Blockade of Prolymphangiogenic VEGF-C suppresses Dry Eye Disease

Sunali Goyal MD
Mentor: Reza Dana, MD, MPH, MSc

Claes Dohlman Chair in Ophthalmology
Director, Cornea & Refractive Surgery
Massachusetts Eye and Ear Infirmary
Laboratory of Immunology, Schepens Eye Research Institute
Harvard Department of Ophthalmology

Disclosures

- Anti-VEGF-C Ab provided by Vegenics
- Schepens Eye Research Institute has rights for use of lymphatic blockade in Dry Eye Disease
Dry eye is a multifactorial disease of the tears and the ocular surface.
Dry Eye Disease (DED)

Epidemiology

- ~ 5 million people > 50 yrs in US affected with severe dry eyes (WHS, PHS)

- DED severely affects the quality of life

- Current therapeutic options limited and costly
  - Topical cyclosporine-A (Restasis®) approved treatment for DED in US only
Immunopathogenesis of DED

- Pathogenesis not fully understood

- Ocular surface inflammation sustained by ongoing activation and infiltration of pathogenic immune cells

- Most recently strong evidence of involvement of lymph nodes and CD4+ T cells
Immunopathogenesis of DED

Hypothesis- 1

DED leads to development of lymphatic vessels in the cornea
EXPERIMENT 1

Demonstration of lymphatics
Evidence of Corneal Lymphangiogenesis in DED

Goyal et al; Corneal Lymphangiogenesis in Dry Eye Disease. Arch Ophthalmol. 2010

CD31, LYVE-1 staining (Epifluorescent Microscope)
Expression levels of VEGFs and VEGFRs in DE corneas using Real-Time PCR

Increased transcript expression of VEGF-C, VEGF-D and VEGFR-3

Goyal et al, Corneal Lymphangiogenesis in Dry Eye Disease. Arch Ophthalmol. 2010
Evidence of Corneal Lymphangiogenesis in Dry Eye Disease

A Potential Link to Adaptive Immunity?

Sunali Goyal, MD; Sunil K. Chauhan, PhD; Jaafar El Annan, MD; Nambi Nallasamy, AB; Qiang Zhang, MD; Reza Dana, MD, MSc, MPH

**Conclusion:** Low-grade inflammation associated with DED is an inducer of lymphangiogenesis without accompanying hemangiogenesis.

*Arch Ophthalmol.* 2010;128(7):819-824
The VEGF Family and Its Receptors: Central Mediator of Angiogenesis

PIGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D

VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), VEGFR-3 (Flt-4)

Angiogenesis, Lymphangiogenesis, Lymphangiogenesis

PIGF = placenta growth factor; VEGFR = VEGF receptor.
Hypothesis- 2

Targeting VEGF-C/D can have therapeutic implications in DED
METHODS
Induction of Dry Eye Disease
Induction of Dry Eye Disease

Experimental Dry Eye Murine Model
Antivascular endothelial growth factor (Anti-VEGF-C) treatment regimen

Experimental Design

• Three groups-
  • 1) Normal
  • 2) DE group treated with saline IP (control)
  • 3) DE group treated with Anti-VEGF-C Ab

• Daily IP anti-VEGF-C antibody / Normal saline from day -1 to day 13

• Dose: 400 μg (20mg/kg) in 100 μl of Normal Saline

Methods
Assessment of Corneal Surface

Corneal Fluorescein Staining

- National Eye Institute grading system (NEI)
- Total score 0-15
Methods

♦ Immunohistochemistry

• Monocyte/macrophage marker - rat anti-mouse CD11b-FITC (1:100)
• Pan-endothelial marker - goat anti-mouse CD31 FITC (1:100)
• Lymphatic endothelial marker - rabbit anti-mouse LYVE-1 (1:400)

✓ Blood vessels identified as CD31$^{\text{hi}}$/LYVE-1$^{-}$
✓ Lymph vessels identified as CD31$^{\text{lo}}$/LYVE-1$^{\text{hi}}$
✓ Macrophages can be LYVE-1 positive

♦ Morphometry of Lymphangiogenesis

• Automated image analysis program written using Mat lab
• Lymphatic Area (LA) - total surface area of the lymphatic vessels when projected into the plane of the image
• Lymphatic Caliber (LC) - measure of the diameters of the lymphatic vessels
RESULTS
Effect of anti-VEGF-C on lymphatics in DED

CD31, LYVE-1 staining (Epifluorescent Microscope)

Anti-VEGF-C decreased growth of corneal neo-lymphatics in DED
Anti-VEGF-C significantly decreased lymphatic caliber (LC) and lymphatic area (LA) in DED
Anti-VEGF-C significantly decreased VEGF-C, VEGF-D and VEGFR-3 in DED Corneas

Results

![Bar chart showing mRNA expression levels of VEGF-A, VEGF-C, VEGF-D, VEGFR-2, and VEGFR-3.](image)

- P = 0.023 for VEGFR-3
- P = 0.014 for VEGF-D
- P = 0.002 for VEGF-C

Comparison between Untreated and Anti-VEGF-C treatments.
Anti-VEGF-C significantly decreased inflammatory cytokines in Conjunctiva

**Results**

- IL1-α: P = 0.003
- IL1-β: P = 0.025
- IL-6: P = 0.005
- IL-17
Anti-VEGF-C treatment significantly decreased corneal fluorescein staining in DED.
Conclusions

- VEGF-C blockade led to significant improvement in DED reflected by:
  - Suppression of lymphatic growth
  - Decrease in expression of lymphangiogenic growth factors and receptors (VEGF-C, -D, -R3) in DE corneas
  - Decrease in corneal staining/epitheliopathy
  - Decrease in levels of inflammatory cytokines in the conjunctiva
Clinical Implications

- Targeting prolymphangiogenic factors/receptors potential therapeutic targets in DED

- What next?
Acknowledgements

• Research Support:
  – National Eye Institute/NIH
  – Research to Prevent Blindness
  – Vegenics

• Dr. Reza Dana
• Dr. Laurie Barber
• Dr. Richard A Harper
Objective: To determine whether blocking prolymphangiogenic factors such as vascular endothelial growth factor C (VEGF-C) would suppress alloimmunity in dry eye disease using a murine model.

Methods: The effects of intraperitoneal injections of 400 μg of anti–VEGF-C antibody (treated group) and intraperitoneal normal saline (untreated group) were evaluated in murine dry eyes induced by exposure to high-flow desiccated air in a controlled-environment chamber. Growth of lymphatic vessels and infiltration of macrophages were evaluated by immunohistochemistry using CD31 (panendothelial marker), lymphatic vessel endothelial receptor 1 (lymphatic endothelial marker), and CD11b (monocyte and macrophage marker). Real-time polymerase chain reaction was performed to quantify expression of different inflammatory cytokine transcripts in the conjunctiva and lymph nodes as well as vascular endothelial growth factors and their receptors (VEGF-A, VEGF-C, VEGF-D, VEGFR-2, and VEGFR-3) in the cornea.

Results: Blocking VEGF-C led to significant reductions in lymphatic caliber (P = .02) and lymphatic area (P = .006) in the corneas of mice with dry eye disease. In addition to significantly decreasing CD11b+ cells (P = .005), anti–VEGF-C treatment significantly decreased transcript levels of VEGF-C (P = .002), VEGF-D (P = .01), and VEGFR-3 (P = .02) in the corneas of the treated group. A significant decrease in expression of inflammatory cytokines in the conjunctiva (interleukin 1α, P = .001; interleukin 1β, P = .02; interleukin 6, P = .005) and lymph nodes (interferon γ, P = .008; interleukin 17, P = .003) was also seen with anti–VEGF-C treatment.

Conclusion: Treatment with anti–VEGF-C led to significant improvement in dry eye disease, reflected by a decrease in inflammation at the clinical, molecular, and cellular levels.

Clinical Relevance: Targeting prolymphangiogenic growth factors or their receptors could inhibit the trafficking of antigen-presenting cells to the draining lymph nodes and hence prove to be a potential therapeutic target for dry eye disease.