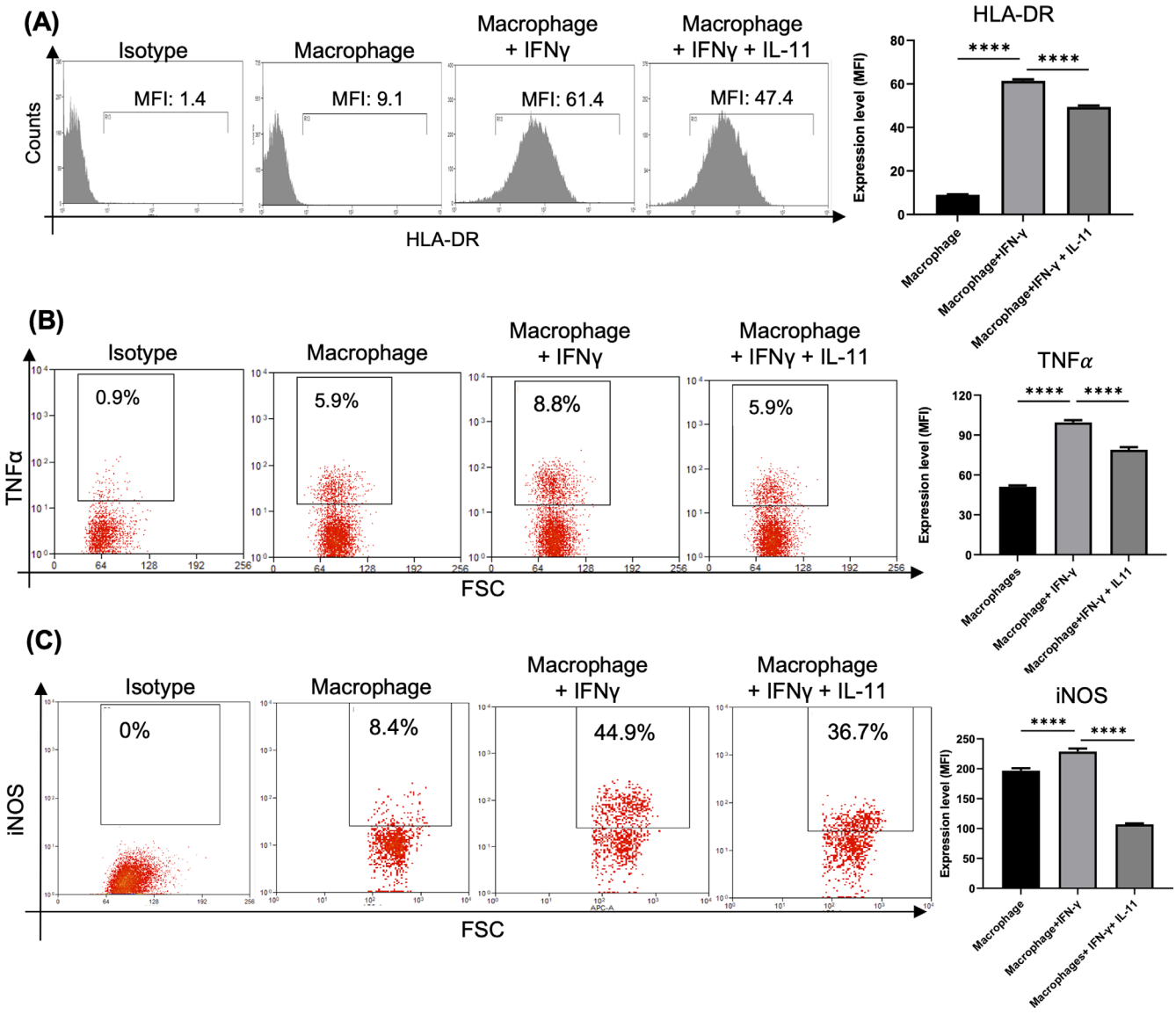

Figure 4. IL-11 downregulates activation of human macrophages. Human macrophages derived from THP-1 cell line were stimulated with IFNγ (10 ng/mL) and cultured with or without IL-11 (1 µg/mL) for 24 hours. Cells cultured in media alone served as control. (**A**) Representative histogram (*left*) showing expression of human macrophage activation marker HLA-DR. Bar chart (*right*) quantifying HLA-DR expression (MFI). (**B**) Representative dot plots (*left*) and bar chart (*right*) quantifying expression of TNFα in control and treatment groups. (**C**) Representative flow cytometry dot plots (*left*) and bar chart (*right*) depicting differential expression (MFI) of iNOS by macrophages in indicated groups. Representative data from three independent experiments are shown, expressed as mean ± SD (*error bar*). Data in each group are from triplicate wells. One-way ANOVA. ****$P < 0.0001$.

Topical IL-11 Treatment Accelerates Wound Healing Following Corneal Injury

Given that controlled inflammation often leads to faster wound healing, we investigated the effect of IL-11 on corneal wound healing. Faster reepithelization was observed in IL-11–treated eyes, compared to MSA-treated eyes (Fig. 6A). On day 2 of treatment, there was a 17% reduction in epithelial defect (green area) in IL-11–treated eyes, whereas only a 2% reduction was observed in control-treated eyes ($P < 0.05$; Fig. 6A). By day 4, 79% of the epithelial defect had healed in the IL-11–treated group, compared to a 9.5% reduction in the control-treated group ($P < 0.001$; Fig. 6A). Treatments were administered until day 6 (Fig. 5A), by which nearly 90% of the epithelium had reepithelized following IL-11 treatment; however, MSA control treatment resulted in approximately 40% reepithelization ($P < 0.001$; Fig. 6A).

Additionally, corneas were harvested on day 6 and frequencies of CD45$^+$ immune cells were analyzed. Flow cytometry analysis showed a 2.5-fold lower infiltration of immune cells in IL-11–treated corneas, relative to MSA-treated controls ($P < 0.01$; Fig. 6B). To examine the tissue morphology, cross sections of harvested corneas were stained with H&E. IL-11–treated corneas showed comparable corneal architecture to naive corneas (Fig. 6C). Furthermore, less immune cell foci and edema were observed in IL-11–treated corneas, compared to MSA-treated controls (Fig. 6C). Our data suggest that IL-11 suppresses injury-induced inflammation and promotes faster wound healing to restore corneal tissue homeostasis.

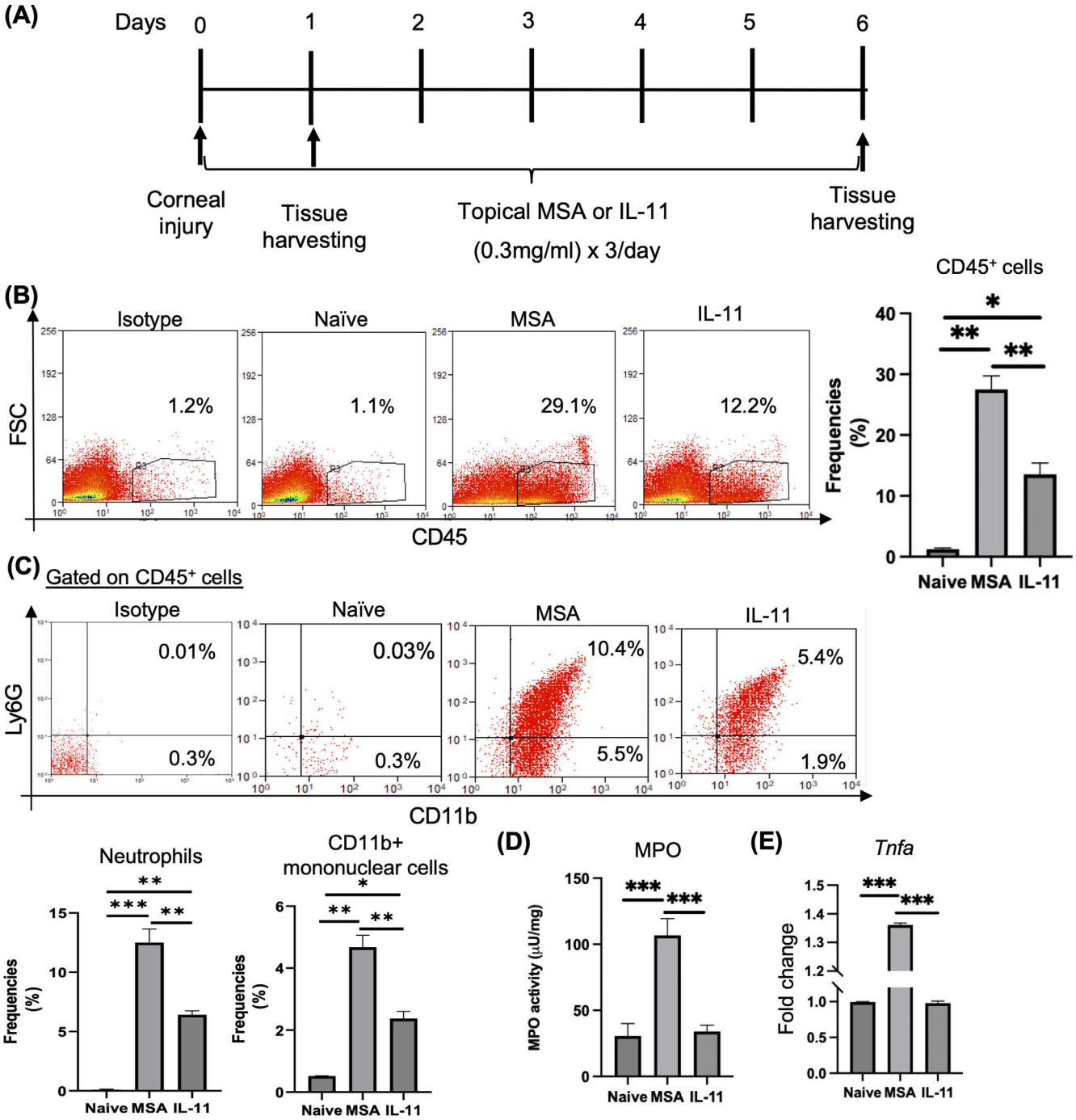

Figure 5. IL-11 reduces infiltration of immune cells following corneal injury. (**A**) Schematic of the experimental design indicating dose and frequencies of IL-11 and MSA administration following injury. Corneas were harvested 24 hours postinjury, and single-cell suspensions were prepared to study immune cell infiltration using flow cytometry. (**B**) Representative dot plots (*left*) showing frequencies of total $CD45^+$ cells in naive, MSA-treated, and IL-11–treated mice. Cumulative bar chart (*right*) quantifying frequencies of total $CD45^+$ leukocytes in the cornea. (**C**) Representative dot plots (*upper panel*) indicating frequencies of $CD11b^+Ly6G^+$neutrophils and $CD11b^+$ $Ly6G^-$ mononuclear cells (gated on $CD45^+$ cells) in corneas of indicated treatment groups. Cumulative bar chart (*lower panels*) quantifying frequencies of neutrophils and $CD11b^+$ mononuclear cells (macrophages and monocytes) in the corneas harvested from indicated groups of mice. (**D**) Bar chart depicting MPO levels in corneas harvested 24 hours postinjury. (**E**) Fold change in mRNA expression of TNFα in corneas of indicated mice groups. Fold change was calculated from naive mice. Data from three independent experiments are shown. Each experiment consisted of 4 to 6 animals per group. The values are expressed as mean ± SD (*error bar*). One-way ANOVA. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

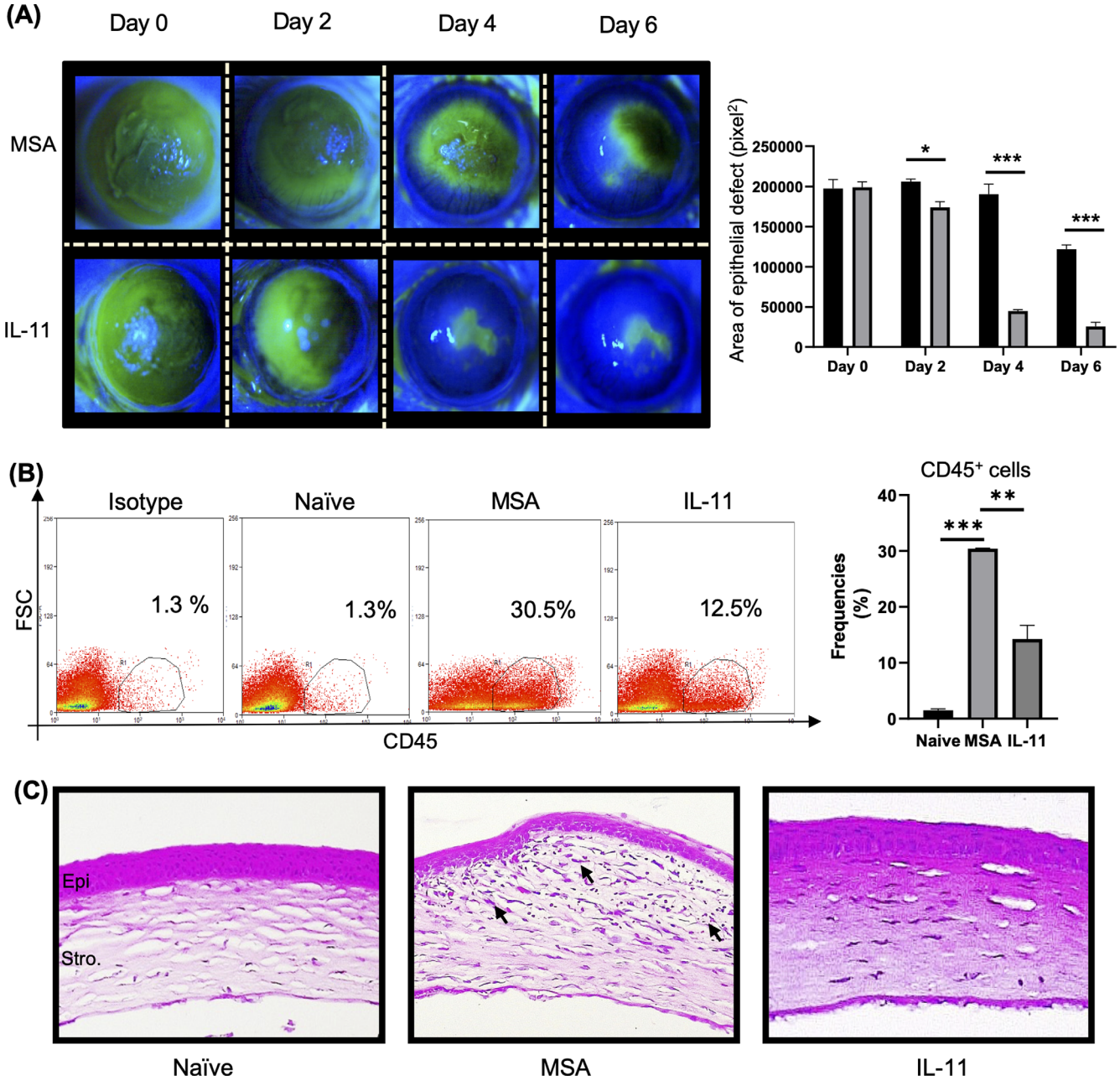

Figure 6. Topical supplementation of IL-11 accelerates wound healing. (**A**) Corneal fluorescein staining was performed to assess for epithelial defects (stained *green*). Area of epithelial defects was quantified using ImageJ software. Representative images of fluorescein-stained corneas (*left*), captured using slit-lamp biomicroscopy under cobalt blue light, and cumulative bar chart (*right*) showing the area of epithelial defect in pixel^2 at indicated time points. (**B**) Single-cell suspensions of corneas harvested on day 6 postinjury were analyzed for immune cell infiltration using flow cytometry. Representative dot plots (*left*) showing frequencies of total CD45$^+$ cells in naive, MSA-treated, and IL-11–treated mice. Cumulative bar chart (*right*) quantifying frequencies of total CD45$^+$ leukocytes in corneas of different treatment groups. (**C**) H&E corneal cross sections showing corneal tissue architecture, edema, and inflammatory cells (*black arrow*). Data from three independent experiments are shown, and each experiment consisted of 6 to 8 animals/group. The values are shown as mean ± SD (*error bar*). Student *t*-test and one-way ANOVA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Epi., epithelium; Stro., stroma.

Discussion

IL-11 is a multifunctional cytokine involved in the pathology of different inflammatory and noninflammatory systemic diseases.[29–31] However, the principal function of IL-11 in ocular pathologies is unknown thus far. Given this gap in knowledge, we investigated the function of IL-11 in mediating ocular inflammation and subsequent wound healing following corneal injury. Our study demonstrates that IL-11 is significantly expressed in the cornea, specifically in the stroma, following injury and that infiltrating immune cells, including neutrophils and macrophages, express IL-11 receptors. Exogenous supplementation of IL-11 suppresses the activation of immune cells in both in vitro and in

vivo settings. Lastly, topical IL-11 treatment following injury accelerates corneal wound healing and suppresses injury-induced inflammation.

IL-11, originally detected in the conditioned medium of primate bone marrow stromal cells, has been shown to enhance lymphohematopoietic stem cell proliferation and differentiation.[6] More recently, it has been studied in different systemic diseases and has been broadly described to be anti-inflammatory; however, very little is known about its role in the eye.[32,33] Beyond a single study by Nagineni et al.[13] reporting expression of IL-11 in primary cultures of human retinal pigment epithelial cells and corneal fibroblast cells in vitro, no study has investigated the role of IL-11 in corneal diseases and inflammation.

In our study, we first investigated the expression of IL-11 in the cornea in the naive and inflammatory states using a murine model of corneal injury. High levels of IL-11 were observed at both transcript and protein levels in the inflamed state. Specifically, IL-11 was highly expressed in the corneal stroma, suggesting the source of this cytokine to be localized cells, namely, the fibroblasts and infiltrating immune cells. Upon injury, quiescent keratocytes transform into active fibroblasts, which aid in repair and regeneration.[34] In conjunction, bone marrow–derived immune cells traffic to the corneal stroma to partake in the inflammatory response, a critical component of the wound-healing cascade.[35,36] Thus, to delineate the cellular source of IL-11, their levels in human fibroblasts and monocytic cells were measured. Fibroblasts constitutively expressed high levels of IL-11, while monocytic cells did not secrete detectable levels of IL-11. Our data suggest corneal fibroblasts are the primary source of IL-11 in the cornea. Next, to determine the target of IL-11, we assessed IL-11 receptor expression on immune cells—namely, neutrophils and $CD11b^+$ mononuclear cells (monocytes and macrophages)—which drive corneal inflammation following injury. Interestingly, sizable expression of IL-11 receptor was found on both neutrophils and $CD11b^+$ mononuclear cells.

Infiltrating immune cells secrete different proinflammatory factors, including MPO by neutrophils and TNFα and iNOS by $CD11b^+$ mononuclear cells, that mediate the degradation of extracellular matrix and keratocyte apoptosis following injury.[14,37] Not only did topical IL-11 treatment suppress infiltration of innate immune cells, but the immune cells cultured in the presence of IL-11 showed dampened activation, as demonstrated by significantly lower expression of their activation marker CD11b by neutrophils and MHC II by macrophages. Moreover, these immune cells cultured in the presence of IL-11 expressed significantly lower levels of tissue-damaging molecules, MPO by neutrophils, and TNFα and iNOS by human macrophages and mouse $CD11b^+$ mononuclear cells—macrophages and monocytes . Additionally, topical treatment with IL-11 led to faster epithelial wound healing and better restoration of tissue architecture, demonstrating the regulatory effect of IL-11 on corneal inflammation.

In conclusion, the present study demonstrates that cells of the corneal stroma express substantial levels of IL-11, which can significantly suppress the infiltration and activation of innate immune cells following injury. Exogenous supplementation of IL-11 promotes faster corneal wound healing and containment of inflammation, underlining its favorable role in regulating the injury-induced inflammatory cascade. In sum, our findings suggest that IL-11 can act as a key immunosuppressive cytokine and provide a novel scope of therapy for promoting corneal wound healing.

Acknowledgments

Supported by the National Institutes of Health grants R01EY024602 and P30EY003790.

Disclosure: **A. Singh**, None; **W.J. Cho**, None; **V.K. Pulimamidi**, None; **S.K. Mittal**, None; **S.K. Chauhan**, None

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