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Original Article

$CD8^+$ $CD103^+$ regulatory T cells attenuate desiccating stress-induced dry eye disease by suppressing $CD4^+$ T cell-mediated inflammation and preserving ocular surface integrity in mice

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Abstract: Objectives: This study aimed to investigate the immunomodulatory and protective role of $CD8^+CD103^+$ T cells in desiccating stress (DS)-induced dry eye disease (DED), focusing on tear secretion, corneal barrier function, and conjunctival goblet cell preservation. Methods: Female C57BL/6 mice were exposed to DS and administered anticholinergic agents to induce experimental DED. $CD8^+CD103^+$ T cells were isolated, purified, and adoptively transferred into DS mice. Tear volume was assessed at multiple circadian phases, corneal epithelial integrity was evaluated by fluorescein staining and MMP expression, conjunctival goblet cells were quantified histologically, and apoptosis markers were analyzed. Flow cytometry was performed to assess $CD4^+$ T cell infiltration and cytokine expression profiles. Results: Adoptive transfer of $CD8^+CD103^+$ T cells significantly improved tear secretion across circadian phases, prevented corneal barrier disruption, and preserved goblet cell density. These effects were associated with reduced infiltration of $CD4^+$ T cells in conjunctiva and draining lymph nodes, decreased levels of pro-inflammatory cytokines IFN-γ and IL-17A, and downregulation of MMP-3 and MMP-9. Conversely, IL-13 levels were elevated, correlating with goblet cell protection. Importantly, safety assessments revealed no adverse effects on body weight, intraocular pressure, corneal sensitivity, or corneal nerve density. Conclusions: $CD8^+CD103^+$ T cells exert immunomodulatory and tissue-protective effects in DS-induced DED by suppressing pathogenic $CD4^+$ T cell responses and restoring a favorable cytokine balance. These findings highlight their potential therapeutic role in ocular surface inflammation and immune-mediated dry eye disease.

Keywords: $CD8^+CD103^+$ T cells, dry eye disease, ocular surface, immunomodulation, inflammation

Introduction

Dry eye disease (DED) is a multifactorial, chronic condition involving inflammation and damage to the ocular surface, often driven by T cell-mediated immune responses. Among the key players in the immune response are $CD4^+$ T cells, particularly Th1 and Th17 subsets, which have been shown to contribute significantly to the pathogenesis of DED through the release of pro-inflammatory cytokines such as IFN-γ and IL-17A. Recent studies suggest that $CD8^+CD103^+$ T cells may also play a crucial role in regulating immune responses within inflammatory settings. These cells exhibit characteristics reminiscent of regulatory T cells (Tregs), such as their ability to migrate to inflamed tissues and suppress effector T cell responses [1-3].

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However, it remains to be determined whether $CD8^+CD103^+$ T cells fully meet the criteria for Tregs, including expression of typical inhibitory markers like IL-10, TGF-β, CTLA-4, and PD-1. The present study investigates their potential for immune modulation in the context of DED, though further in vitro assays are needed to conclusively characterize these cells. However, while these cells can modulate local inflammation, their full regulatory identity remains unclear, as they do not fully meet the criteria for Tregs, including expression of typical inhibitory markers like IL-10 and TGF-β. This study investigates their potential for immune modulation in the context of DED, although further in vitro assays are required to conclusively characterize these cells.

The pathogenesis of DED involves a complex interplay among environmental stressors, neurosensory dysfunction, tear hyperosmolarity, and immune-mediated inflammation [4-6]. Among these, T cell-driven immune responses, especially those mediated by $CD4^+$ T helper (Th) cells, are central contributors to disease initiation and chronicity. Experimental models of DED have shown that desiccating stress (DS) triggers activation and expansion of $CD4^+$ T cells, particularly Th1 and Th17 subsets, which infiltrate ocular tissues and release pro-inflammatory cytokines such as interferon-γ (IFN-γ) and interleukin-17A (IL-17A) [7, 8]. These cytokines drive epithelial cell apoptosis, goblet cell loss, and MMP overexpression, leading to corneal barrier disruption and reduced mucin production, two key pathophysiological features of DED.

The pivotal role of $CD4^+$ T cells in DED is highlighted by adoptive transfer experiments, where $CD4^+$ T cells from DS-exposed mice induce DED-like symptoms in immunodeficient recipients [7]. Therefore, immunomodulation of $CD4^+$ T cell-mediated responses presents a promising therapeutic strategy. Traditionally, regulatory T cell (Treg) research has focused on the $CD4^+FoxP3^+$ subset, but growing evidence highlights the immunosuppressive capacity of $CD8^+$ Tregs, particularly the $CD8^+CD103^+$ population, which are increasingly recognized for their roles in maintaining mucosal immune homeostasis [9-11].

CD103, also known as integrin αEβ7, binds to E-cadherin and facilitates the retention of lymphocytes within epithelial tissues. $CD8^+CD103^+$ T cells, even those lacking FoxP3 expression, have been shown to exert regulatory functions in various mucosal environments, including the gastrointestinal tract, lungs, kidneys, and skin [12-14]. These cells suppress pathogenic immune responses through cytokine secretion (e.g., IL-10, TGF-β), cell-cell contact-dependent inhibition, and modulation of dendritic cell function. Their immunosuppressive roles have been described in multiple disease models, including inflammatory bowel disease, lupus nephritis, and graft-versus-host disease [12-14].

In the ocular setting, relatively little is known about the function of $CD8^+CD103^+$ Tregs in immune regulation during DED. Recent studies have indicated their increased presence in the cervical lymph nodes and conjunctival tissues of DS-exposed mice [15-17], suggesting a potential regulatory role. Their ability to migrate to inflamed ocular tissues and their localization within epithelial compartments make them well-positioned to modulate immune responses at the ocular surface - a feature underexplored in previous research. Moreover, the specific effects of $CD8^+CD103^+$ T cells on pathogenic $CD4^+$ T cell activity, cytokine production, and tissue damage during DED remain to be comprehensively investigated.

The present study addresses this critical knowledge gap by evaluating the immunomodulatory and tissue-protective functions of $CD8^+CD103^+$ T cells in a DS-induced murine model of DED. Specifically, we examined the effects of adoptively transferred $CD8^+CD103^+$ T cells on tear production, conjunctival goblet cell density, corneal epithelial barrier function, MMP expression, and ocular surface apoptosis. Furthermore, we assessed their influence on $CD4^+$ T cell infiltration, cytokine production (IL-17A, IFN-γ, and IL-13), and lymphocyte dynamics in draining cervical lymph nodes. By integrating functional, histological, and molecular analyses, this study aims to determine whether $CD8^+CD103^+$ T cells can suppress $CD4^+$ T cell-driven inflammation and preserve ocular surface integrity under DS conditions. Our findings provide new insights into mucosal immune regulation in the eye and support the therapeutic potential of $CD8^+CD103^+$ T cells for DED and other ocular inflammatory diseases.

Materials and methods

Animal ethics and experimental subjects

All animal experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health (NIH) guidelines. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jinan 2nd People's Hospital (Approval No. JsPH/JC/2024/415, dated 18 September 2023).

Female C57BL/6 (B6) mice, aged 6-8 weeks, were obtained from the Shanghai SLAC Laboratory Animal Center (Shanghai, China). Animals were housed in a specific pathogen-free facility under standard laboratory conditions, and all procedures were performed in compliance with institutional ethical requirements and international standards for the care and use of laboratory animals.

At the end of the experiment, mice were euthanized by CO_2 inhalation followed by cervical dislocation to ensure death. All procedures were carried out in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020).

Induction of experimental dry eye

Experimental dry eye was induced using a well-established desiccating stress (DS) model. Mice received subcutaneous injections of scopolamine hydrobromide (0.5 mg/0.2 mL; MB5860, Melonepharma, Dalian, China) four times daily (08:00, 12:00, 15:00, and 18:00) and were simultaneously exposed to continuous airflow under low-humidity conditions (< 40%) for five consecutive days. Age- and sex-matched mice not exposed to DS served as non-stressed (NS) controls.

Isolation and sorting of $CD8^+CD103^+$ T cells

$CD8^+CD103^+$ T cells were isolated from the spleens and superficial cervical lymph nodes (CLNs) of desiccating stress (DS)-exposed mice using anti-CD8 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) followed by CD103-APC re-sorting. The purity of the sorted population was determined by flow cytometry, and the final $CD8^+CD103^+$ T cell population had a purity of 95% following two rounds of sorting. The remaining cell population consisted of 2% $CD4^+$ T cells, 1% γδ T cells, 1% NK cells, and 1% ILCs.

Flow cytometry gating strategy

Flow cytometry was performed using a BD LSRII cytometer (BD Biosciences) and data were analyzed with FlowJo software (TreeStar Inc.). The gating strategy involved initial exclusion of dead cells using propidium iodide, followed by gating on the $CD8^+$ population. The $CD8^+$ cells were then further analyzed for CD103 expression. To control for non-specific binding, FMO (fluorescence-minus-one) and isotype controls were used for all antibodies, ensuring that the observed signals were due to specific binding and not background fluorescence.

Tear volume measurement and circadian control

Tear volume was measured using phenol red-impregnated cotton threads (Zone-Quick; FCI Ophthalmics, USA) placed in the lateral canthus of the eye for 60 seconds. To account for potential circadian influences on tear secretion, measurements were performed at four time points across the light-dark cycle: 8:00 a.m. (lights on), 2:00 p.m. (light phase), 8:00 p.m. (lights off), and 2:00 a.m. (dark phase). All mice were housed under a 12-hour light/12-hour dark cycle (lights on 7:00 a.m., lights off 7:00 p.m.), and measurements were carried out under dim red light during dark phases to minimize disruption. For each mouse, tear volumes were measured in both eyes, and the mean was recorded.

Assessment of corneal epithelial permeability

Corneal barrier function was evaluated using Oregon Green Dextran (OGD; 70,000 MW; D7172, Invitrogen, Eugene, OR, USA). A 0.5 µL drop of a 50 mg/mL OGD solution was applied to the ocular surface one minute before euthanasia. Corneas were rinsed five times with sterile saline and imaged using a fluorescence stereomicroscope (AZ100, Nikon, Tokyo, Japan). The mean fluorescence intensity within a 2 mm central corneal area was measured with NIS Elements software (version 4.1, Nikon).

Table 1. Mouse primer sequences used for qRT-PCR

Gene	Sense primer	Anti-sense primer
MMP-3	CCTTTTGATGGGCCTGGAAC	GAGTGGCCAAGTTCATGAGC
MMP-9	CAATCCTTGCAATGTGGATG	AGTAAGGAAGGGGCCCTGTA
IL-13	GCAGCATGGTATGGAGTGT	TATCCTCTGGGTCCTGTAGATG
IL-17A	CGCAATGAAGACCCTGATAGAT	CTCTTGCTGGATGAGAACAGAA
IFN-γ	AAATCCTGCAGAGCCAGATTAT	GCTGTTGCTGAAGAAGGTAGTA
β-actin	CCTAAGGCCAACCGTGAAAAG	AGGCATACAGGGACAGCACAG

Histological analysis and goblet cell quantification

Eyes and adnexa were excised and processed either as paraffin-embedded or OCT-embedded specimens. Paraffin sections (5 μm) were stained with periodic acid-Schiff (PAS) reagent (395B-1KT, Sigma, St. Louis, MO, USA) to identify goblet cells. Goblet cells across the entire conjunctival area were quantified using NIS Elements imaging software.

Immunofluorescence staining

Frozen tissue sections (6 μm) were fixed in cold acetone at -20°C for 10 minutes. Sections were incubated overnight at 4°C with the following primary antibodies: goat anti-MMP-3 (1:50; sc-6839), goat anti-MMP-9 (1:50; sc-6840), rabbit anti-active caspase-3 (1:250; ab52181), and rabbit anti-active caspase-8 (1:50; sc-7890). After PBS washes, sections were incubated with Alexa Fluor 488-conjugated donkey anti-goat or anti-rabbit secondary antibodies (1:300; Invitrogen) for 1 hour at room temperature in the dark. Nuclei were counterstained with DAPI (H-1200; Vector Laboratories, Burlingame, CA, USA), and images were captured using a Leica DM2500 microscope. Fluorescence intensity was analyzed with NIS Elements software.

TUNEL assay for apoptosis

Apoptotic cells were detected on paraffin-embedded sections using the DeadEnd Fluorometric TUNEL System (G3250; Promega, Madison, WI, USA) according to the manufacturer's instructions. Slides were counterstained with DAPI, and representative images were acquired with a Leica upright microscope. TUNEL-positive cells were manually counted.

Immunohistochemistry for $CD4^+$ T cells

Cryosections were stained with rat anti-mouse CD4 (1:50; 553647, BD Pharmingen) followed by a goat anti-rat secondary antibody (1:25; 559286, BD Pharmingen). Sections were visualized using the Vectastain Elite ABC Kit with NovaRed chromogen (PK-6100; Vector Laboratories). $CD4^+$ cells were counted in the conjunctiva using digital images analyzed with NIS Elements software.

Flow cytometry of cervical lymph nodes

Single-cell suspensions from cervical lymph nodes were blocked with anti-CD16/32 and stained with FITC-conjugated anti-CD4 (clone GK1.5, BD Pharmingen). Propidium iodide was used to exclude dead cells. Flow cytometry was performed on a BD LSRII cytometer, and data were analyzed with FlowJo software (TreeStar Inc.).

RNA isolation and quantitative RT-PCR

Corneal epithelium and conjunctival tissues were harvested, and total RNA was extracted using the PicoPure RNA Isolation Kit (KIT0204; Arcturus, USA). Complementary DNA was synthesized with a reverse transcription kit (RR047A; TaKaRa, Shiga, Japan). qRT-PCR was performed using SYBR Premix Ex Taq (RR420A; TaKaRa) on a StepOne™ Real-Time PCR System (Applied Biosystems). Expression levels of IL-17A, IFN-γ, IL-13, MMP-3, and MMP-9 were normalized to β-actin and analyzed with the 2^-ΔΔCt method. Primer sequences are listed in **Table 1**.

ELISA for cytokine quantification

Protein was extracted from conjunctival tissues using cold RIPA buffer (R0278; Sigma). Total protein concentrations were determined by BCA assay (23225; ThermoFisher Scientific, MA, USA). ELISA kits for mouse IL-17A (BMS6001), IL-13 (BMS6015), and IFN-γ (BMS606) were used according to the manufacturer's protocols (eBioscience, San Diego, CA, USA). Absorbance at 450 nm was measured with a BioTek ELx800 microplate reader,

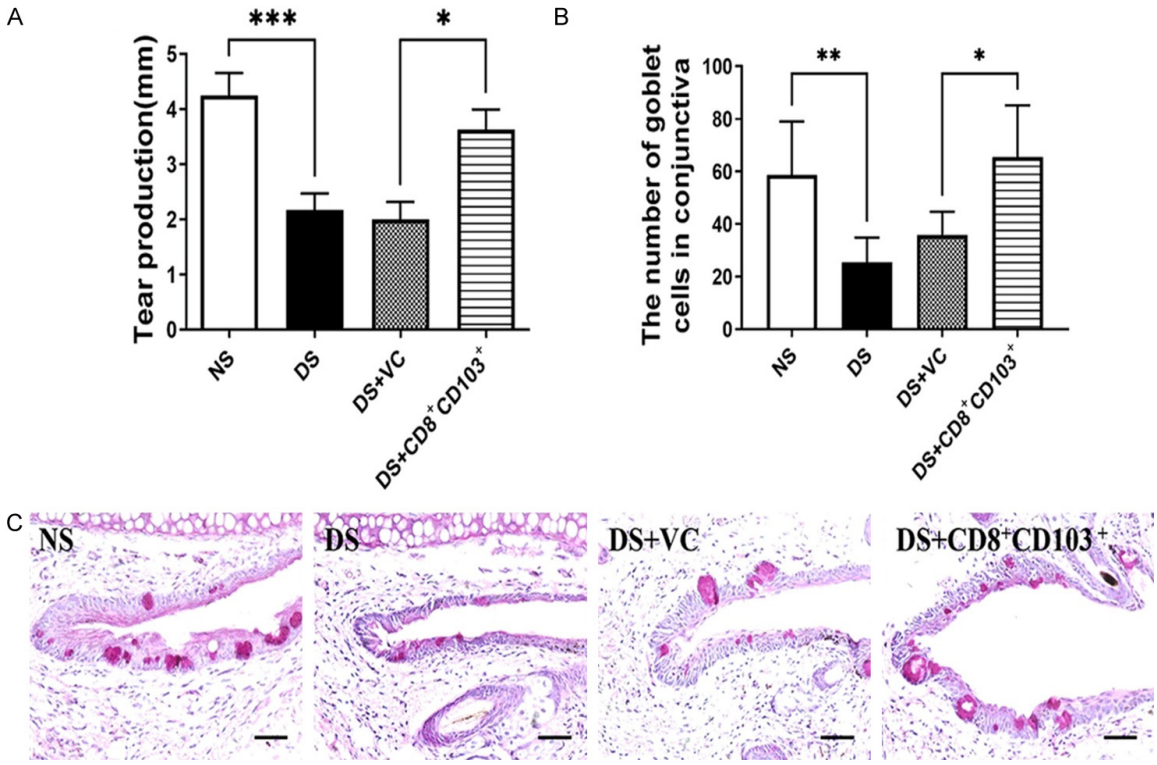

Figure 1. The effects of adoptive transfer of $CD8^+CD103^+$ T cells on tear production and goblet cell loss during DS. Tear production measured by phenol red thread test (A). The mean goblet cell numbers (B) and representative images of PAS staining (C) in conjunctiva. Data were shown as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: 50 μm.

and cytokine concentrations were calculated based on standard curves.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). A *p*-value ≤ 0.05 was considered statistically significant.

Results

$CD8^+CD103^+$ T cell transfer enhances tear production under desiccating stress

To evaluate the functional effects of adoptively transferred $CD8^+CD103^+$ T cells on lacrimal gland activity during dry eye induction, tear secretion was quantified using the phenol red thread test. Mice subjected to desiccating stress (DS) showed a significant reduction in tear production compared with non-stressed (NS) controls (**Figure 1A**; P < 0.01). However, adoptive transfer of $CD8^+CD103^+$ T cells significantly restored tear production in DS-exposed mice (P < 0.01 vs. DS group), indicating improved lacrimal function.

$CD8^+CD103^+$ T cells preserve conjunctival goblet cells during DS

PAS staining was performed to assess conjunctival goblet cell density, a key marker of ocular surface health. DS exposure resulted in significant goblet cell loss compared with NS controls (**Figure 1B**, **1C**; P < 0.05). In contrast, mice receiving $CD8^+CD103^+$ T cell transfer exhibited marked preservation of goblet cell numbers (P < 0.01 vs. DS group), indicating protection of conjunctival mucin-secreting cells under environmental stress.

$CD8^+CD103^+$ T cells maintain corneal barrier integrity

To assess corneal epithelial integrity, OGD staining was conducted. DS exposure signifi-

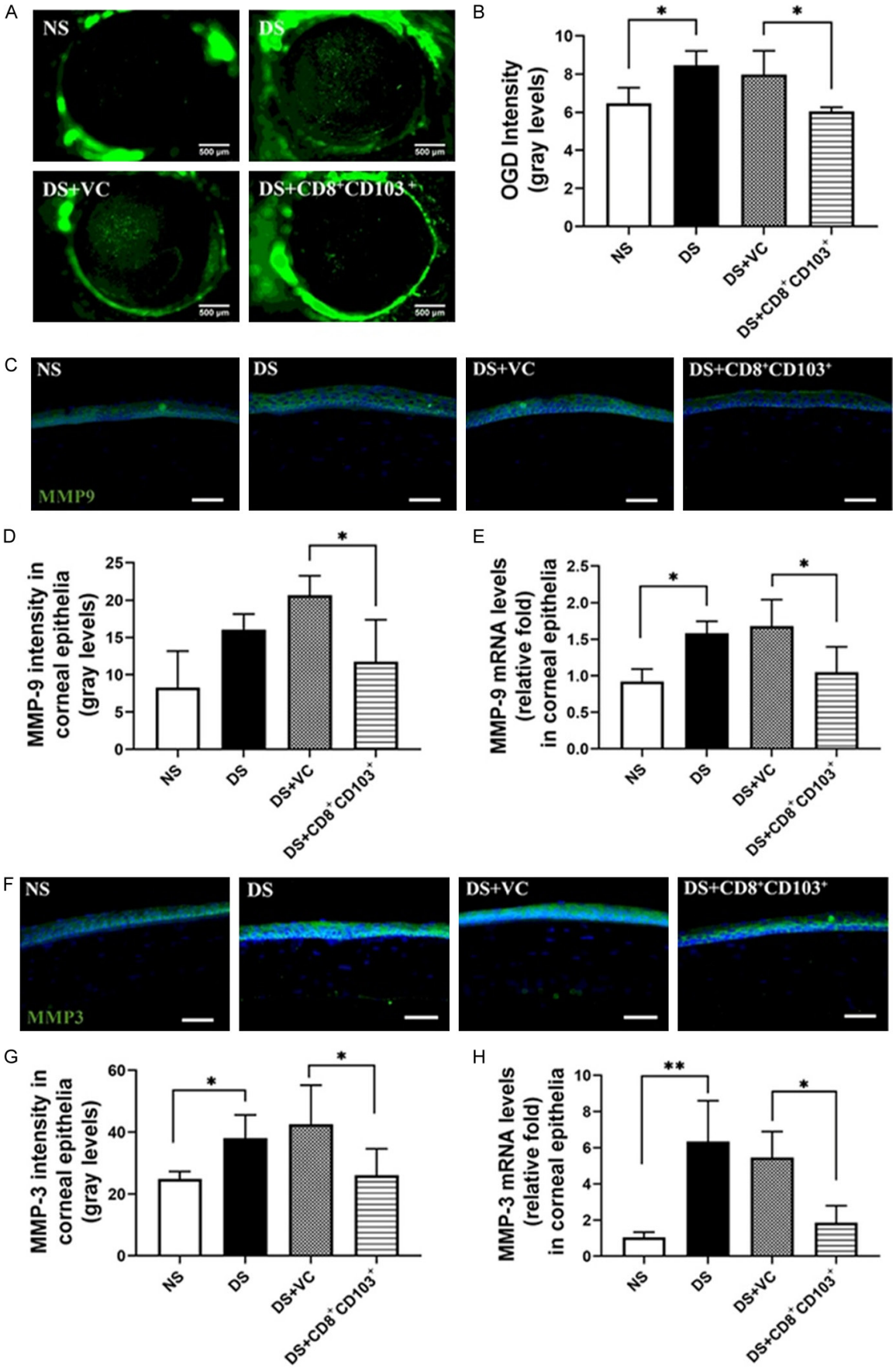
A
NS
DS
DS+VC
DS+CD8+CD103+
500 μm
B
OGD Intensity (gray levels)
C
MMP9
D
MMP-9 intensity in corneal epithelia (gray levels)
E
MMP-9 mRNA levels (relative fold) in corneal epithelia
F
MMP3
G
MMP-3 intensity in corneal epithelia (gray levels)
H
MMP-3 mRNA levels (relative fold) in corneal epithelia

Figure 2. The effects of $CD8^+CD103^+$ T cells treatment on corneal barrier function during DS. Representative images (A) and mean intensity (B) of OGD staining. Representative images (C) and mean intensity (D) of MMP-9 immunofluorescence staining. The level of MMP-9 mRNA (E) of corneal epithelium. Representative images (F) and mean intensity (G) of MMP-3 immunofluorescence staining. The level of MMP-3 mRNA (H) of corneal epithelium. Data was shown as mean ± SD. *P < 0.05, **P < 0.01. Scale bars: 50 μm.

cantly increased corneal permeability compared with NS controls (**Figure 2A**, **2B**; P < 0.05), indicating barrier disruption. $CD8^+CD103^+$ T cell-treated mice showed significantly reduced corneal permeability, as reflected by decreased OGD staining intensity (P < 0.01 vs. DS group).

Since matrix metalloproteinases (MMPs) are implicated in corneal barrier breakdown, the expression of MMP-3 and MMP-9 was assessed using immunofluorescence and qRT-PCR. DS exposure caused a significant upregulation of both MMP-3 and MMP-9 protein and mRNA expression, while $CD8^+CD103^+$ T cell transfer significantly suppressed these increases (**Figure 2C-H**; P < 0.01), supporting a protective effect on epithelial homeostasis.

$CD8^+CD103^+$ t cells attenuate ocular surface apoptosis

To evaluate the anti-apoptotic effects of $CD8^+CD103^+$ T cells, TUNEL staining was performed on ocular surface tissues. DS resulted in increased apoptosis in the corneal epithelium and conjunctiva, with a higher number of TUNEL-positive cells (**Figure 3A-C**; P < 0.01). $CD8^+CD103^+$ T cell treatment significantly reduced apoptotic cell numbers (P < 0.01).

Consistent with these findings, immunofluorescence staining for cleaved caspase-3 and caspase-8 revealed significantly decreased signals in $CD8^+CD103^+$ T cell-treated mice compared to DS controls (**Figure 3D-I**; P < 0.05 to P < 0.001), indicating suppression of both intrinsic and extrinsic apoptotic pathways.

$CD8^+CD103^+$ T cells suppress $CD4^+$ T cell infiltration and activation

To investigate the immunoregulatory effects of $CD8^+CD103^+$ T cells, $CD4^+$ T cell infiltration in the conjunctiva and cervical lymph nodes was examined. Immunohistochemistry revealed a significant increase in $CD4^+$ T cells in the conjunctiva of DS mice, which was markedly reduced by $CD8^+CD103^+$ T cell transfer (**Figure 4A**, **4B**; P < 0.01). Flow cytometry of draining cervical lymph nodes also showed a significant reduction in $CD4^+$ T cell frequencies in the treated group (**Figure 4C**, **4D**; P < 0.01).

$CD8^+CD103^+$ T cells modulate cytokine production at the ocular surface and in lymphoid tissues

Given the importance of cytokines in DED pathogenesis, we measured IL-17A, IFN-γ, and IL-13 levels in conjunctival tissues and cervical lymph nodes using qRT-PCR and ELISA. DS exposure significantly increased IL-17A and IFN-γ expression while reducing IL-13 levels, reflecting a shift toward a pro-inflammatory Th1/Th17 response (**Figure 5A-I**).

Adoptive transfer of $CD8^+CD103^+$ T cells reversed this imbalance, significantly downregulating IL-17A and IFN-γ (P < 0.01) and upregulating IL-13 (P < 0.05 to P < 0.01) in both conjunctival tissues and draining lymph nodes. These findings suggest that $CD8^+CD103^+$ T cells promote a regulatory cytokine environment that supports ocular surface homeostasis.

Adverse reaction indicators

To evaluate the safety of $CD8^+CD103^+$ T cell therapy, we monitored body weight, signs of infection, intraocular pressure, corneal sensitivity, and corneal nerve density. As shown in **Table 2**, no significant differences were observed in any of these parameters among the groups. Body weight remained stable throughout the experiment, and no clinical signs of ocular or systemic infection were detected in the $CD8^+CD103^+$ T cell-treated mice. IOP, corneal sensitivity, and corneal nerve density (βIII-tubulin staining) were comparable across all groups, indicating that adoptive transfer of $CD8^+CD103^+$ T cells did not induce adverse reactions.

Tear volume and circadian variation

To determine whether circadian rhythm influenced tear secretion, we measured tear volume at four time points across the 24-hour

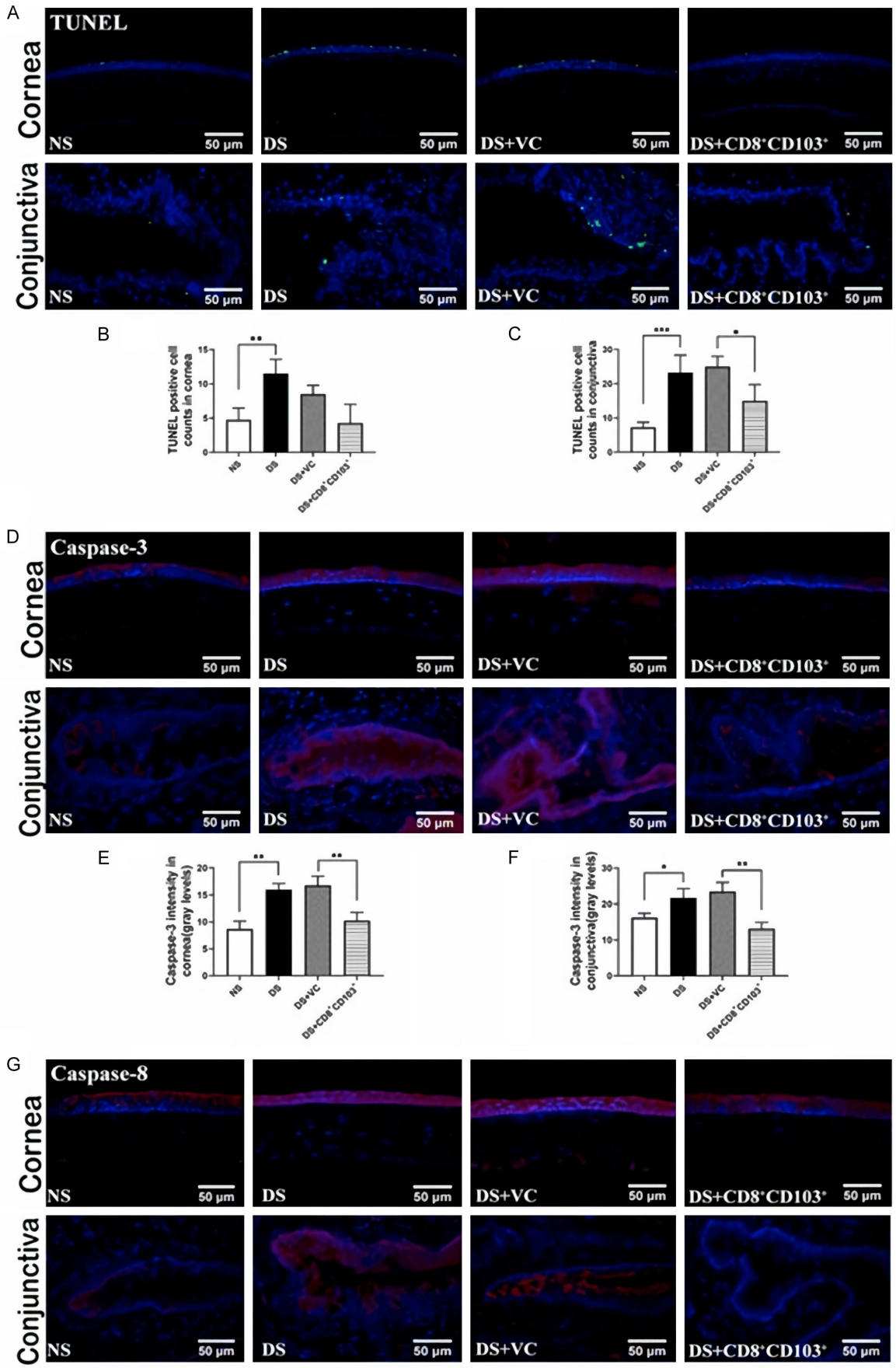
A
TUNEL
Cornea
Conjunctiva
NS
DS
DS+VC
DS+CD8+CD103+
50 μm
B
TUNEL positive cell counts in cornea
C
TUNEL positive cell counts in conjunctiva
D
Caspase-3
E
Caspase-3 intensity in corneal(gray levels)
F
Caspase-3 intensity in conjunctival(gray levels)
G
Caspase-8

Figure 3. The effects of $CD8^+CD103^+$ T cells treatment on DS-induced apoptosis in ocular surface. (A) Representative images for TUNEL staining in corneal epithelium and conjunctiva; The number of TUNEL-positive cells in corneal epithelium (B) and conjunctiva (C); (D) Representative merged images of active (AC)-Caspase-3 (red) immunofluorescent staining in corneal epithelium and conjunctiva with DAPI counterstaining (blue) in nucleus; The immunofluorescence intensity of AC-Caspase-3 in corneal epithelium (E) and conjunctiva (F); (G) Representative merged images of AC-Caspase-8 (red) immunofluorescent staining in corneal epithelium and conjunctiva with DAPI counterstaining (blue) in nucleus; The immunofluorescence intensity of AC-Caspase-8 in corneal epithelium (H) and conjunctiva. (I) Data was shown as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: 50 μm.

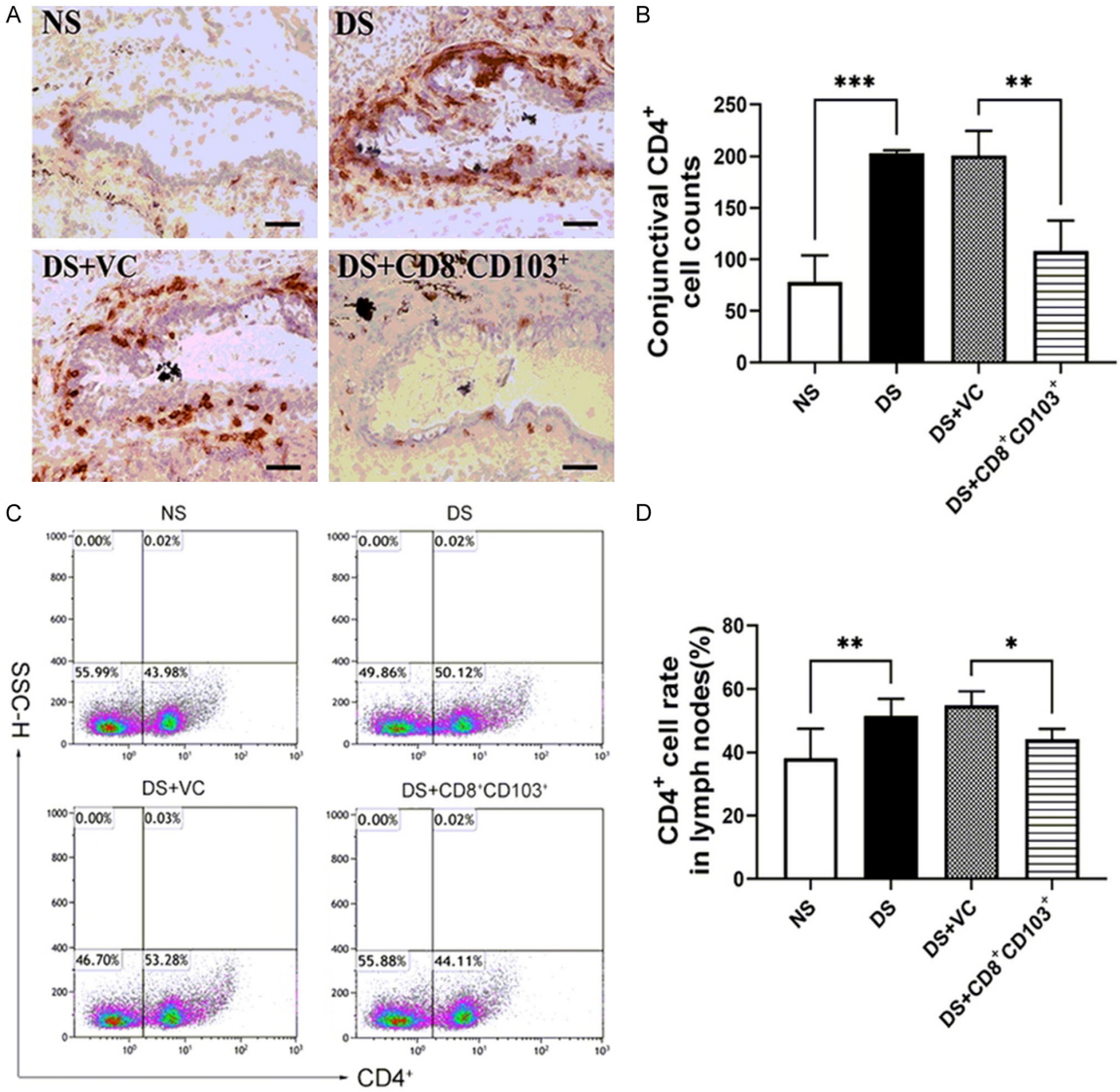

Figure 4. The effects of $CD8^+CD103^+$ T cells treatment on $CD4^+$ T cells infiltration in conjunctiva and generation in the draining cervical lymph nodes. Representative images of CD4 staining (A) and the number of $CD4^+$ T cells (B) in conjunctiva. (C) Representative dot plots of $CD4^+$ lymphocytes in draining CLN. (D) Mean ± SD of flow cytometry analysis of $CD4^+$ lymphocytes in CLN. Data was shown as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars: 50 μm.

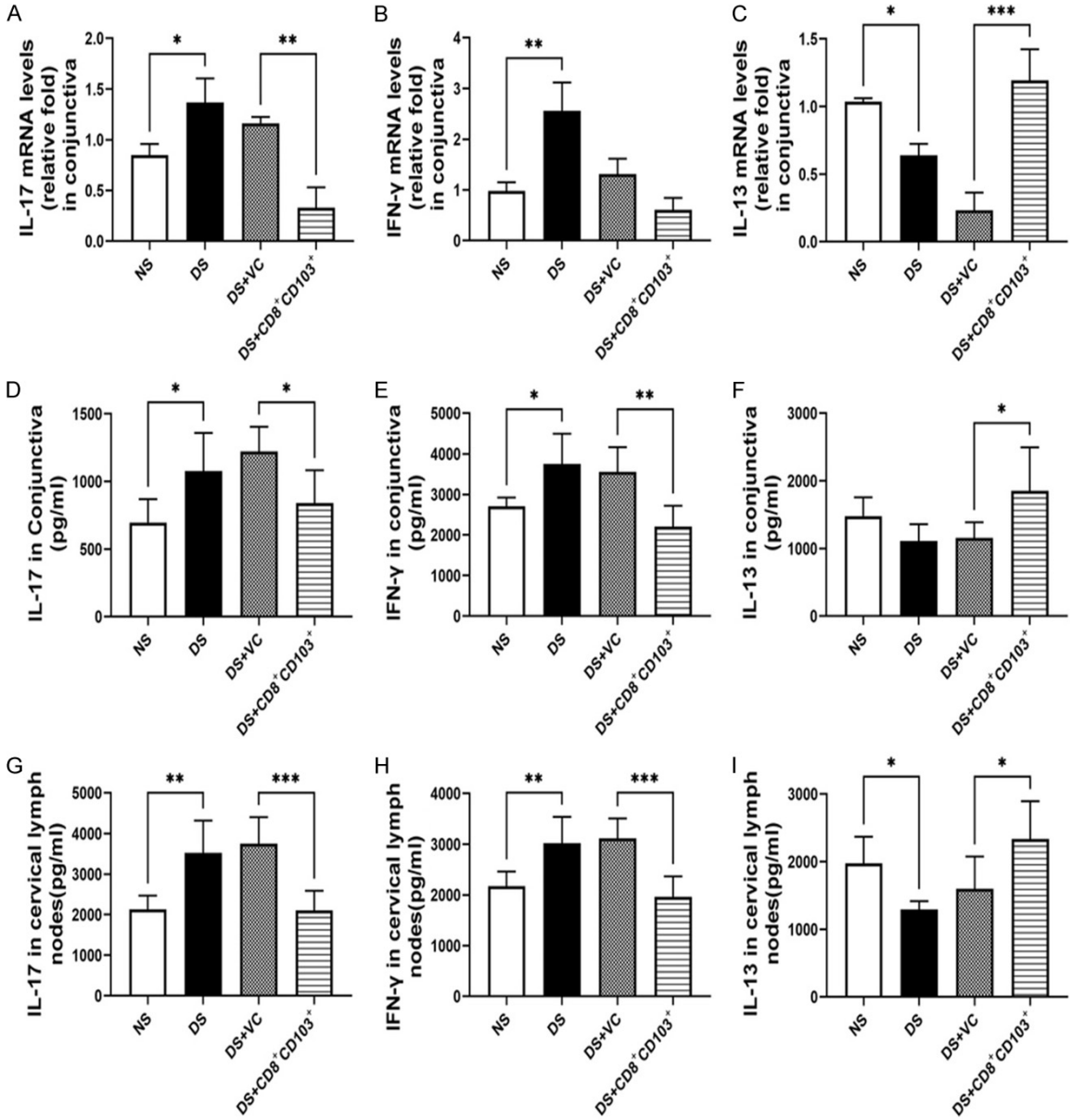

Figure 5. The effects of $CD8^+CD103^+$ T cells treatment on Th cytokines production in conjunctiva and cervical lymph nodes. The mRNA levels of IL-17A (A), IFN-γ (B) and IL-13 (C) in conjunctiva; The protein levels of IL-17A (D), IFN-γ (E) and IL-13 (F) in conjunctiva; The protein levels of IL-17A (G), IFN-γ (H) and IL-13 (I) in CLN. Data was shown as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

light-dark cycle. The tear secretion in normal control mice remained stable throughout the circadian cycle, with no significant intra-group fluctuations. In contrast, DS-exposed mice demonstrated significantly reduced tear secretion compared with controls at all time points (P < 0.01). Importantly, adoptive transfer of $CD8^+CD103^+$ T cells significantly restored tear

Table 2. Adverse reaction indicators in mice following $CD8^+CD103^+$ T cell transfer under desiccating stress conditions

Parameter	Normal Saline (NS) Control (Mean ± SD)	DS + Vehicle (Mean ± SD)	DS + $CD8^+CD103^+$ T Cell Transfer (Mean ± SD)	*P* value (vs. DS + Vehicle)
Body Weight (g)	22.3 ± 1.1	21.8 ± 1.3	22.0 ± 1.2	0.64
Signs of Infection	None observed	Mild periocular redness (n=2)	None observed	-
Intraocular Pressure (mmHg)	15.1 ± 1.0	14.9 ± 1.2	15.0 ± 1.1	0.72
Corneal Sensitivity (mm filament, Cochet-Bonnet)	5.9 ± 0.3	5.7 ± 0.4	5.8 ± 0.4	0.58
Corneal Nerve Density (βIII-tubulin$^+$ fibers/mm^2)	128 ± 12	124 ± 14	127 ± 13	0.61

Table 3. Tear volume at different circadian time points in DS mice with or without $CD8^+CD103^+$ T cell transfer

Group	8:00 a.m. (lights on)	2:00 p.m. (light phase)	8:00 p.m. (lights off)	2:00 a.m. (dark phase)
Normal Control	1.15 ± 0.10 μL	1.12 ± 0.08 μL	1.14 ± 0.09 μL	1.16 ± 0.11 μL
DS + Vehicle	0.62 ± 0.07 μL	0.59 ± 0.08 μL	0.61 ± 0.09 μL	0.58 ± 0.07 μL
DS + $CD8^+CD103^+$ T Cell Transfer	0.96 ± 0.09 μL	0.94 ± 0.08 μL	0.95 ± 0.07 μL	0.97 ± 0.08 μL

volume toward normal levels across all circadian phases ($P < 0.05$ vs. DS + Vehicle). No significant circadian variation was detected within the DS + $CD8^+CD103^+$ T cell transfer group, indicating that the therapeutic effect of these cells on tear secretion was independent of circadian rhythm (**Table 3**).

Discussion

This study provides compelling evidence that $CD8^+CD103^+$ T cells exert a protective and immunomodulatory role in desiccating stress (DS)-induced DED. Through adoptive transfer experiments combined with detailed immunohistochemical and molecular analyses, we demonstrate that $CD8^+CD103^+$ T cells suppress $CD4^+$ T cell-mediated inflammation, preserve ocular surface architecture, and promote a cytokine profile that supports epithelial and goblet cell homeostasis. These findings provide mechanistic insights and suggest that $CD8^+CD103^+$ T cells may be a promising immunotherapeutic approach for DED and other T cell-mediated ocular surface disorders, as has been previously suggested in autoimmune and inflammatory conditions [9, 10, 21-24].

Chronic inflammation driven by autoreactive $CD4^+$ T cells is a well-established contributor to DED pathogenesis. In particular, Th1 and Th17 subsets secrete pro-inflammatory cytokines such as IFN-γ and IL-17A, which disrupt epithelial tight junctions, promote apoptosis, and enhance MMP expression, leading to corneal barrier dysfunction [7, 18, 26-29]. In our DS model, $CD8^+CD103^+$ T cell adoptive transfer significantly reduced IL-17A and IFN-γ levels in the conjunctiva and draining cervical lymph nodes, accompanied by suppression of MMP-3 and MMP-9 expression in the corneal epithelium. These findings suggest that $CD8^+CD103^+$ T cells can mitigate downstream effector pathways of Th1/Th17 responses, thereby supporting epithelial integrity in DS-induced DED. Similar mechanisms have been proposed in other inflammatory diseases, such as graft-versus-host disease and lupus nephritis, where $CD8^+CD103^+$ T cells modulate inflammatory responses and protect tissue integrity [12, 14, 30].

Furthermore, $CD8^+CD103^+$ T cells reduced ocular surface apoptosis, as evidenced by decreased TUNEL staining and reduced activation of caspase-3 and caspase-8. This aligns with prior studies demonstrating that IFN-γ promotes ocular epithelial apoptosis via caspase-8-dependent mechanisms in murine dry eye models [22, 37-39]. Our findings reinforce the central role of IFN-γ modulation by $CD8^+CD103^+$ T cells in mediating their anti-apoptotic and tissue-protective effects. The suppression of caspase-mediated apoptosis, a key feature of dry eye pathology, has also been shown to be critical in other immune-driven disorders [16, 17].

Another hallmark of DED is conjunctival goblet cell depletion, which compromises mucin production and destabilizes the tear film. This process is linked to an imbalance between IL-13 and IFN-γ, with the latter promoting goblet cell apoptosis and interfering with IL-13-mediated differentiation pathways [31-36]. In our study, $CD8^+CD103^+$ T cell therapy restored IL-13 levels while reducing IFN-γ, correlating with significant preservation of goblet cell density. These data indicate that $CD8^+CD103^+$ T cells help re-establish a Th2/Th1 cytokine balance and support the mucin-secreting cell populations essential for ocular surface health. Similar findings have been reported in autoimmune models, where modulation of IL-13 was critical for maintaining goblet cell integrity and promoting tissue healing [6, 28].

Our findings suggest that MMP downregulation correlates with barrier protection, and IL-13 upregulation correlates with goblet cell retention. However, while these associations were observed in our study, causal validation of these relationships was not performed. The observed MMP downregulation may protect the corneal barrier by preventing extracellular matrix degradation, and the upregulation of IL-13 may help maintain goblet cell integrity. Nevertheless, these relationships are correlational, and further experiments are needed to establish causal links. Specifically, functional inhibition of MMPs and IL-13, or using gene knockout models, will be necessary to confirm whether MMP suppression directly leads to barrier protection and whether IL-13 upregulation is causally linked to goblet cell preservation. Previous studies on matrix metalloproteinases have shown their critical role in barrier disruption, and similar causal tests should be conducted in future studies to confirm these findings [19, 20].

Given the promising nature of these findings, we propose that future studies include experiments that test the causal relationships by inhibiting MMPs or IL-13 and measuring the direct impact on barrier integrity and goblet cell retention. Gene knockout models or neutralizing antibodies targeting these pathways could be particularly useful in validating the causal role of these molecules. We also recommend utilizing in vivo models that allow for precise control over MMP and IL-13 levels to more definitively determine their roles in the observed protective effects. This approach has been successful in models of other immune-mediated diseases, including allergic inflammation and autoimmune diseases [12, 15, 24].

Importantly, we observed that $CD8^+CD103^+$ T cells decreased $CD4^+$ T cell infiltration in the conjunctiva and reduced $CD4^+$ T cell frequencies in cervical lymph nodes, suggesting regulatory effects not only locally at the ocular surface but also systemically through modulation of T cell priming or expansion in secondary lymphoid organs. These results align with prior reports that $CD8^+CD103^+$ Tregs can suppress effector T cell responses both in peripheral and tissue compartments [15, 24, 25]. Their ability to localize to epithelial tissues via CD103 (integrin αE) expression further supports their role as tissue-resident immune regulators. Their enrichment at mucosal surfaces allows them to exert contact-dependent and cytokine-mediated suppression of pathogenic effector T cells within inflammatory microenvironments. While most ocular Treg research has focused on classical $CD4^+FoxP3^+$ cells, our findings highlight the unique and underexplored immunoregulatory potential of $CD8^+CD103^+$ T cells in ocular autoimmunity [9, 10].

Our findings suggest that $CD8^+CD103^+$ T cells may exert immunomodulatory effects, similar to Tregs, by suppressing $CD4^+$ T cell infiltration and modulating cytokine profiles. These cells showed promising results in ameliorating inflammation and protecting ocular surface integrity in our DED model. However, it is important to note that in vitro assays examining classical Treg markers such as IL-10, TGF-β, CTLA-4, PD-1, and CD39/CD73 are required to fully classify these cells as regulatory T cells. Additionally, these cells are likely tissue-resident memory cells (TRM), which act locally in the tissue to provide immune surveillance and modulation, rather than exhibiting the broad regulatory functions typical of Tregs. The present study provides preliminary evidence for the immunomodulatory role of $CD8^+CD103^+$ T cells, but further molecular studies are necessary to delineate their full regulatory capacity [9, 11, 12].

Limitations

Our findings suggest that $CD8^+CD103^+$ T cells may exert immunomodulatory effects, similar

to Tregs, by suppressing $CD4^+$ T cell infiltration and modulating cytokine profiles. These cells showed promising results in ameliorating inflammation and protecting ocular surface integrity in our dry eye disease model. However, it is important to note that in vitro assays examining classical Treg markers such as IL-10, TGF-β, CTLA-4, PD-1, and CD39/CD73 are required to fully classify these cells as regulatory T cells. The present study provides preliminary evidence for the immune-regulatory role of $CD8^+CD103^+$ T cells, but further molecular studies are necessary to delineate their full regulatory capacity and better understand their mechanistic pathways in immune regulation.

Despite these promising findings, several limitations must be acknowledged. First, this study was performed in a murine DS model, which may not fully recapitulate the chronicity, heterogeneity, and immunological complexity of human DED, particularly autoimmune forms such as Sjögren's syndrome. Second, while we demonstrated the immunosuppressive activity of $CD8^+CD103^+$ T cells, we did not perform functional inhibition or depletion experiments (e.g., blocking CD103 or TGF-β pathways) to definitively identify their mechanisms of action. Third, we did not track the in vivo migration and persistence of transferred T cells, leaving their homing patterns and tissue residency undetermined. Additionally, cytokine profiling was limited to a focused panel (IL-17A, IFN-γ, and IL-13), and a broader analysis of other immune mediators would provide a more comprehensive understanding of their immunomodulatory profile. Finally, the absence of human samples or patient-derived immune cells limits the immediate translational relevance of these results and warrants follow-up studies in human systems.

Future directions

To advance our understanding of $CD8^+CD103^+$ regulatory T cells in ocular immunity and dry eye pathogenesis, future studies should delineate the molecular and cellular mechanisms by which these cells exert their immunosuppressive effects. This includes characterization of their cytokine secretion profiles, their modes of interaction with effector T cells and antigen-presenting cells, and their dependence on mediators such as IL-10, TGF-β, and PD-1/PD-L1. In vivo tracking using fluorescent or reporter-labeled cells will be essential to confirm their migration patterns and persistence at the ocular surface. Studies involving human ocular tissues or patient-derived T cell populations are critical to validate their clinical relevance. Moreover, therapeutic strategies to enhance $CD8^+CD103^+$ Treg function - whether through adoptive transfer, pharmacological induction, or gene editing - should be explored. High-dimensional immune profiling, including single-cell transcriptomics and spatial mapping, may provide further insight into their integration within ocular immune networks. Finally, evaluating the combination of $CD8^+CD103^+$ T cell-based interventions with current DED therapies may help optimize treatment efficacy and establish long-term immune tolerance.

Conclusion

This study demonstrates that adoptive transfer of $CD8^+CD103^+$ regulatory T cells confers robust protection against desiccating stress-induced dry eye disease in a murine model. These cells effectively restored tear production, preserved conjunctival goblet cell density, maintained corneal epithelial barrier integrity, and reduced ocular surface apoptosis. Mechanistically, $CD8^+CD103^+$ T cells modulated the local and systemic immune environment by suppressing $CD4^+$ T cell infiltration and rebalancing cytokine profiles, attenuating pro-inflammatory mediators such as IL-17A and IFN-γ while upregulating the protective cytokine IL-13. These findings provide strong evidence for the immunoregulatory role of $CD8^+CD103^+$ T cells in mitigating $CD4^+$ T cell-mediated ocular inflammation and preserving epithelial homeostasis. Collectively, this work supports $CD8^+CD103^+$ T cells as a promising immunotherapeutic target for dry eye disease and other mucosal autoimmune disorders. Further studies are warranted to clarify their mechanisms of action and explore their translational potential in human ocular surface diseases.

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Disclosure of conflict of interest

None.

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