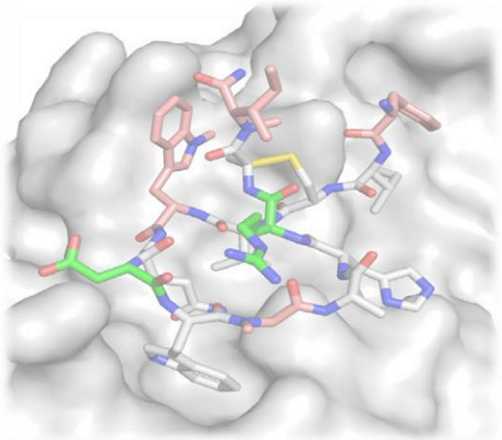
A primer to complement biology and pathology…
…in a rapidly evolving clinical landscape



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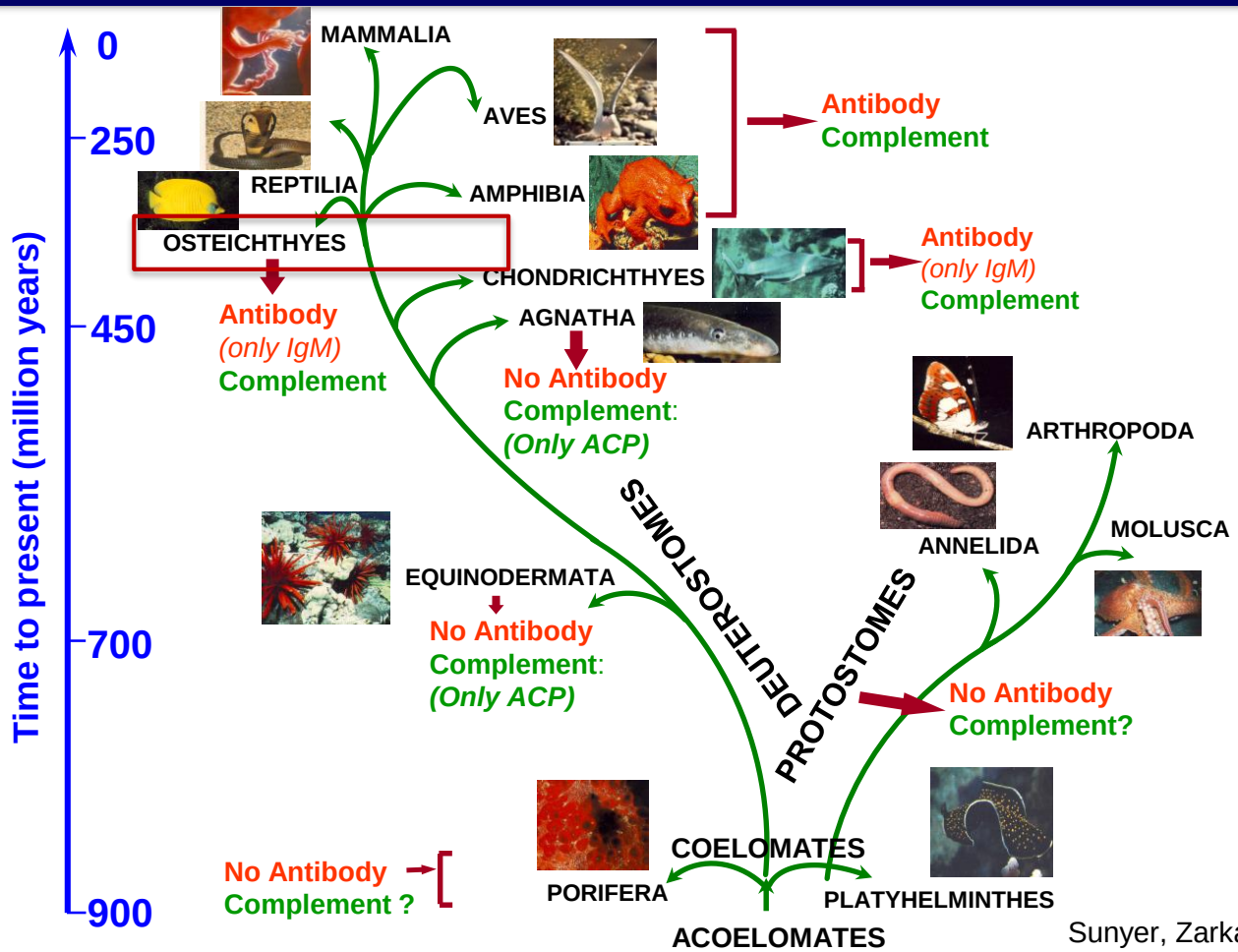
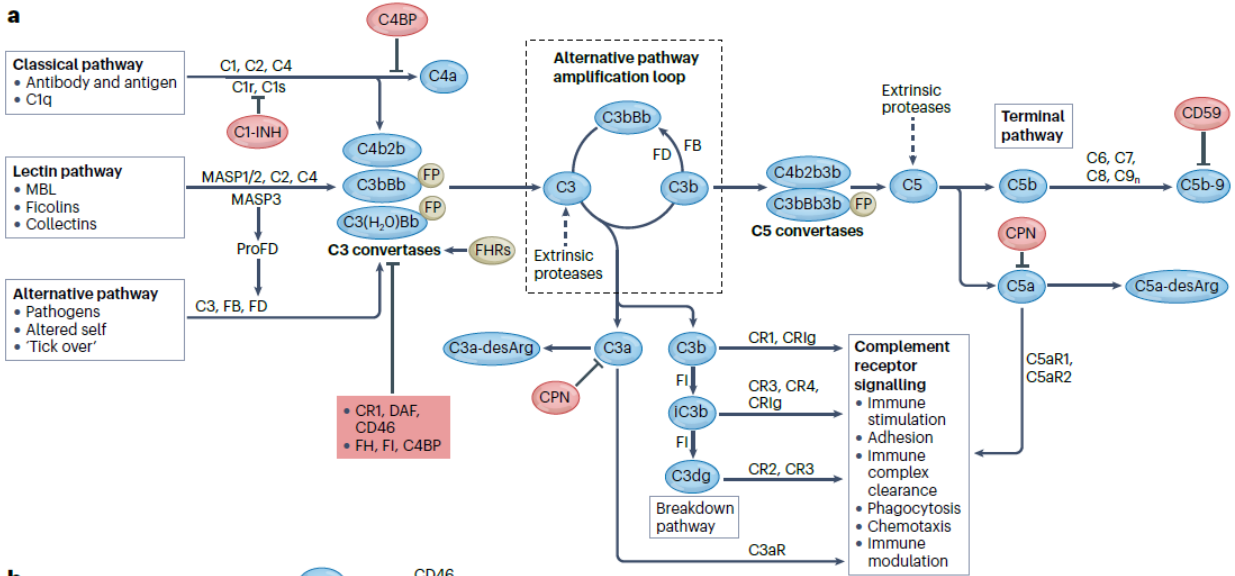
Complement: an evolutionarily ancient sentinel of innate immunity



- A primordial complement system emerged **~900 M** yrs ago
- Multiple isoforms of complement proteins generate immune diversity in species that lack a well developed adaptive immune response

Sunyer, Zarkadis, Lambris, Immunol Today, 1998

Complement: A key effector of innate immunity and inflammation



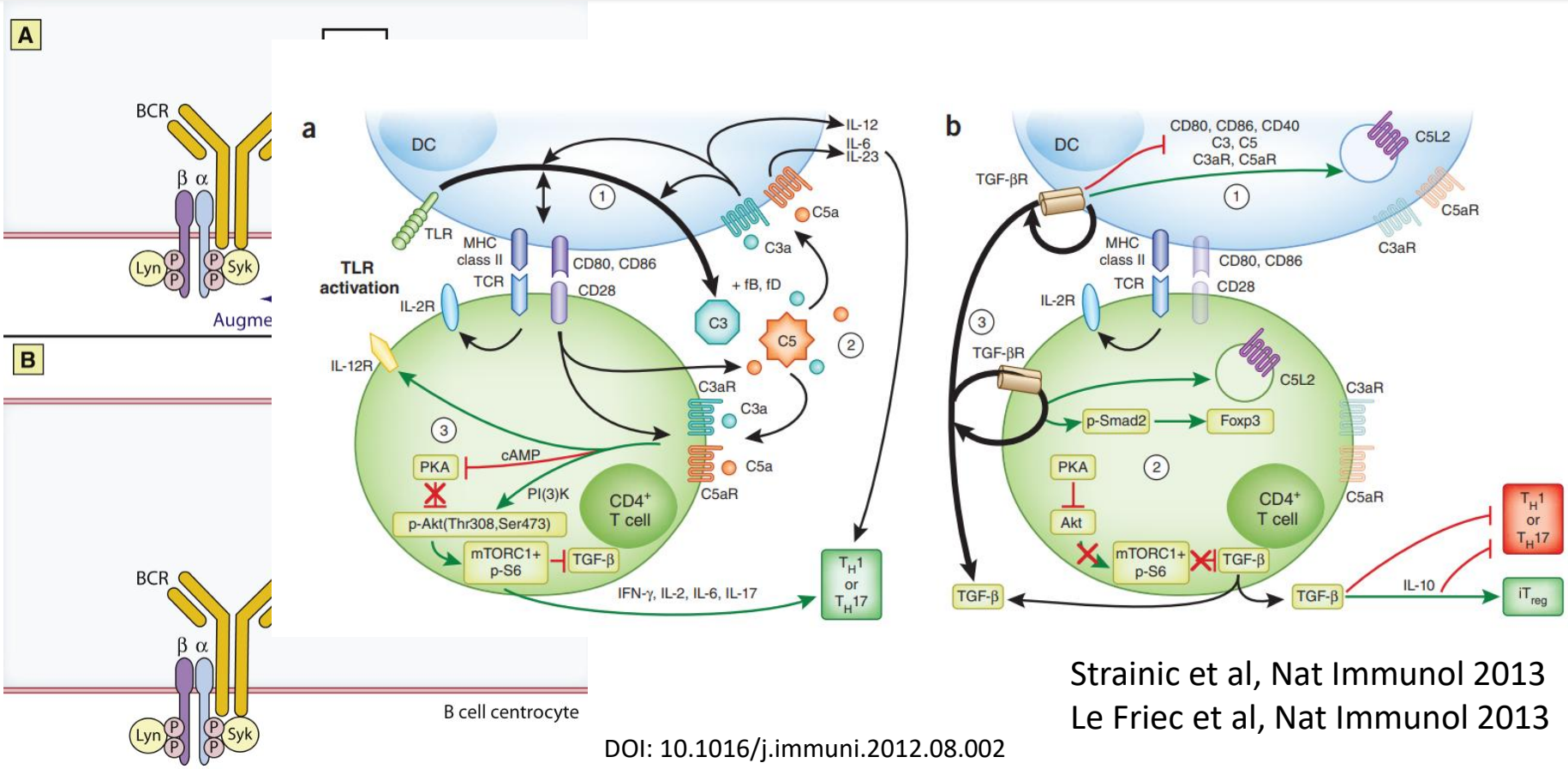
- Innate immune recognition-danger sensing (PRPs) and tissue immunosurveillance
- Modulator of inflammation and adaptive immune stimulation
- **Complement dysregulation** fuels a wide spectrum of **immune-mediated** and **inflammatory** diseases

nature reviews immunology
https://doi.org/10.1038/s41577-023-00926-1
Review article
Check for updates

A guide to complement biology, pathology and therapeutic opportunity

Dimitrios C. Mastellos[1], George Hajishengallis[2] & John D. Lambris[3]

Complement 'bridges' innate and adaptive immunity: Finetuning B and T cell responses

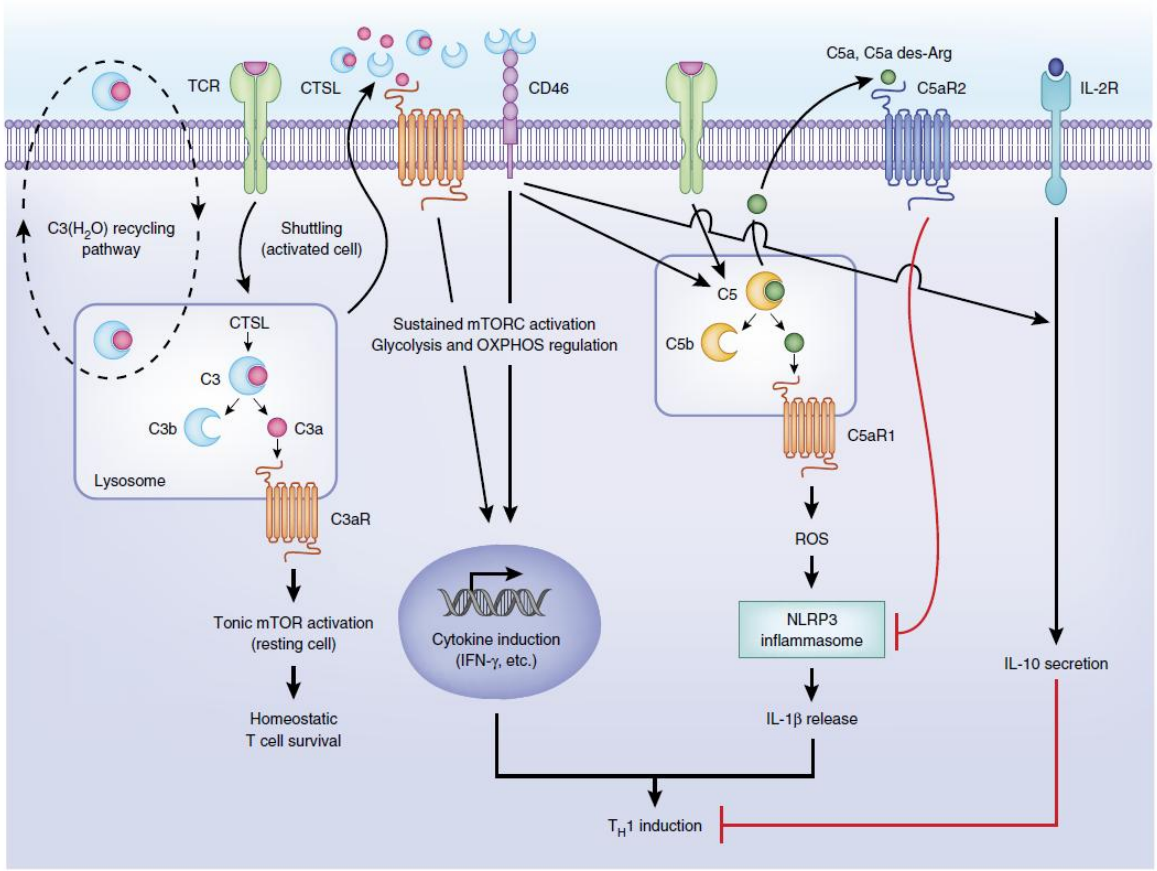


Strainic et al, Nat Immunol 2013
Le Friec et al, Nat Immunol 2013

DOI: 10.1016/j.immuni.2012.08.002

Paradigm Shifts in Complement Biology

Intracellular complement: Guarding homeostasis ‘from within’



‘Umbrella’ term: distinguishes functions of complement proteins that take place **intracellularly**

Roles:

- T cell homeostasis
- Cell differentiation
- Tumor progression
- Autophagy regulation
- Metabolic reprogramming
- Pathogen sensing
- Inflammation

Controversies:

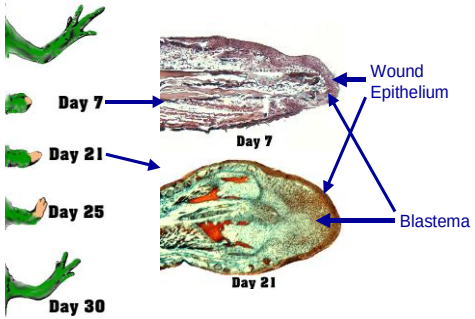
Origin of proteins, compartments, assembly of fully functional C’ pathways

Hajishengallis G, Reis ES, Mastellos DC, Ricklin D, Lambris JD. (2017), *Nat Immunol*, 2017

Complement modulation of tissue regeneration: A homeostatic function conserved throughout evolution

- **Amphibians: De/Trans-differentiation & morphogenesis**

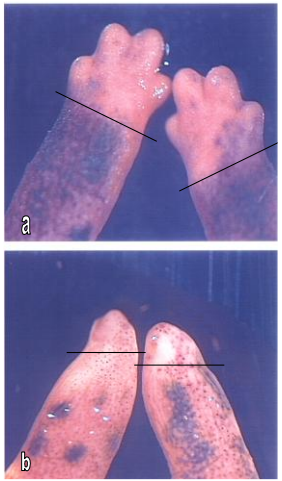
Limb Regeneration



C3 is expressed mainly in limb blastema and C5 in wound epithelium

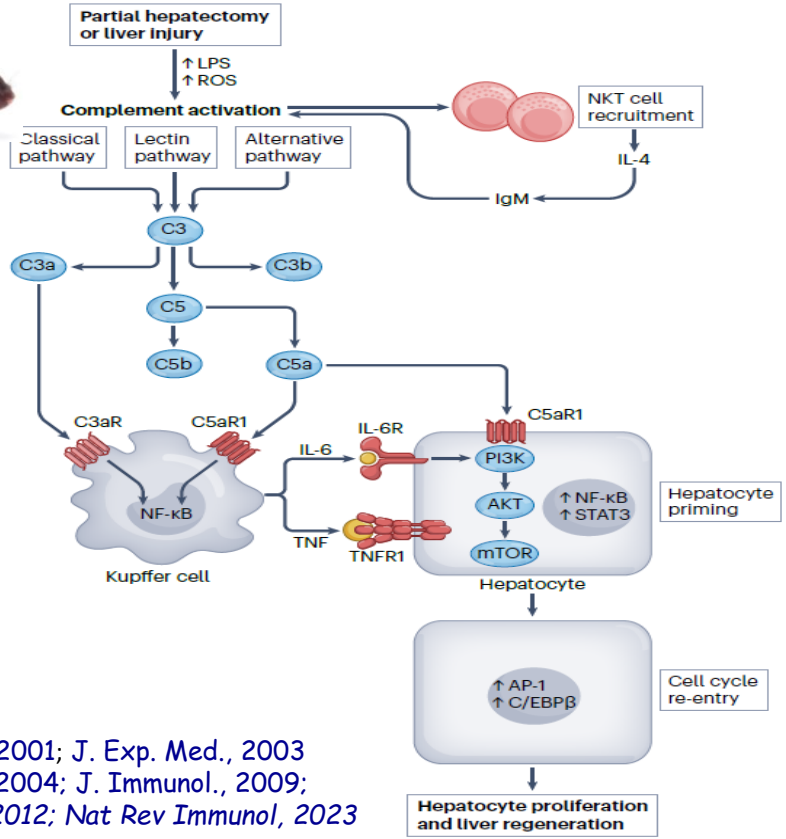
J Immunol. 161:6819, *J Immunol.* 170: 2331

Control limbs at digit stage



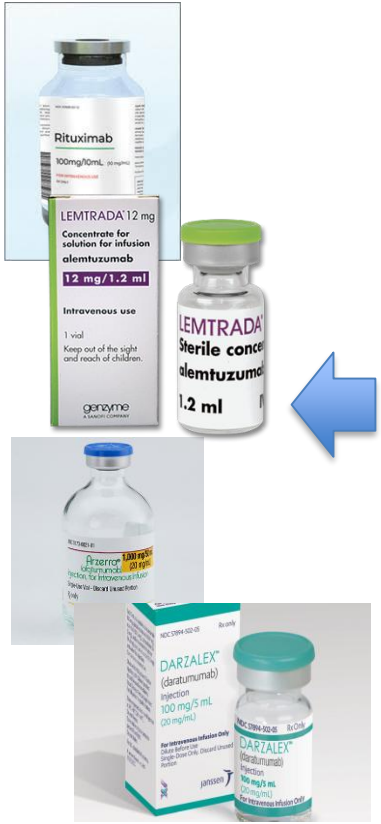
Regenerating limbs injected with cobra venom

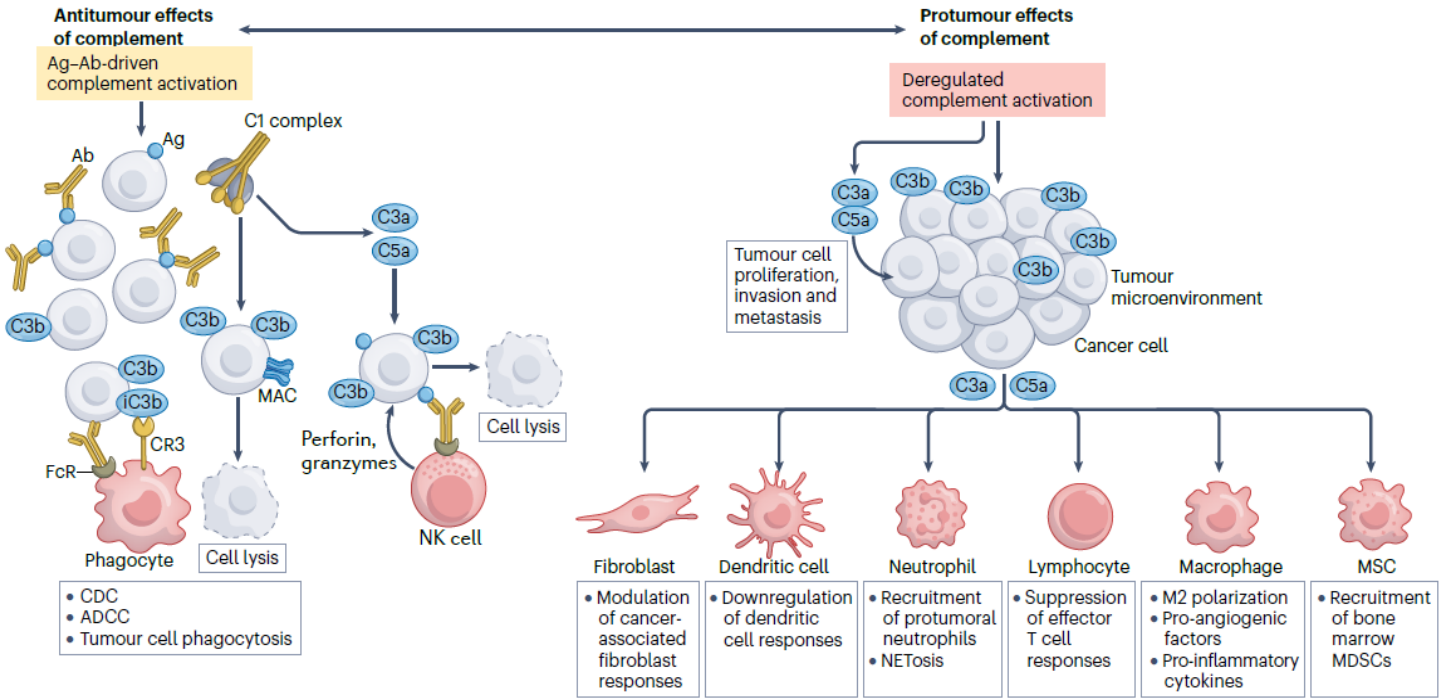
- **Mammals: Liver regeneration**



J. Immunol.; 2001; J. Exp. Med., 2003
J. Immunol., 2004; J. Immunol., 2009;
J. Immunol. *2012; Nat Rev Immunol, 2023*

Complement modulates anti-tumor immunity and promotes tumorigenesis through diverse immunosuppressive mechanisms

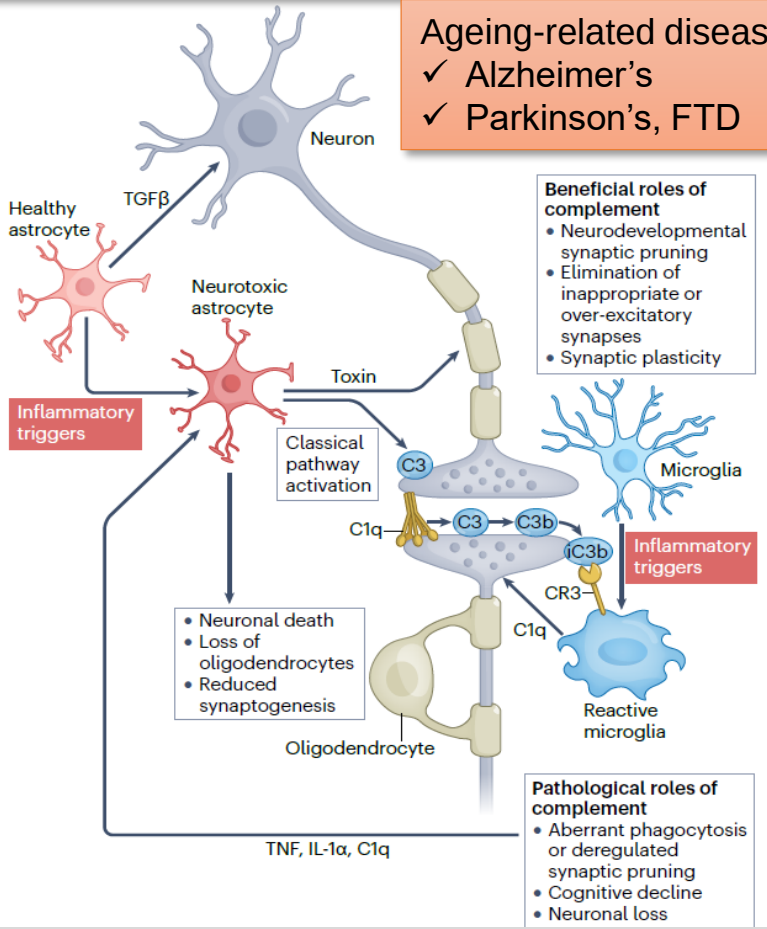


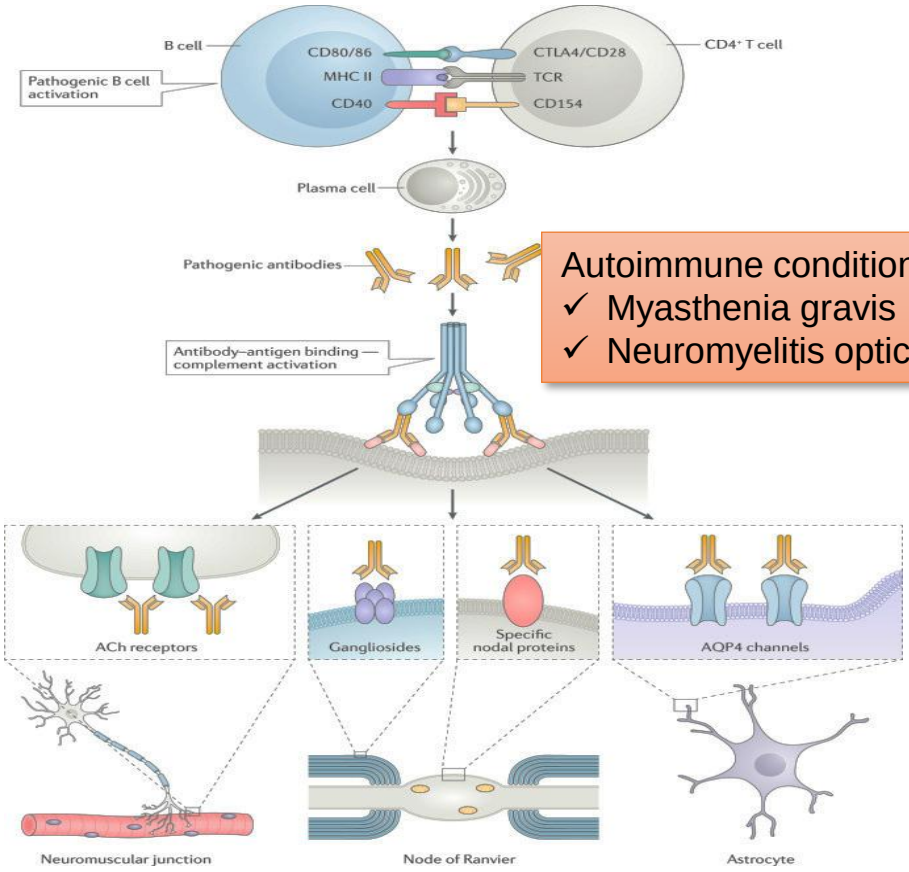


Reis, Mastellos, Ricklin & Lambris. *Nat Rev Immunol*, 2018; Mastellos, Hajishengallis & Lambris, *Nat Rev Immunol*, 2023

Complement as a therapeutic target in neuroinflammatory diseases

Ageing-related diseases:
- ✓ Alzheimer's
- ✓ Parkinson's, FTD

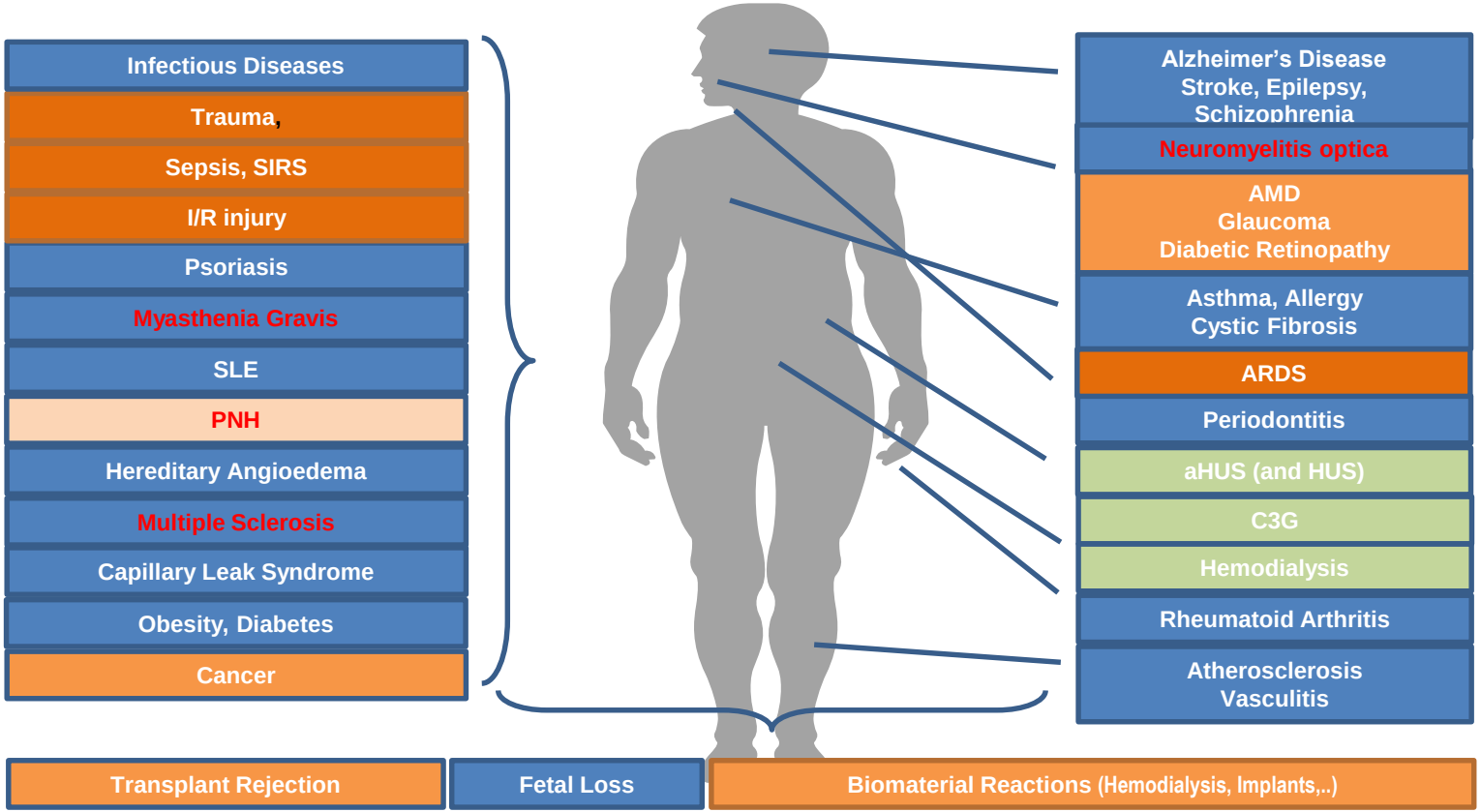




Autoimmune conditions:
- ✓ Myasthenia gravis
- ✓ Neuromyelitis optica

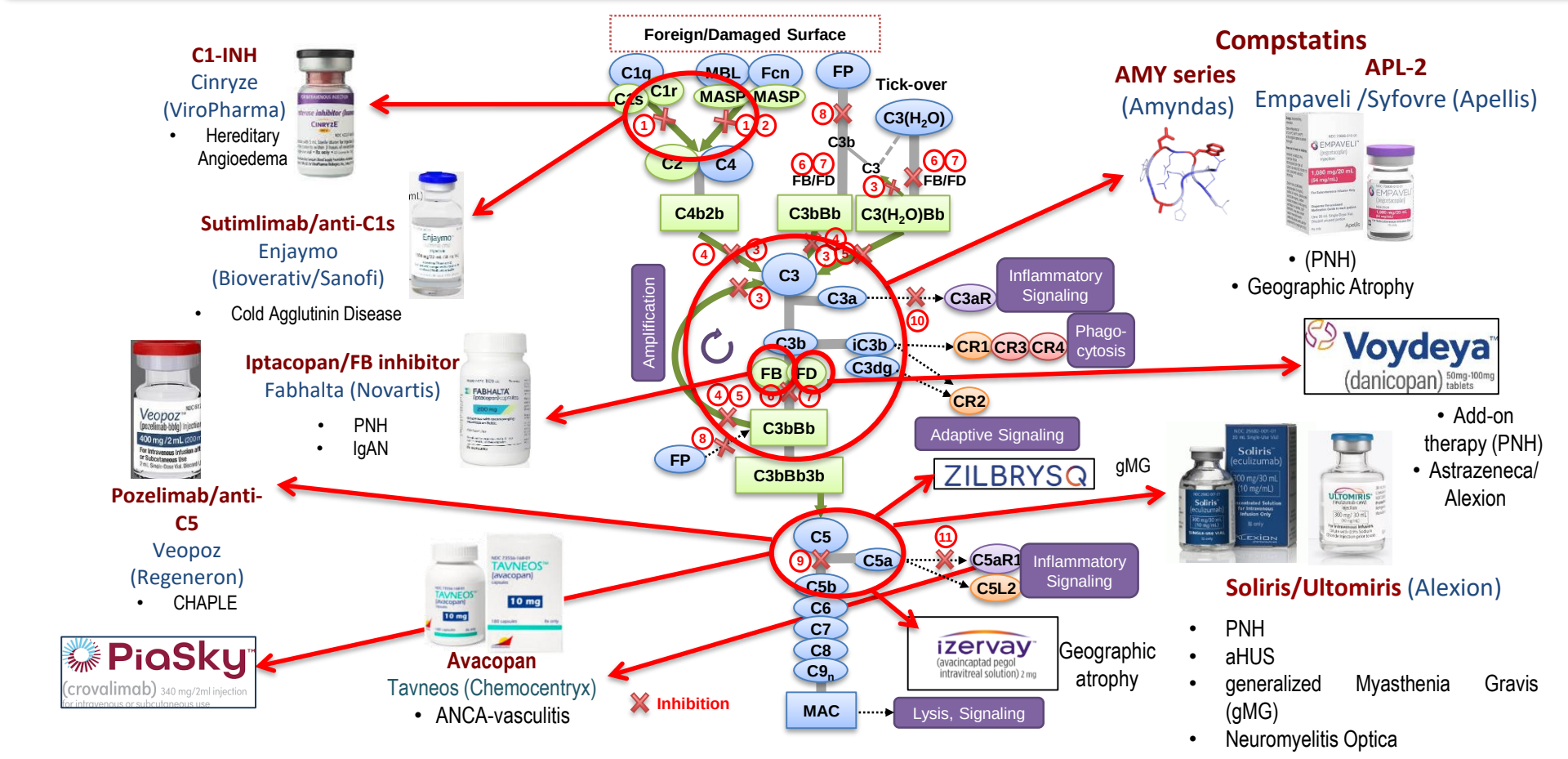
Hajishengallis G, et al *Nat Immunol*, 2017; Mastellos, Hajishengallis & Lambris, Nat Rev Immunol 2023; Dalakas MC et al, *Nat Rev Neurol*. 2020

A Growing List of Complement-Associated Diseases



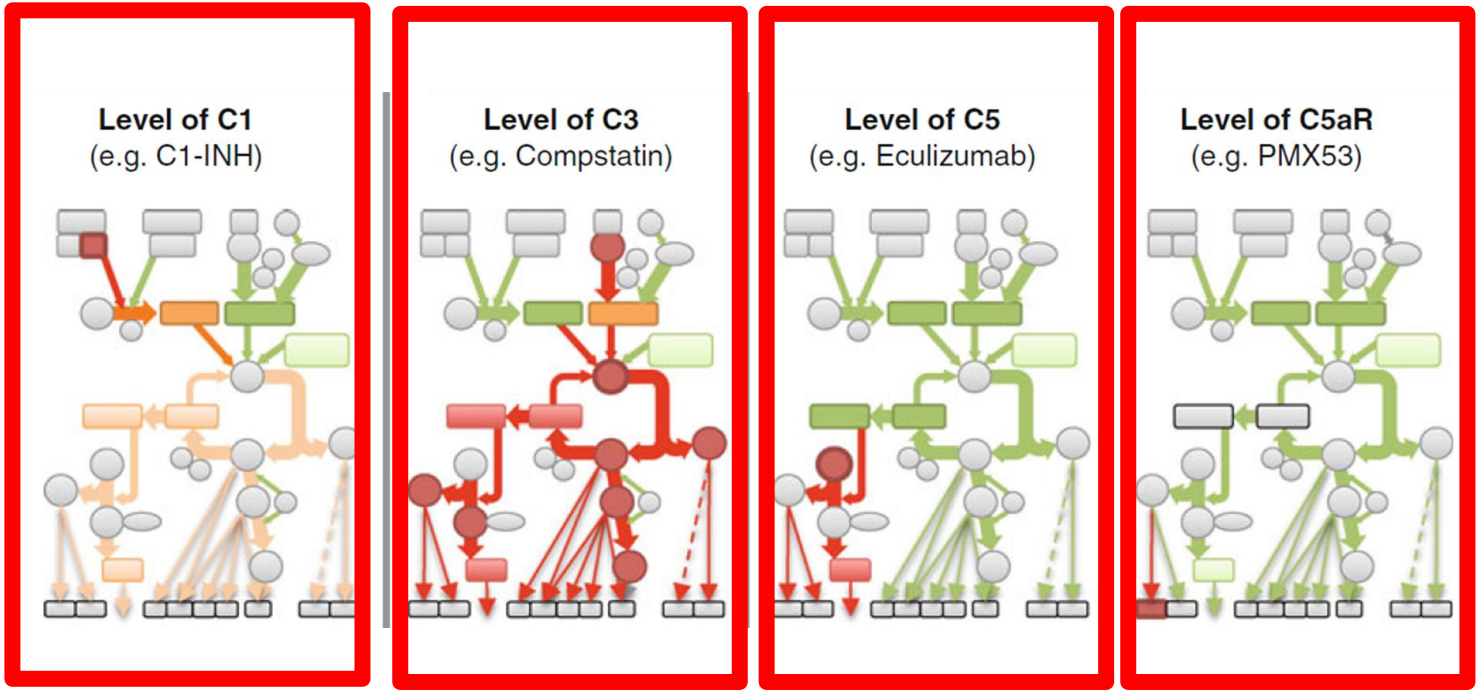
Nat. Biotechnol., 2007; J. Immunol, 2013, Semin Immunol. 2016, Nat. Rev. Neph, 2017, Nat. Rev. Drug. Disc. 2019

2021-present: A true resurgence of clinical complement inhibitors



Nat Biotechnol, 2007; J. Immunol., 2013; Nat Rev Nephrol, 2017; Nat Rev Drug Discov, 2019; Nat Rev Immunol, 2023

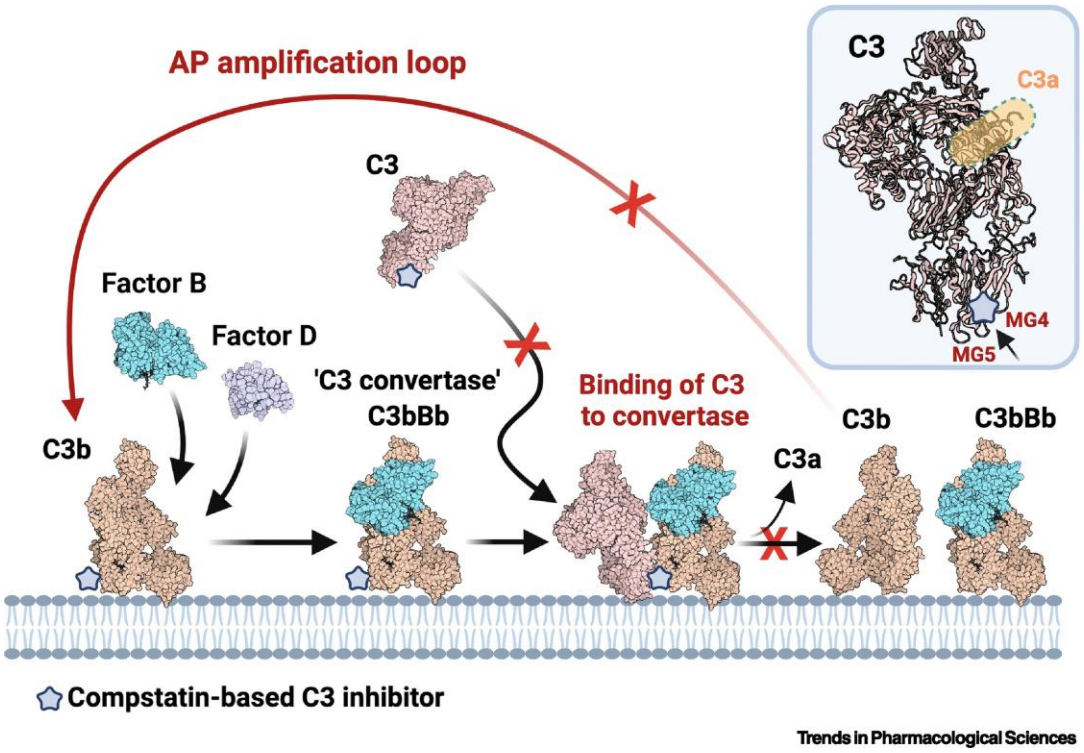
Choosing the optimal point of therapeutic intervention



- ‘One size fits all’ approach? **NO**
- Complement modulation should be tailored to disease pathophysiology & guided by pathway and target ‘penetrance’

Ricklin D & Lambris JD. *Adv Exp Med Biol* 2013

The Compstatin Family of peptidic C3-targeted inhibitors

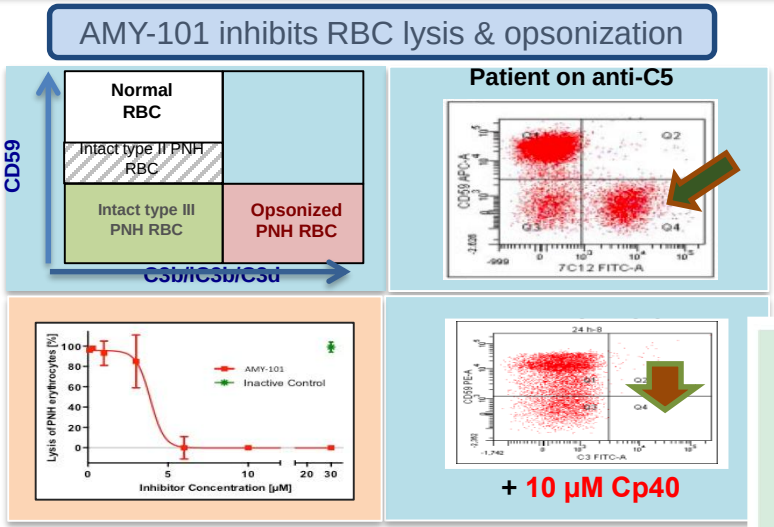


- Distinct C' inhibitor class: series of cyclic peptides of 13-17 aa
- PPI inhibitors blocking the access of C3 to the C3 convertases
- Potent inhibition of C3b opsonization via all major activation pathways
- Species specificity for C3 of humans and non-human primates

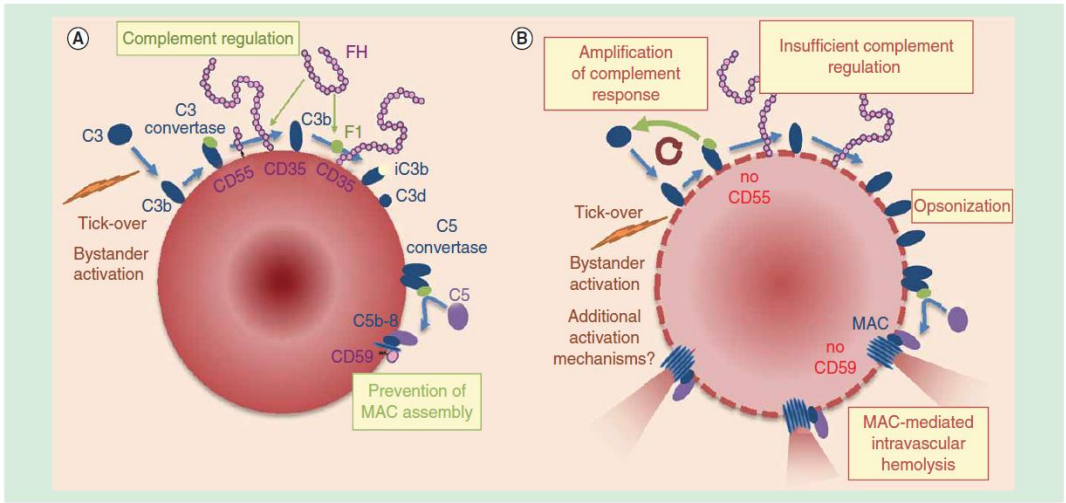
Ricklin & Lambris, Adv. Exp. Med. Biol., 2008; Janssen et al., JBC, 2007; Mastellos et al, 2019; Mastellos and Lambris 2024

AMY-101 (CP40) Targets Complement Component C3 in PNH

Blood (2014) 123:2094-101



C3-mediated extravascular hemolysis

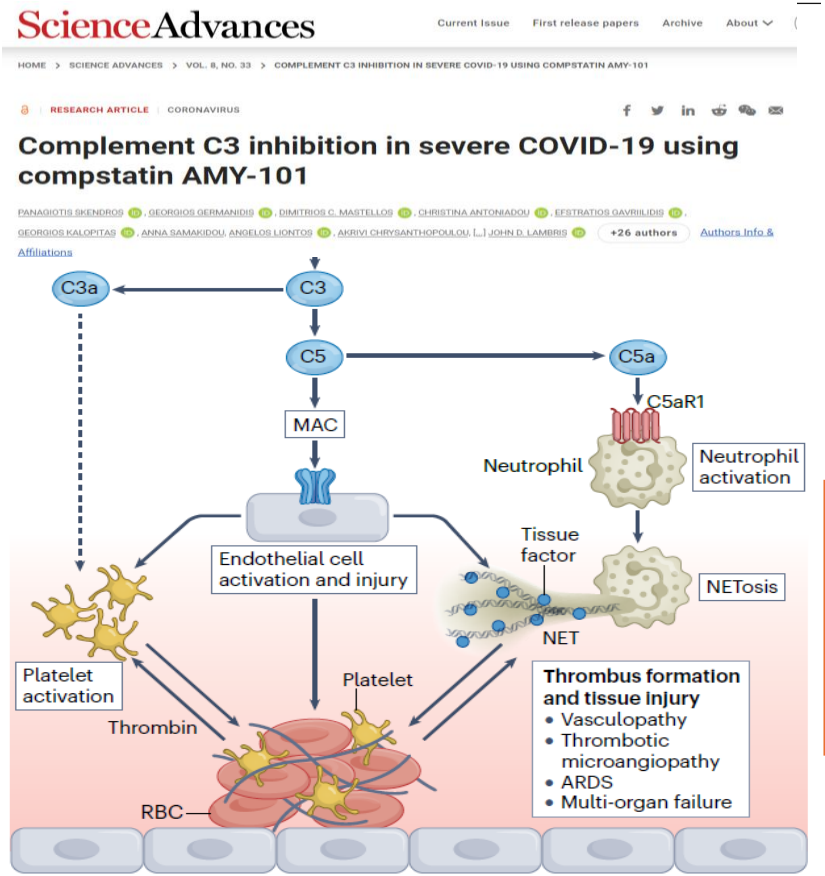


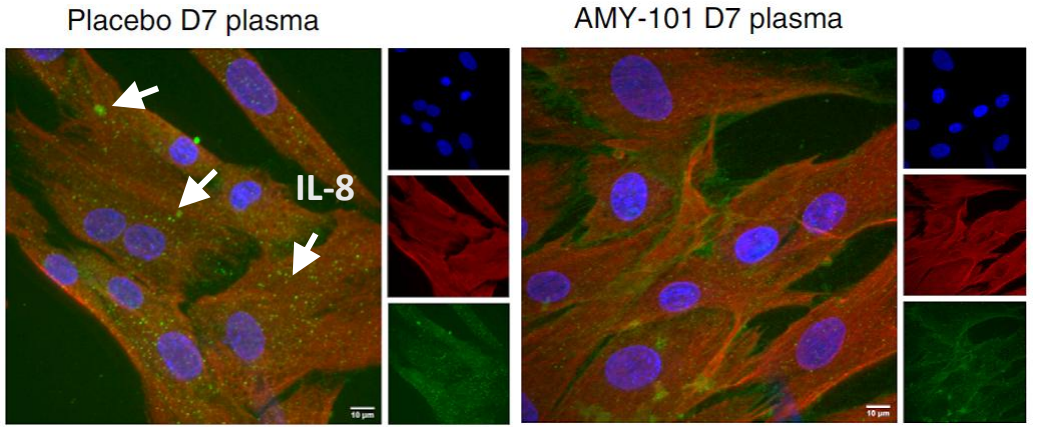
- Cp40 inhibits C3 convertases from cleaving C3 into C3a and C3b
- C3b is needed for both extravascular hemolysis through opsonization and intravascular hemolysis through C5 convertase and MAC complex formation
- **By blocking C3b opsonization, CP40 prevents both intravascular and extravascular hemolysis**

FDA Approval: Successful **Phase III** head-to-comparison of APL-2/pegcetacoplan/Empaveli with Soliris

Blood (2014) 123:2094-101, Expert Rev. Hematol., 7:583-98.; EJCI. 45:423-40

Complement C3: a key driver of COVID-19 immunopathology and fibroblast IL-8-mediated thromboinflammation





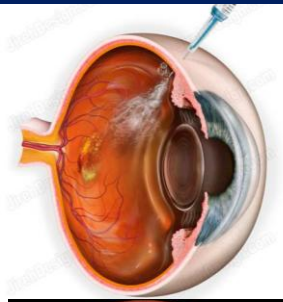
- **AMY-101 dampens plasma IL-8 levels in COVID patients as early as day 7**
- **AMY-101 attenuates IL-8 expression in lung fibroblasts**
- **C3 mediates IL-8-driven neutrophil migration into the lungs**

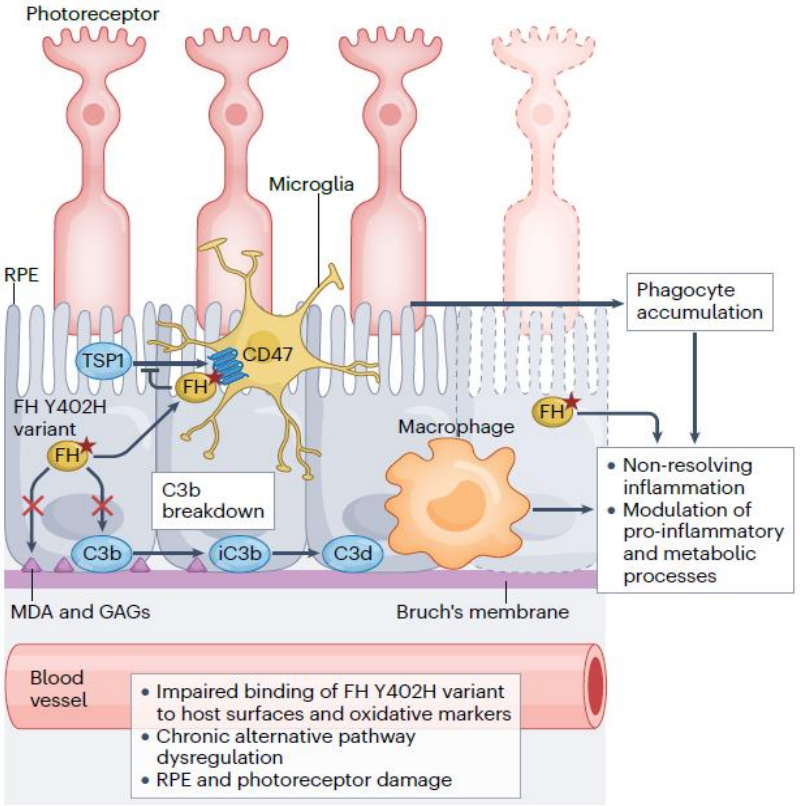
Risitano A. *et al, Nat Rev Immunol,* 20, 343–344 (2020)
Mastellos, Hajishengallis & Lambris. *Nat Rev Immunol* (2024)
Skendros P. et al, 2022; Antoniadou C. *et al*, *Immunology*, 2025

From systemic to… local complement modulation:

Emerging clinical paradigms

Therapeutic complement modulation in age-related macular degeneration

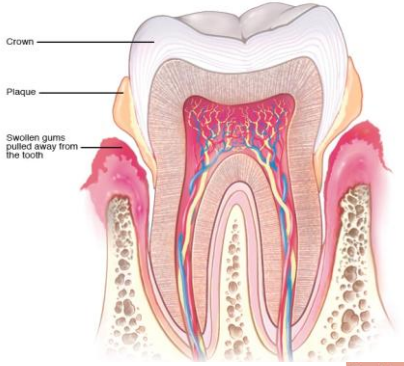


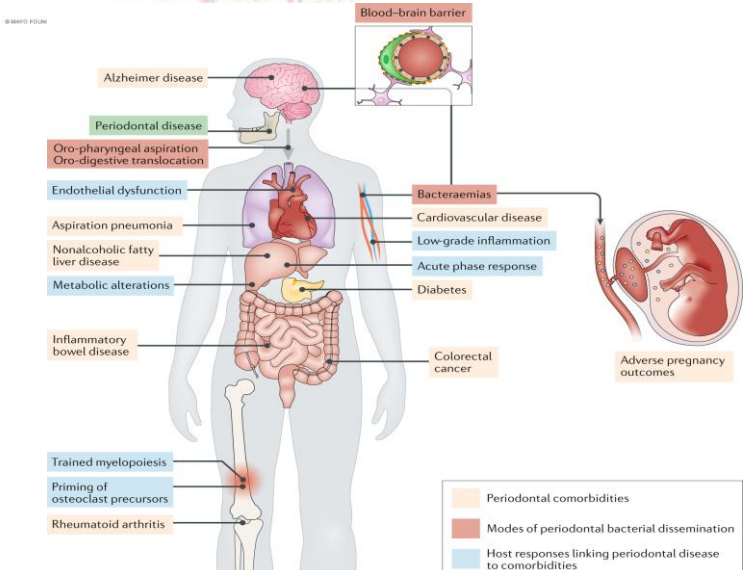


- Chronic inflammatory disease
- Leading cause of irreversible vision loss in the industrialized world
- Highly prevalent condition in the elderly >65 yo
- Approximately **200 million** afflicted with AMD worldwide
- Complement **AP dysregulation**: a key pathogenic driver of AMD

Compstatin-based pegcetacoplan (Syvofre) was approved as the first ever treatment for GA-Recently a C5-targeted RNA aptamer (Izervay) was also approved

Therapeutic C3 modulation in periodontal disease





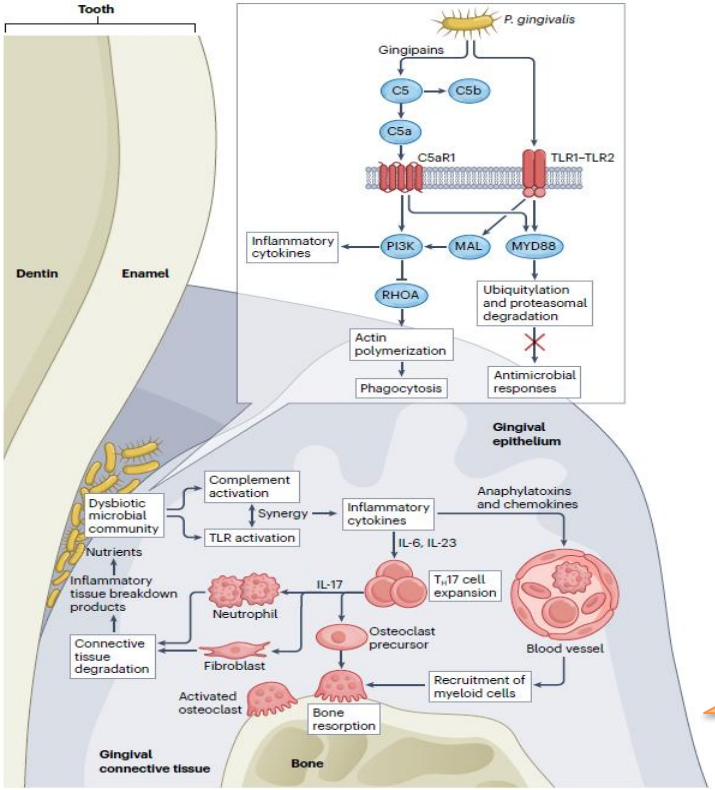
- **Periodontitis** is a **common inflammatory disease** that is induced by tooth-associated biofilms (microbial **dysbiosis**)
- **Destruction of the tissues that surround and support the teeth** (i.e., gingiva, alveolar bone)

- **A highly prevalent chronic disease affecting >47% of US adults.**

- **In its severe form it affects 8.5% of US adults**
- Associated with increased risk for certain **systemic conditions**, such as atherosclerosis, RA, pregnancy complications and diabetes

Hajishengallis and Chavakis, Nat Rev Immunol, 2021

C3 activation takes center stage in the host inflammatory response that perpetuates periodontal inflammation



Bacteria are necessary

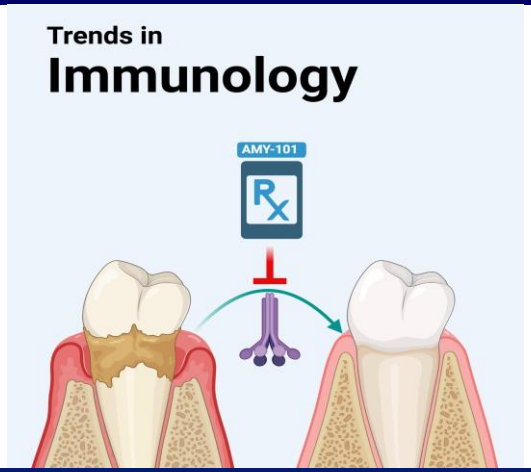
not sufficient to cause disease

It is the host response

that inflicts periodontal tissue damage

- C3 inhibition (AMY-101)

Breaks the vicious cycle of destructive inflammation and dysbiosis

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Phase IIa clinical trial of complement C3 inhibitor AMY-101 in adults with periodontal inflammation

Hatice Hasturk,[1] George Hajishengallis,[2] The Forsyth Institute Center for Clinical and Translational Research staff,[1] John D. Lambris,[3] Dimitrios C. Mastellos,[4] and Despina Yancopoulou[5]

Hajishengallis et al, *Front Immunol*, 2019; Kajikawa T, et al. *Mol Ther Methods Clin Dev*,2017; Maekawa T et al, *J Clin Periodontol* 2016; Maekawa T et al, *J Immunol*, 2014; Wang H, Ideguchi H, et al, *J Immunol*, 2022; Li X, et al. *J Immunol* 2023

Expanding Complement therapies: Challenges lying ahead

- ✓ Personalized medicine approaches warranted for discrete indications - (lessons learnt from PNH i.e., extravascular hemolysis)
- ✓ Reliable biomarkers for informing medical treatment algorithms
- ✓ Optimizing routes of drug administration
- ✓ Duration of intervention (acute-transient vs chronic)
- ✓ BBB-permeable therapeutics tailored to CNS disease modulation
- ✓ Systemic vs local therapeutic modulation
- ✓ Cell-permeable complement inhibitors?
- ✓ 'Single shot' durable treatments? AAV-based gene delivery of C' inhibitors
- ✓ Clinical trial transparency and patient accessibility to new C' drugs

Complement analysis in the clinical lab: key considerations

- ✓ Moving beyond….total C3, C4 levels and CH50/AH50
- ✓ Selection of appropriate **complement functional assays**:Pathway-specific ELISA-based assays (Wieslab/Quidel), liposome-based, mHAM test
- ✓ In screening for an immunodeficiency or complement prot. 'consumption', **total C' activity:** CH50/AH50
- ✓ Specialized assays for individual C' proteins (test **dysfunction**)
- ✓ **Multiplex** analysis (Luminex technology) + **NGS** approaches for C' variants
- ✓ **ELISA** assays do not require high quality RBCs (**lower variability**-advantage for 'standardized' handling across labs)

Thank you for your attention!

How Risky is proximal (C3-Targeted) Therapy…?

- Clinical experience with chronic C3 modulation in PNH pts shows a good safety profile
- Risk mitigation strategy/monitoring in place, similar to anti-C5 therapy
- Acute/transient C3 inhibition does not raise any concerns- ‘phase out’
- FDA/EMA approval of Empaveli solidifies early safety record of C3 therapeutics

- C3-deficient patients do not show uniform phenotype
- Increased susceptibility to infections mainly during childhood

- Pharmacological C3 inhibition does not necessarily phenocopy C3 deficiency after the immune system is fully developed
- Intracellular C3 could provide essential signaling for immunosurveillance
- Small amounts of uninhibited C3 could support opsonization
- Non-complement-dependent mechanisms mediate bactericidal serum activity