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College of Physicians & Surgeons of Columbia University
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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

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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.

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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)

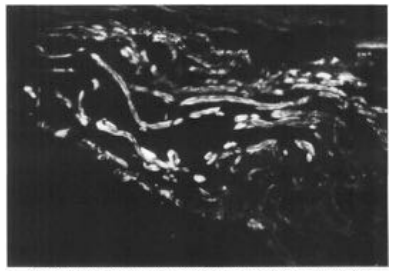
Suggested Mechanisms

- 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

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.

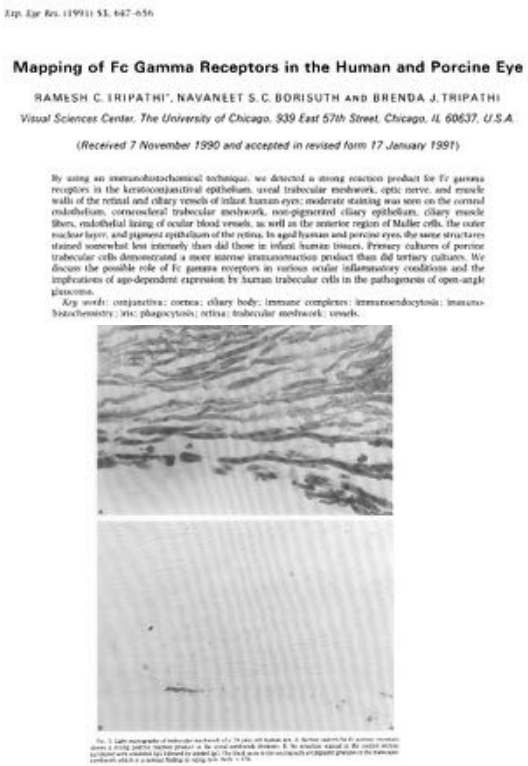


Immunofluorescent studies on the trabecular meshwork in open-angle glaucoma.

M. Bruce Shields, Ralph C. McCoy, 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, immunofluorescence assays on the trabecular meshwork of eyes with open-angle glaucoma were negative for specific immunoglobulins 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.[1, 2] Despite growing evidence for immunogenic mechanisms in many ocular disorders, additional evi-



Exp. Eye Res. (1991) 53, 647–656

Mapping of Fc Gamma Receptors in the Human and Porcine Eye

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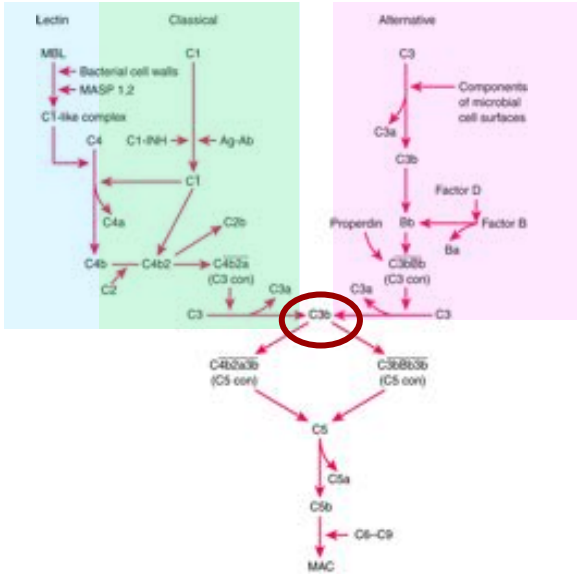

- Complement components are present in the trabecular meshwork
- Trabecular meshwork cells express Fc receptors

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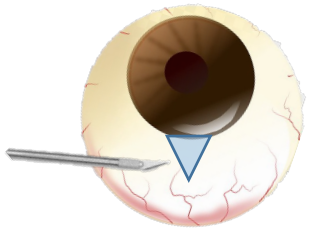
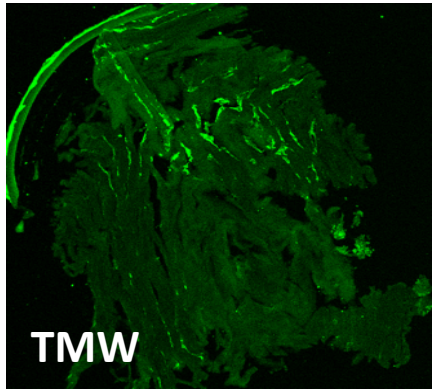
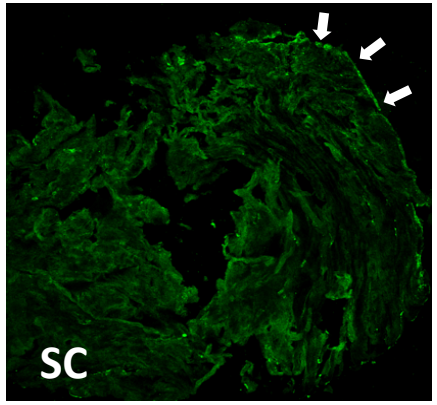
METHODS



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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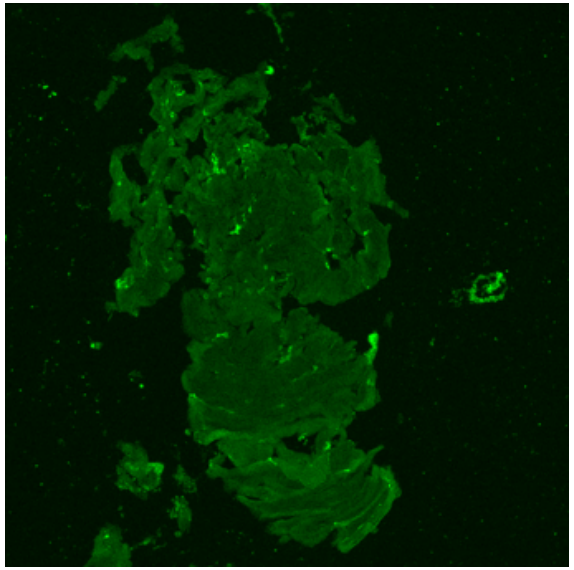
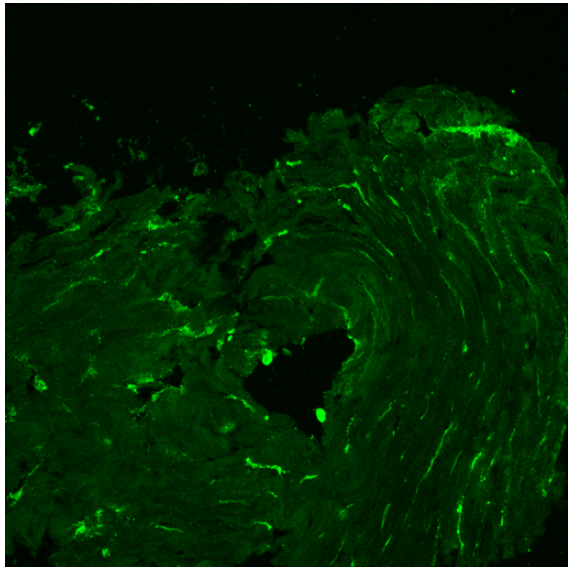
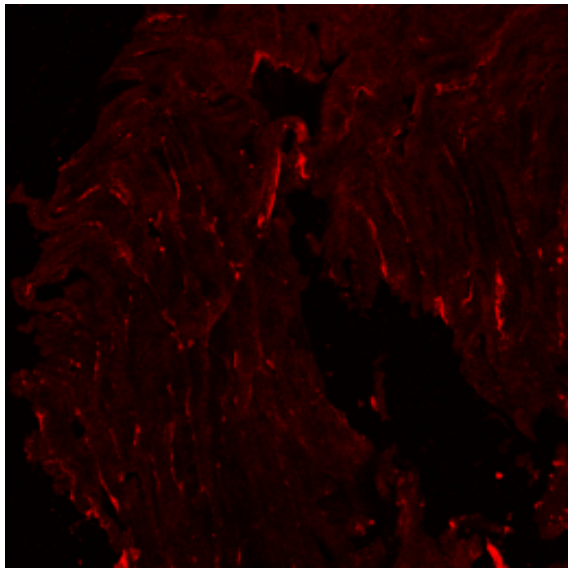
C5b-9 (MAC)

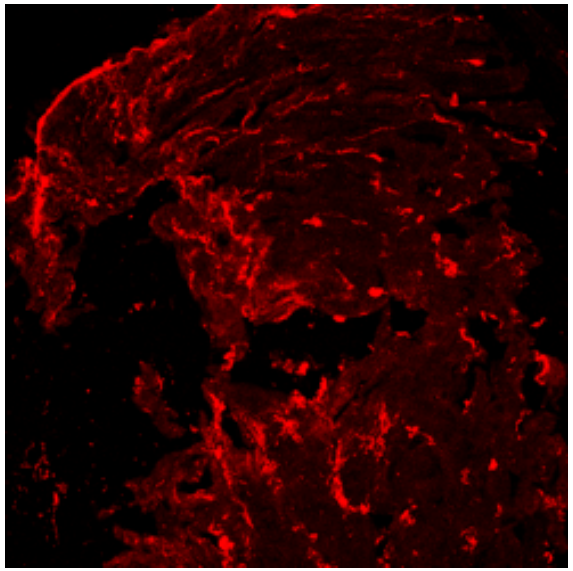
No aVEGF

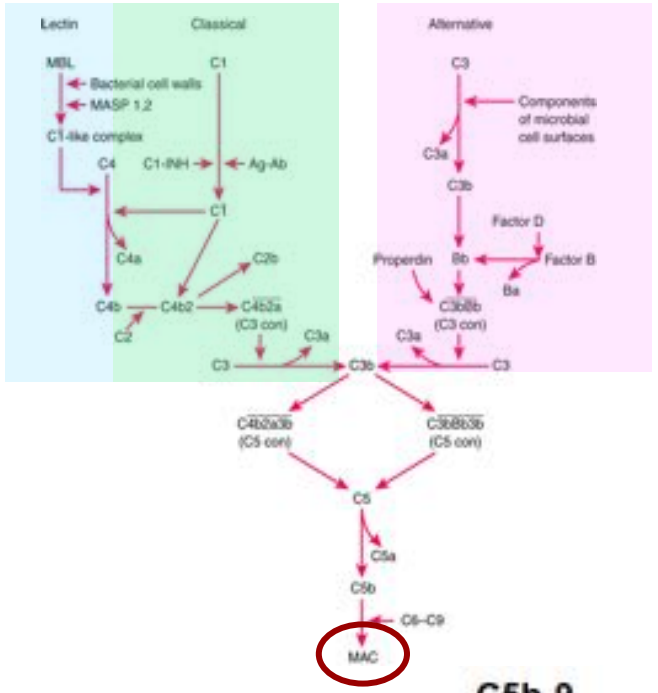
With aVEGF

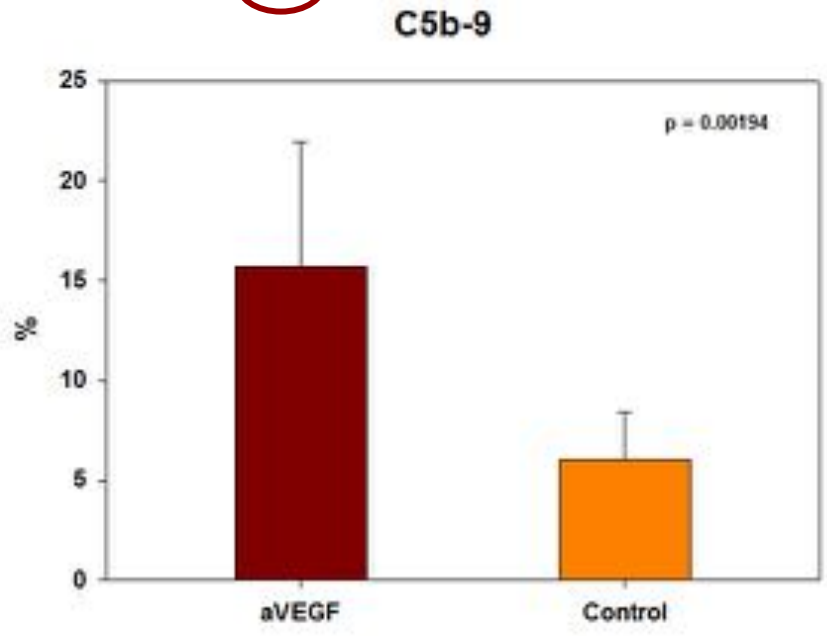








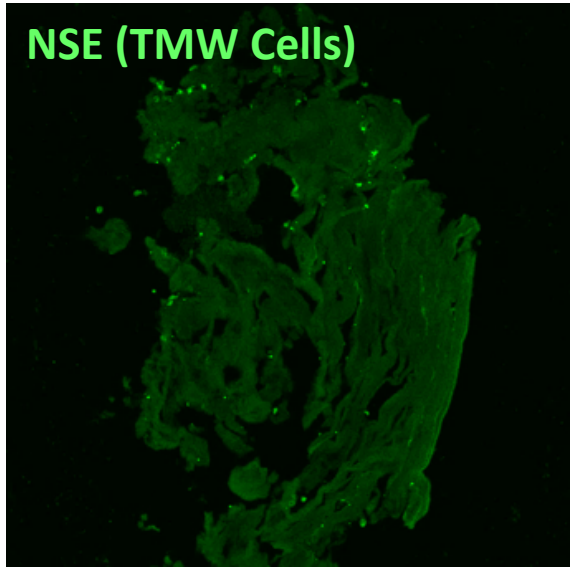


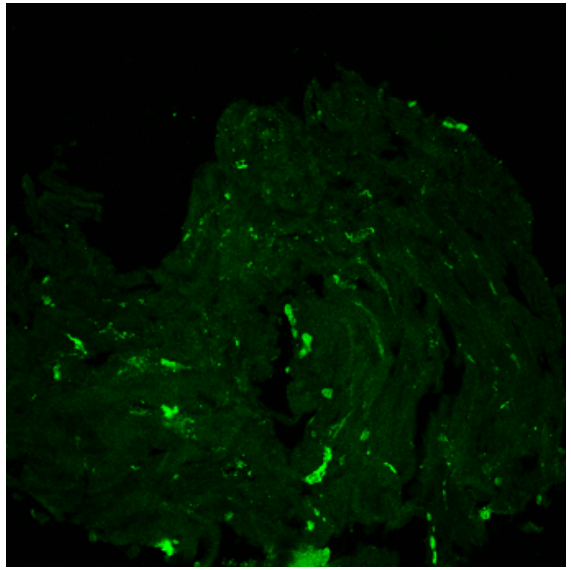


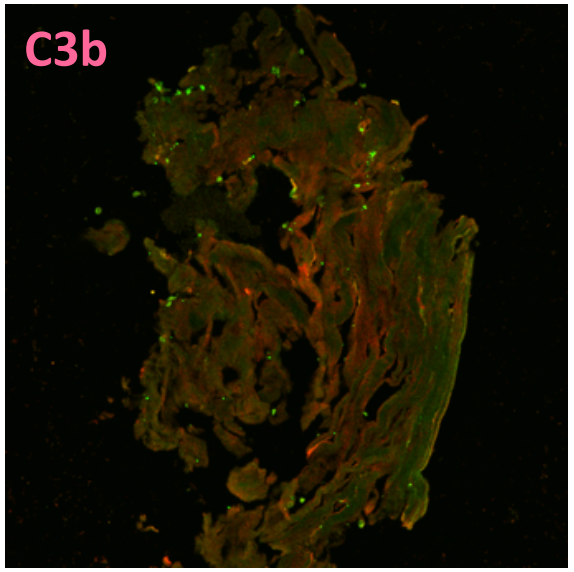
No aVEGF

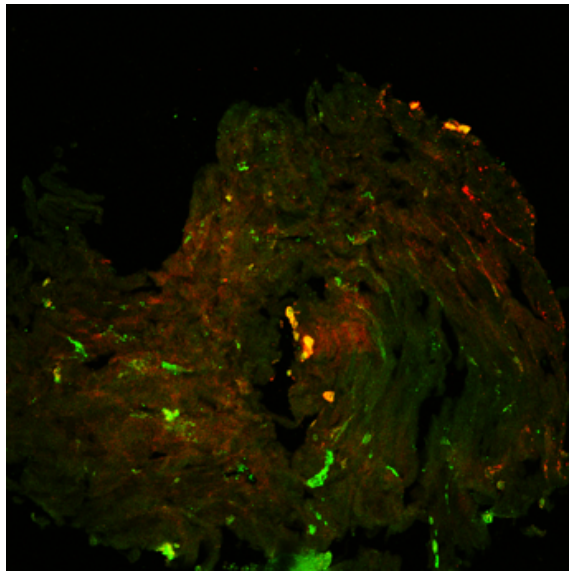
With aVEGF

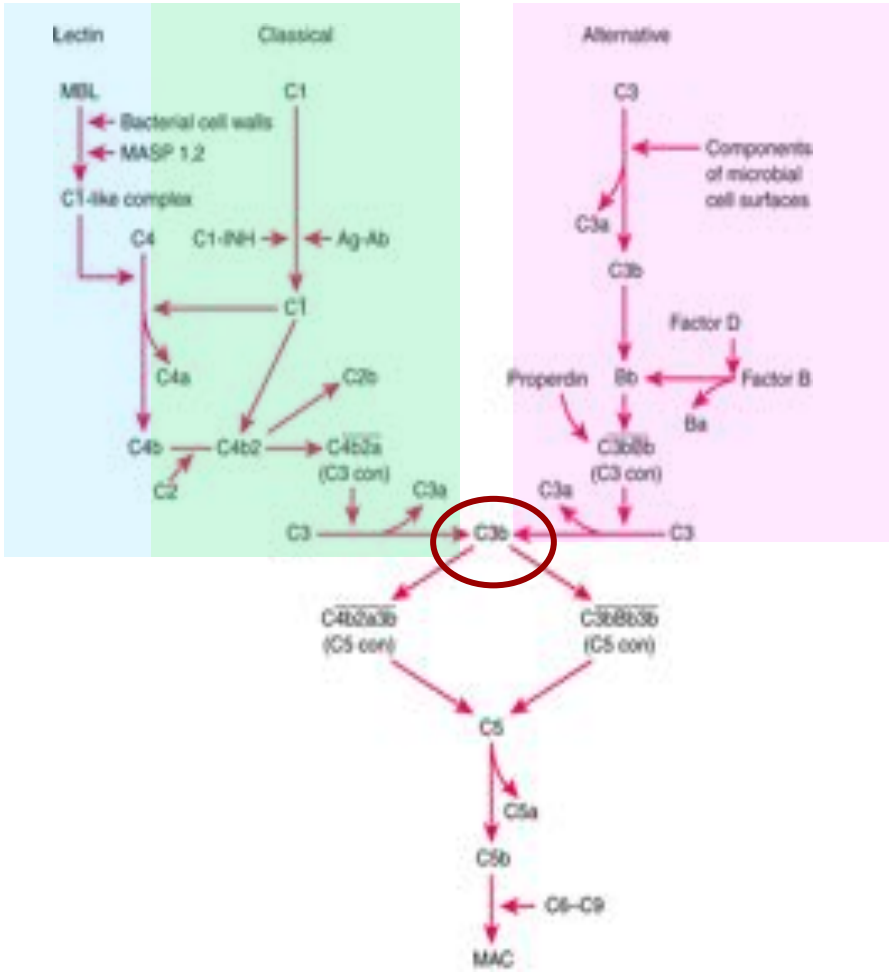
C3b







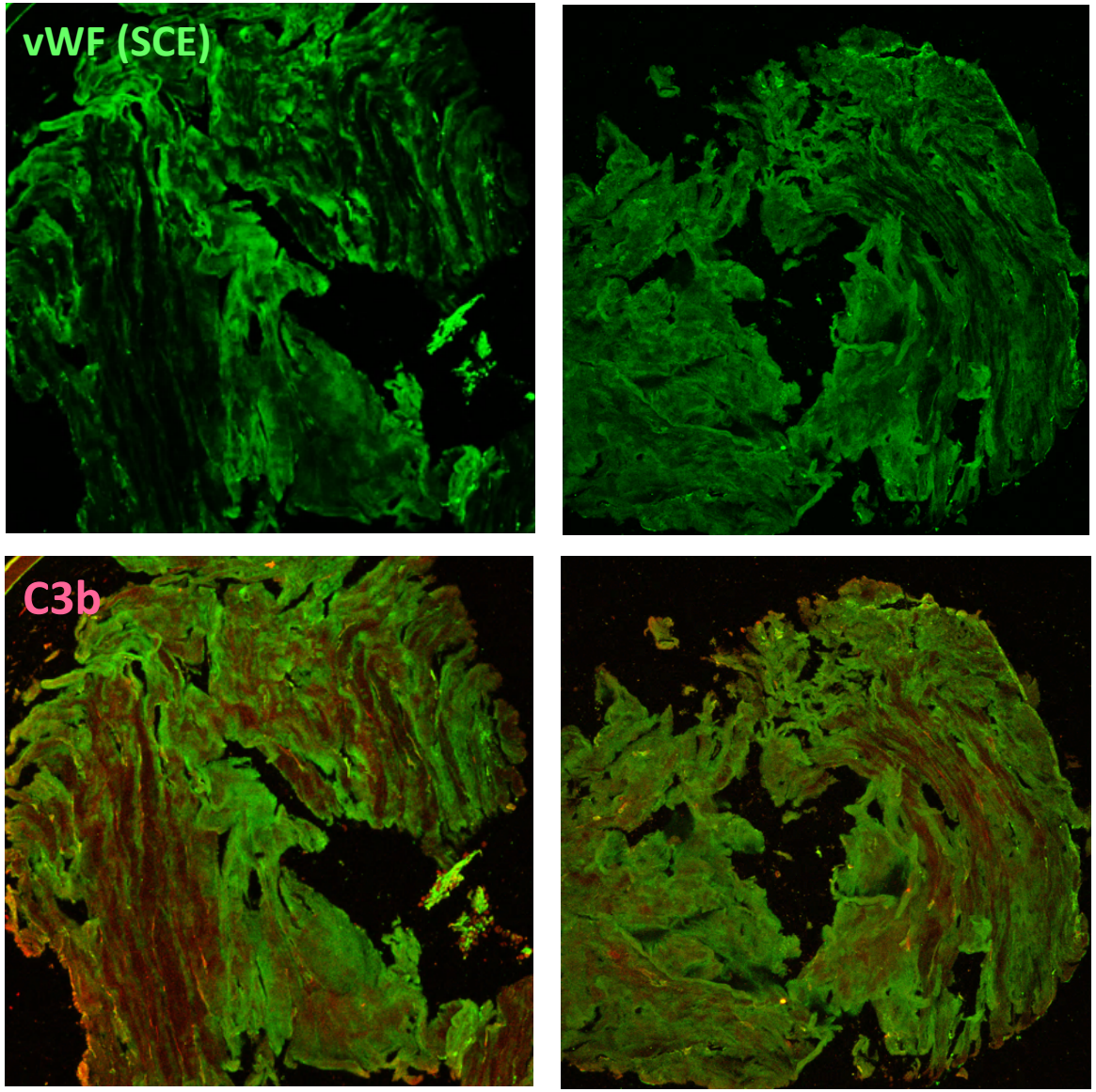


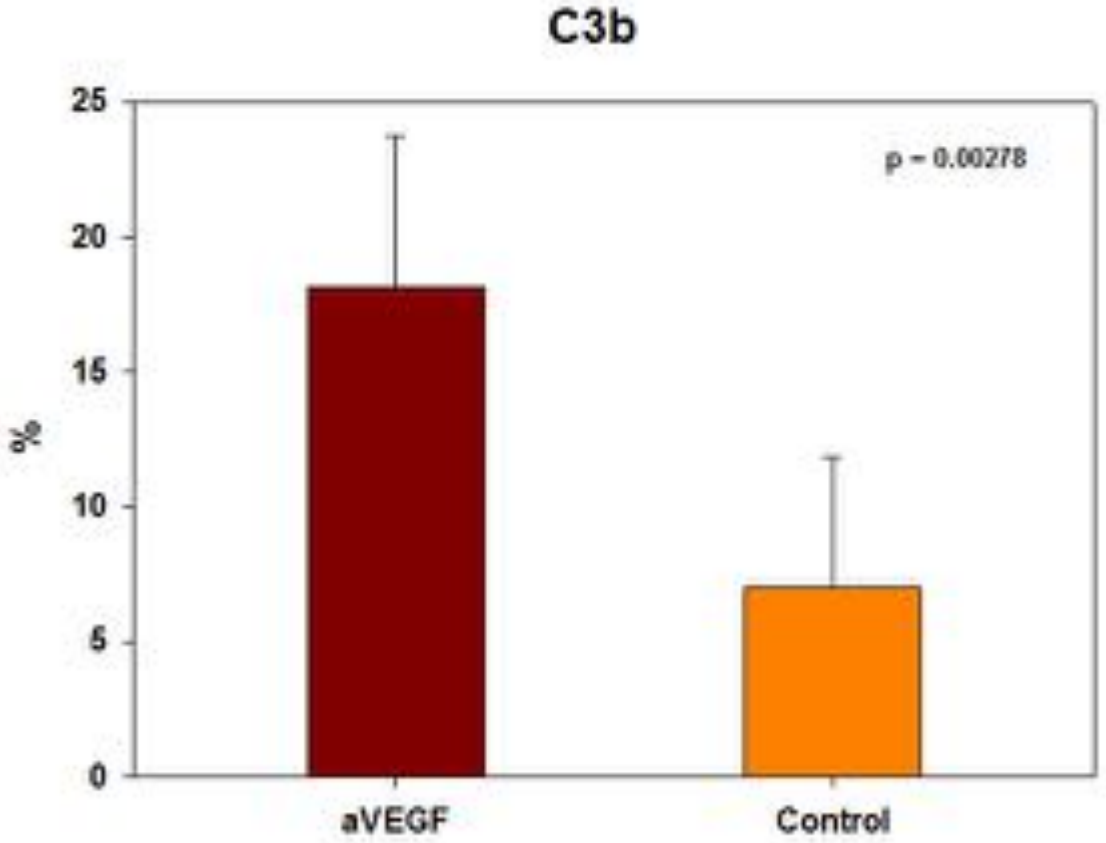


No aVEGF

With aVEGF

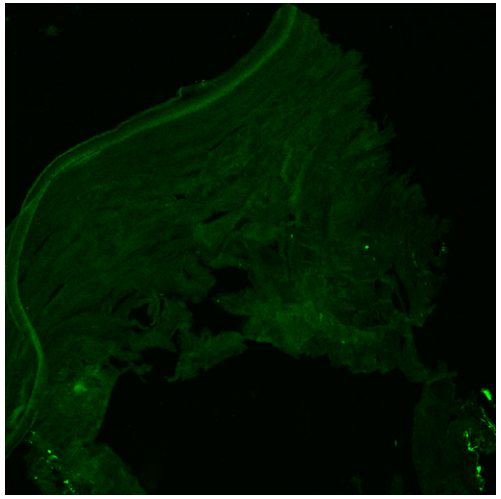
C3b



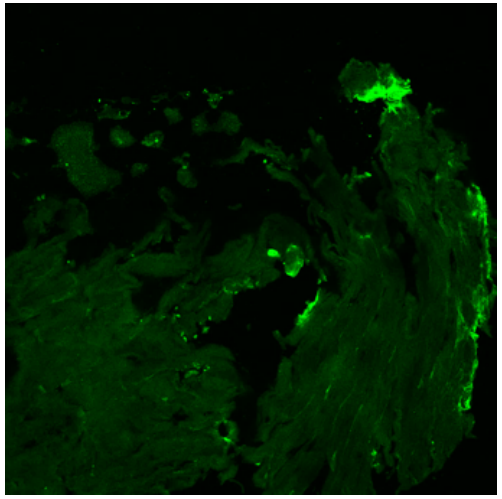


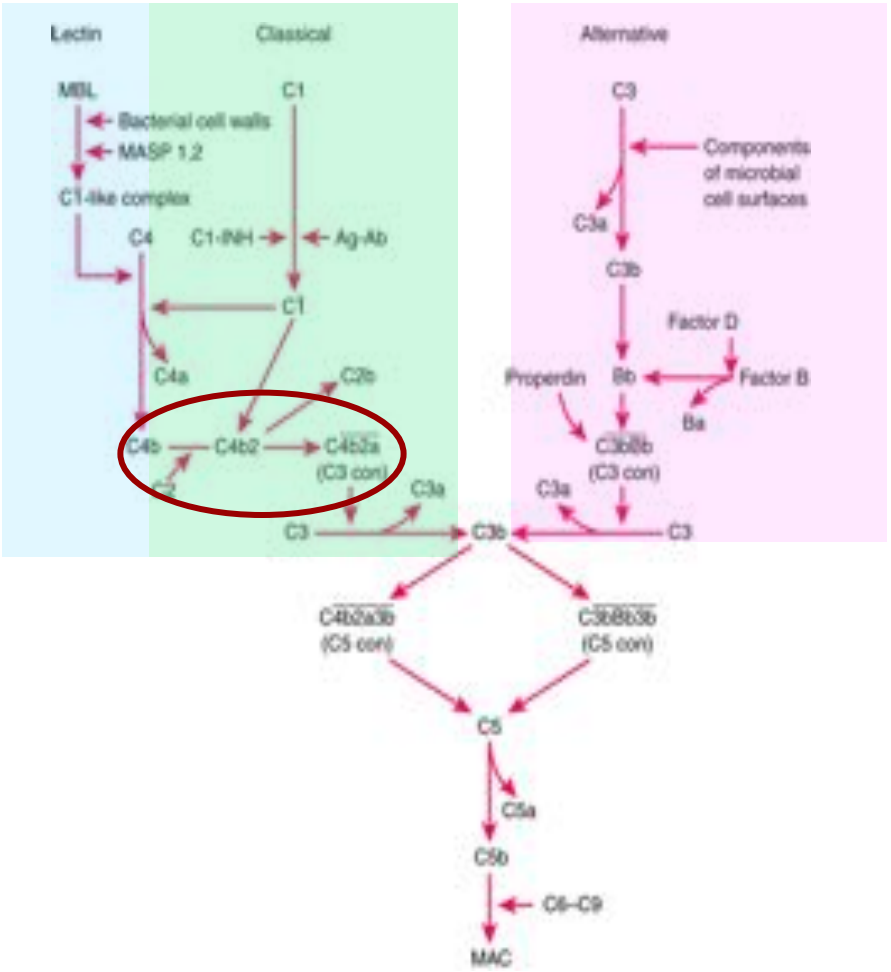
C4d

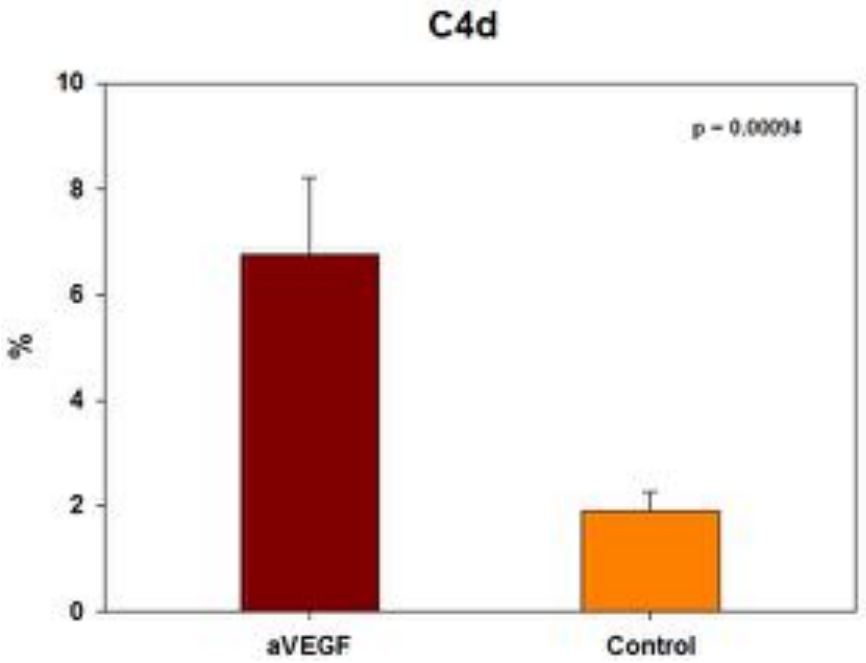
No aVEGF



With aVEGF



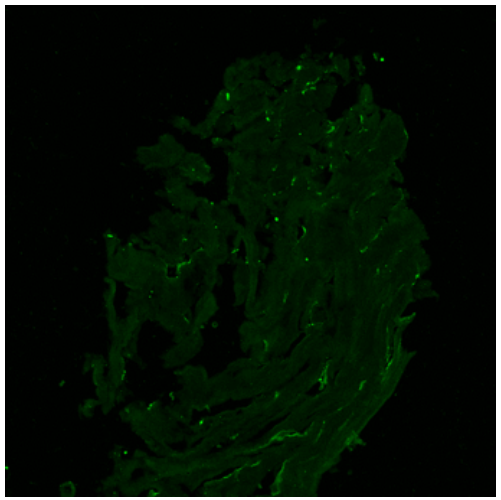


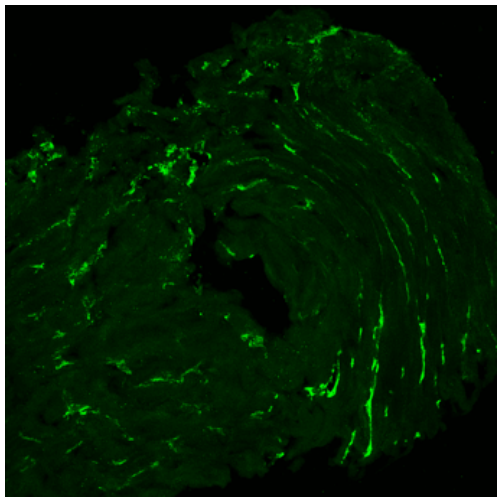


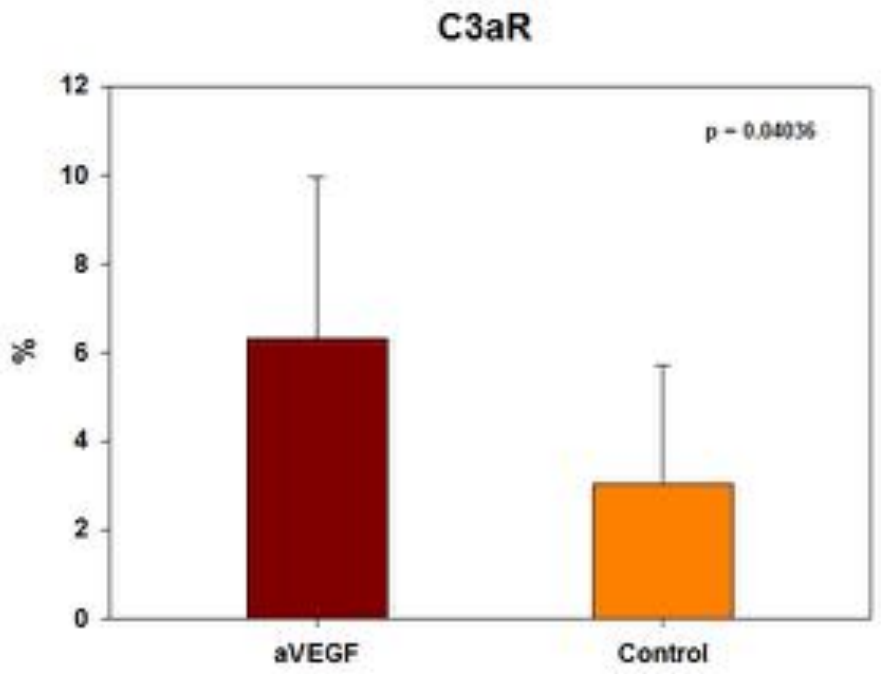
No aVEGF

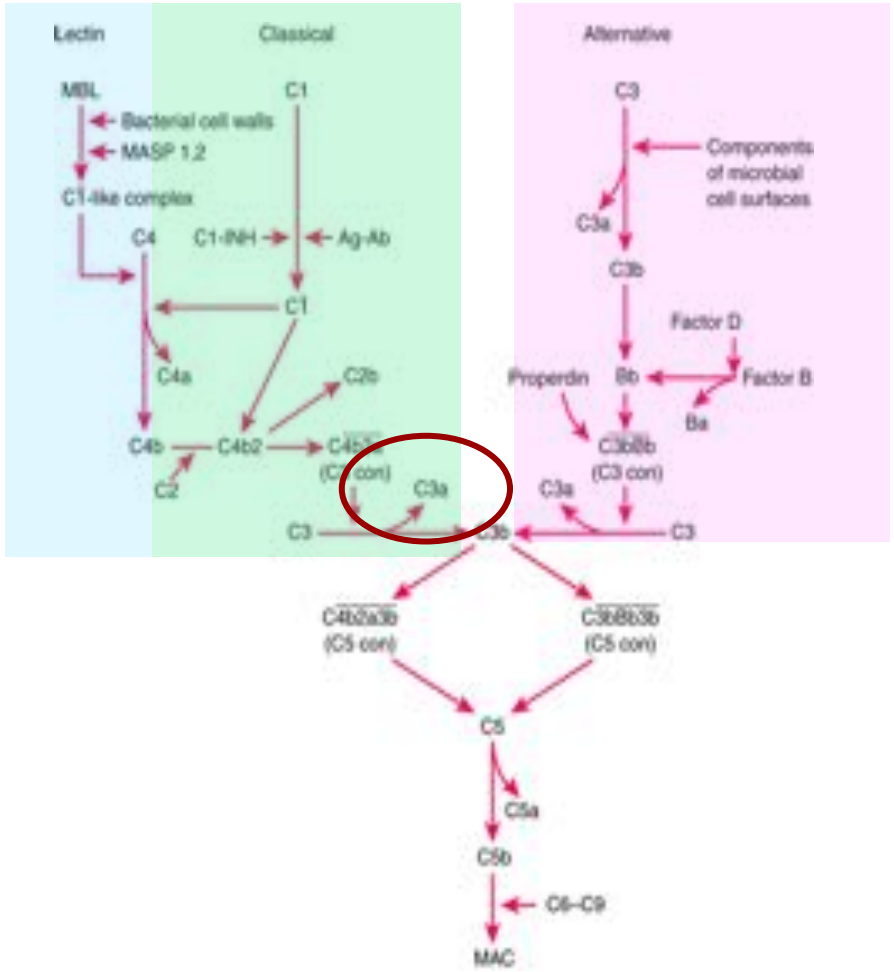
With aVEGF

C3aR

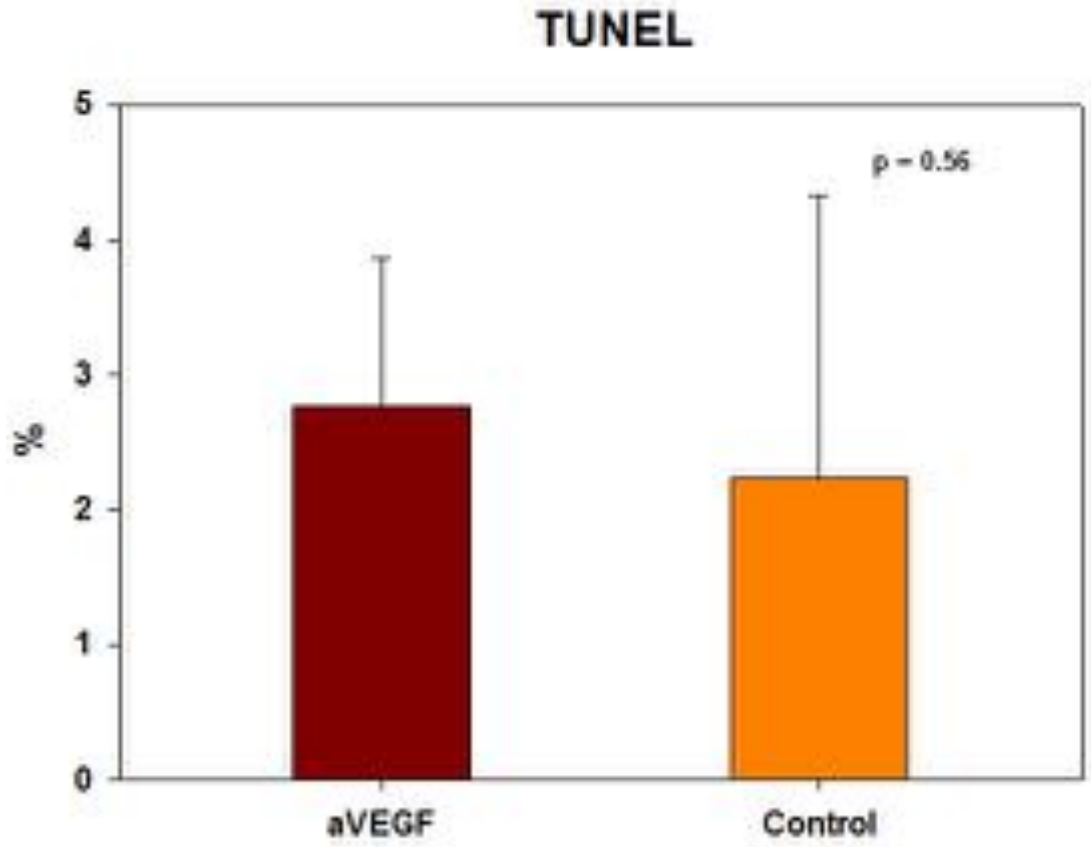


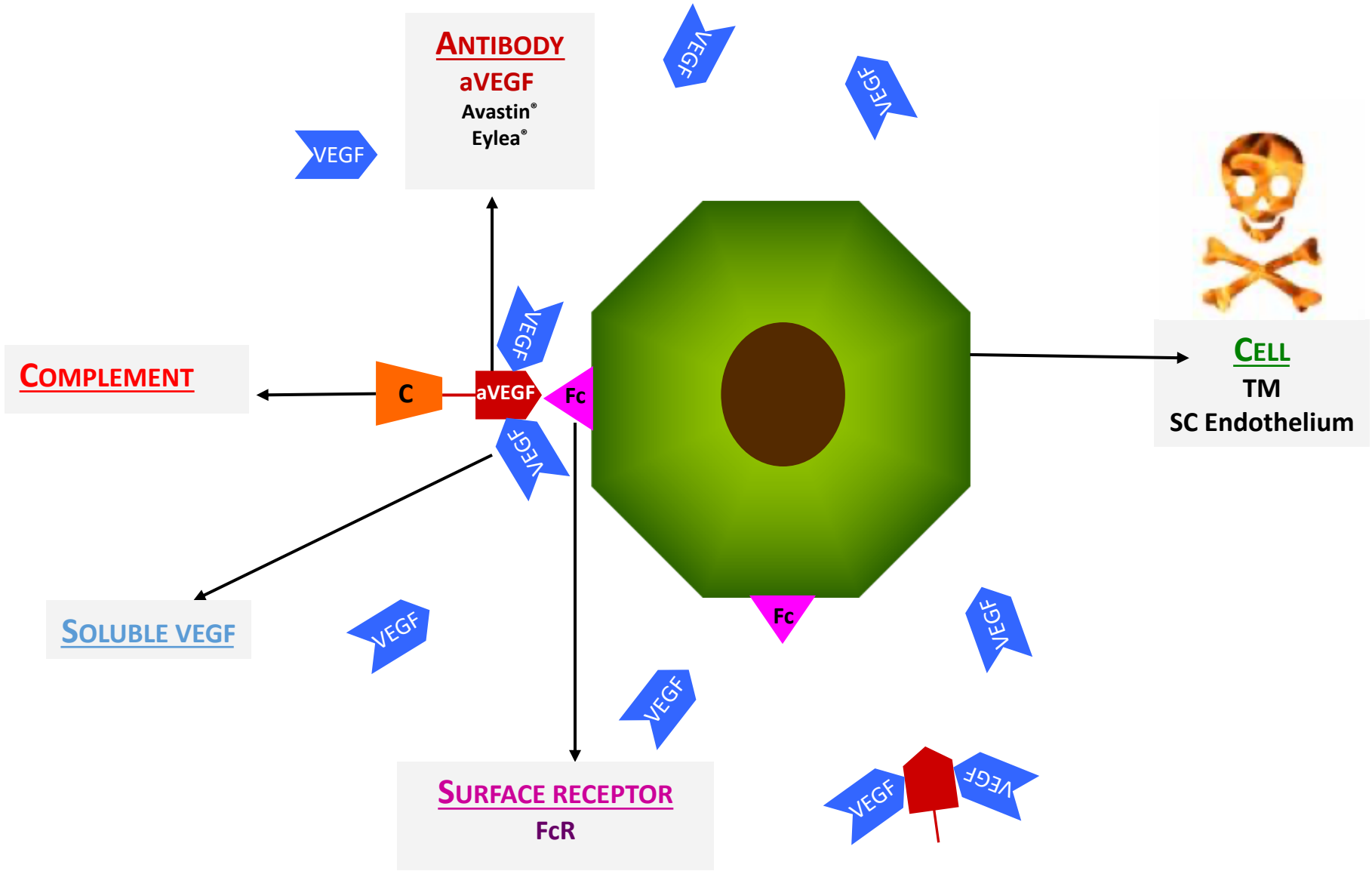






Is it physiological scavenging function of the complement system?




ANTIBODY
aVEGF
Avastin®
Eylea®
VEGF
VEGF
VEGF
VEGF
CELL
TM
SC Endothelium
COMPLEMENT
C
aVEGF
Fc
VEGF
VEGF
Fc
SOLUBLE VEGF
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SURFACE RECEPTOR
FcR

CAN A MONOCLONAL F(AB) FRAGMENT ACTIVATE COMPLEMENT?

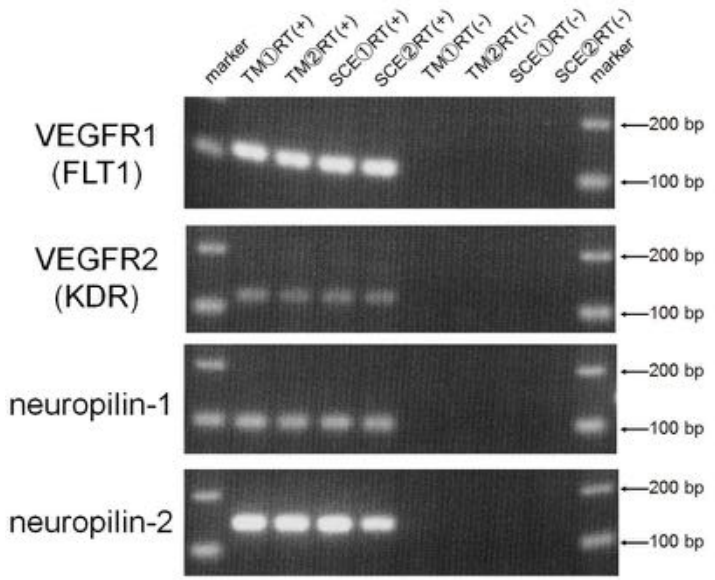

Fig 1. mRNA expression of VEGF receptors in TM and SCE cells. The expression of the mRNAs of four VEGF receptors [VEGFR1 (FLT1), VEGFR2 (KDR), neuropilin-1, and neuropilin-2] in TM and SCE cells was examined by RT-PCR. Samples without reverse transcription [RT(-)] were used as negative controls.

doi:10.1371/journal.pone.0161332.g001

PLOS | ONE

RESEARCH ARTICLE

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

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Department of Ophthalmology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Immunology 1983 42 75

Importance of the integrity of the inter-heavy-chain disulphide bond of rabbit IgG in the activation of the alternative pathway of human complement by the F(ab')$_2$ region of rabbit IgG antibody in immune aggregates

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Accepted for pu

ACTIVATION OF THE ALTERNATIVE COMPLEMENT PATHWAY BY THE IMMUNE PRECIPITATE FORMED WITH F(ab')$_2$ FRAGMENT OF HUMAN IgG ANTIBODY

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(*First received* 13 *January* 1983; *accepted in revised form* 24 *May* 1983)

Abstract—The complement fixing ability of the F(ab')$_2$ fragment of human IgG was studied using an immune precipitate (Ippt) formed between tetanus toxoid and the F(ab')$_2$ of high-titer IgG antibody against tetanus toxin. A major subclass of the specific IgG antibody against tetanus toxin, which was separated by affinity column chromatography, was identified as IgG$_1$. On incubation of normal human serum (NHS) with the Ippt formed at equivalence, a dose-dependent consumption of CH50, C3 and C5 activities was observed without significant loss of the early acting complement components. A similar

HOW IMMUNE COMPLEXES FROM CERTAIN IgG NAbs AND ANY F(ab')$_2$ CAN MEDIATE EXCESSIVE COMPLEMENT ACTIVATION

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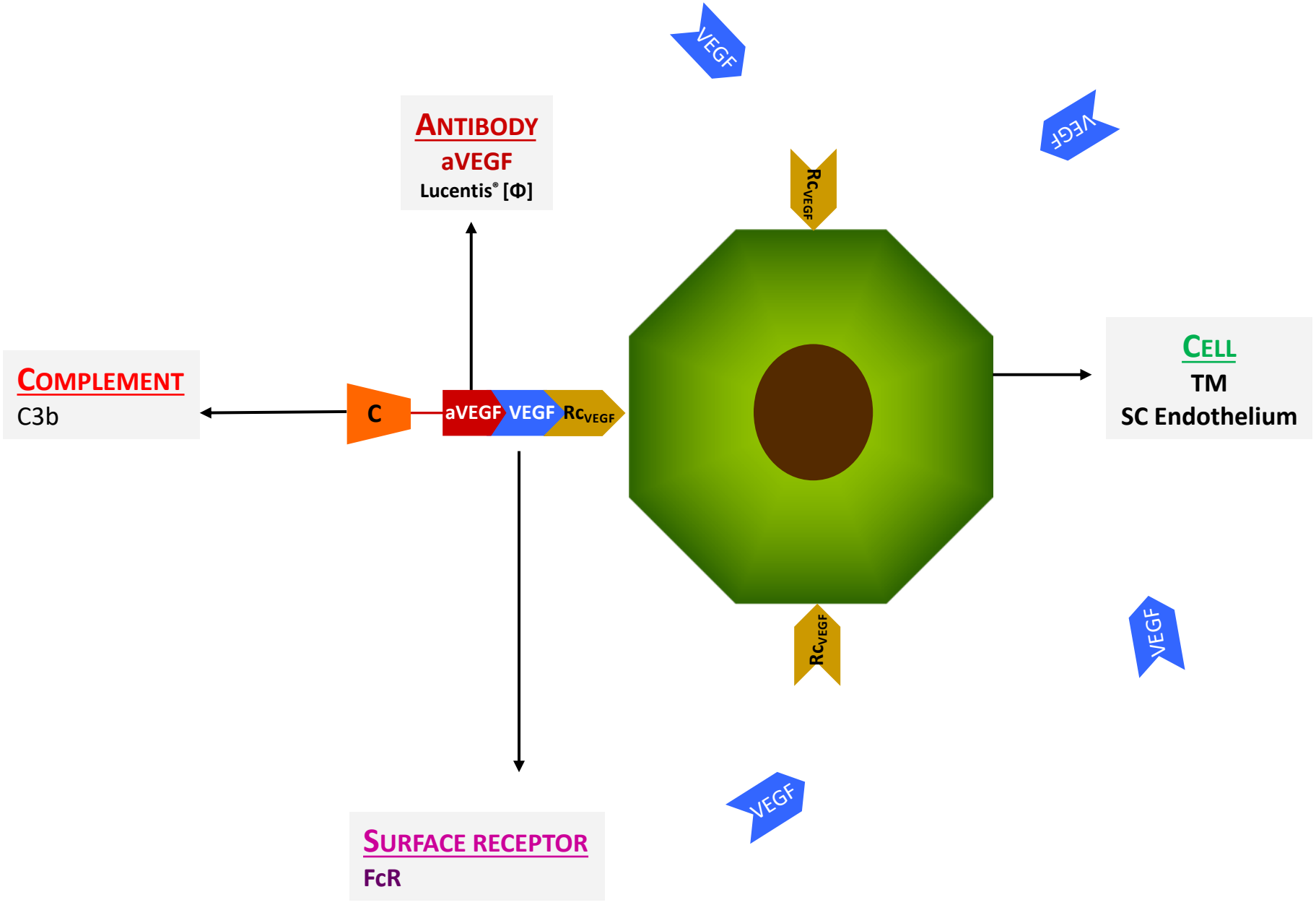
Abstract: In sepsis death follows an excessive inflammatory response involving cytokines and complement that is activated primarily via the amplifying C3/C5 convertase. Excessive stimulation of complement amplification requires IgG-containing or F(ab')$_2$-containing immune complexes (IC) that capture dimeric C3b on one of their heavy chains or heavy chain fragments. The ability of IgG-IC to capture dimeric C3b by the Fab portion is dependent on an affinity for C3 within the Fab portion, but outside the antigen-binding region. This property is rare among IgG NAbs. In contrast to this, the lack of the Fc portion renders the Fab regions of any F(ab')$_2$-IC accessible to nascent C3b, but dimeric C3b deposits only if F(ab')$_2$-IC form secondary IC with anti-hinge NAbs that rigidify the complex and thereby promote deposition of dimeric C3b. Both types of complexes, C3b$_2$-IgG-IC and


VEGF
ANTIBODY
aVEGF
Lucentis® [Φ]
VEGF
RcVEGF
COMPLEMENT
C3b
C
aVEGF
VEGF
RcVEGF
CELL
TM
SC Endothelium
RcVEGF
VEGF
VEGF
SURFACE RECEPTOR
FcR

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**