A POSSIBLE MECHANISM OF LONG-TERM INTRAOCULAR PRESSURE INCREASE AFTER REPEATED ANTI-VEGF INJECTIONS

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NONE OF THE AUTHORS HAVE ANY FINANCIAL INTERESTS OR ASSOCIATIONS RELATED TO THIS PRESENTATION
Summary

- Intravitreal injection of anti-VEGF drugs are known to cause sustained and delayed intraocular pressure increases in a considerable proportion of the patients. Several theories have been proposed to explain the mechanism of this IOP elevation, including, mechanical disruption of the trabecular meshwork during repeated IOP fluctuations; clogging of the trabecular meshwork by impurities or silicone oil droplets, intraocular inflammation or toxic effect of anti-VEGF drugs to trabecular meshwork cells. (PMID: 25905784, 29336897, 24393349, 5719991, 22990314)

- Herein, we investigated whether activation of the complement by anti-VEGF drugs at the trabecular meshwork and subsequent cell death may underlie the observed IOP increases with the long-term use of anti-VEGF agents.

- Surgical trabeculectomy specimens from 8 patients, 2 of whom had been receiving repeated (>6) anti-VEGF (aflibercept and/or bevacizumab) injections for the treatment of exudative macular degeneration for more than a year, were co-immunostained for complement activation (c3b, c4d, c5b-9, C3a) and trabecular meshwork / Schlemm’s canal endothelium-specific markers.

- Masked-quantification of the histological sections revealed a significant increase in complement activation (C5-9b: 15.7±6.3% vs. 6.1±2.4%, p:0.002; C3b: 18.2±7.0% vs. 5.6±4.9%, p:0.003) with aVEGF injection. The main target of complement activation was trabecular meshwork cells. Increase in C4d staining suggested complement activation through classical pathway (C4d: 6.8±1.5% vs. 1.9±0.4%, p:0.001). There was an increased expression of complement anaphylatoxin receptor C3aR expression with anti-VEGF injection (6.3±3.7% vs. 3.0±2.7%, p:0.04) indicating the role of complement in inflammation. Lack of difference in TUNEL staining (2.8±1.1% vs. 2.2±2.1%, p:0.58) showed that activation of the complement system was not related to its physiological scavenging task.

- **Conclusion**: Patients receiving anti-VEGF injection reveal increased complement activation in the trabecular meshwork. Activation of the complement system induces cell death and may incite an inappropriate and damaging inflammatory response through C3a receptor upregulation.
# Long-term IOP Increase with Anti-VEGF Injections

## How Common?

- 23.6% of the patients (vs. 13.6% of the controls) in MARINA and ANCHOR (PMID: 24393349)
- 9.5% of the patients (vs. 3.4% of the controls) in DRCR (PMID: 5719991)
- 2.6% of the injected eyes (vs. 1.5% of the fellow eyes) in IRIS Registry (PMID: 29336897)
- 2.6% - 14.8% of patients in 8 studies included in AAO Report (PMID: 30472176)

## Suggested Mechanisms

- Disruption of the trabecular meshwork by large IOP fluctuations occurring during injection
- Clogging of the trabecular meshwork by impurities and/or silicone oil particles
- Induction of inflammation at the trabecular meshwork
- Toxic effect of anti-VEGF drugs on trabecular meshwork

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**Proposed Alternative Mechanism**

**Hypothesis**

Long-term intraocular pressure increases after repeated injections of anti-VEGF drugs is due to cell death at trabecular meshwork and Schlemm’s canal via activation of the complement system.

- Complement components are present in the trabecular meshwork
- Trabecular meshwork cells express Fc receptors

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**Immunofluorescent studies on the trabecular meshwork in open-angle glaucoma**

M. Bruce Sheldrick, Ralph C. McGue, and John D. Shelburne.

The trabecular meshwork of eyes with open-angle glaucoma has been demonstrated to have an increase in gamma globulin and plasma cells, raising the question of an immunogenic mechanism in this disorder. In the present study, however, immunofluorescent assays on the trabecular meshwork of eyes with open-angle glaucoma were negative for specific immuno globulins and for complement components that would result specifically from an antigen-antibody reaction. The study fails to provide any evidence in support of an immunogenic mechanism in open-angle glaucoma.

Over a decade ago, Becker and co-workers raised the question of an immunogenic mechanism in open-angle glaucoma by demonstrating an increase in gamma globulin and plasma cells in the trabecular meshwork of eyes with this disorder. Despite growing evidence for immunologic mechanisms in many ocular disorders, additional evi-
METHODS

Trabeculectomy specimens from 8 patients, 2 of whom had been receiving repeated (>6) anti-VEGF (aflibercept and/or bevacizumab) injections for the treatment of exudative macular degeneration >1 year.

Immunostaining for markers of complement activation (c3b, c4d, c5b-9, C3a), Schlemm’s Canal Endothelium (von Willebrand factor) and Trabecular Meshwork Cells (neuron-specific enolase).

Masked Quantification and Analyses

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No aVEGF

With aVEGF

C5b-9 (MAC)
NSE (TMW Cells)

No aVEGF

With aVEGF

C3b

C3b
No aVEGF  
With aVEGF

C4d

Diagram showing the relationship between C4d and other components of the complement system.
IS IT PHYSIOLOGICAL SCAVENGING FUNCTION OF THE COMPLEMENT SYSTEM?
**CELL Endothelium**

**Surface Receptor**

**FcR**

**Antibody**

aVEGF
Avastin®
Eylea®

**Complement**

**Soluble VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**VEGF**

**FC**

**CELL**

TM
SC Endothelium
CAN A MONOCLONAL F(AB) FRAGMENT ACTIVATE COMPLEMENT?

VEGFR1 (FLT1)

VEGFR2 (KDR)

neuropilin-1

neuropilin-2

**Vascular Endothelial Growth Factor-A Increases the Aqueous Humor Outflow Facility**

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**Abstract:** In open-angle glaucoma, excessive inflammatory response involving cytokines and complement is activated primarily via the amplifying C3-C5 conversion. Excessive activation of complement amplification requires IgG-containing or F(ab)̊-containing immune complexes (IC) that capture dimeric C3b on one of their heavy chain or heavy chain fragments. The ability of IgG-IC to capture dimeric C3b by the Fab portion depends on an affinity for C3 within the Fab portion, but outside the antigens-binding region. This property is rare among IgG NAbs. In contrast to this, the lack of the Fc region renders the Fab regions of any F(ab)̊-IC accessible to noncovalent C3b, but dimeric C3b deposits only if Fab-γ IC form secondary IC with anti-C3 NAbs that rigidly the complex and thereby promote deposition of dimeric C3b. Both types of complexes, C3b-IgG-IC and
CONCLUSIONS

- A low-grade complement activation can be seen at the trabecular meshwork of patients with open angle glaucoma undergoing filtering surgery.

- Complement activation significantly increases after repeated intravitreal anti-VEGF injections and occurs more often on trabecular meshwork cells compared to Schlemm’s canal endothelium.

- Increased activation of the complement system can directly cause cell death through the formation of membrane attack complex (MAC) and may induce inflammation through C3a receptor upregulation.

- The most possible cause of complement activation is the formation of immune complexes through the interaction of the anti-VEGF drugs with VEGF in the aqueous and binding of these immune complexes to trabecular meshwork cells through Fc or VEGF receptors on the cell membrane.