Complement Biology in PNH

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Disclosures of Ronald P. Taylor

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
True North			X				

I thank the organizers of this meeting for the kind invitation to speak. I also thank them for years of friendship and fruitful scientific collaboration.

What am I going to talk about?

- C3b deposition and the alternative pathway
- Complement-mediated killing of cells
- Complement inhibitors for treatment of PNH
- Malaria
- Sickle Cell Disease

In 1897, **the physicist William Thompson (Lord Kelvin)** looked at all the tremendous advancements in electricity, astronomy and biology that marked his age and concluded:

"There is nothing new to be discovered in physics now.”

After approval of eculizumab for the treatment of PNH, was there anything new to be learned about inhibiting complement in the clinic?

Pay attention to your findings

Louis Pasteur

"In the fields of observation chance favors only the prepared mind."

George Wald
awarded the 1967 Nobel Prize
for his studies of the chemistry of vision

- “When it (scientific research) is going well, it is like a quiet conversation with Nature.
- One asks a question and gets an answer, then one asks the next question and gets the next answer.
- An experiment is a device to make Nature speak intelligibly. After that, one only has to listen.”

Questions we will ask, based on our "fields of observation" as well as those of others

1. The most immediate mechanism of killing of nucleated Chronic Lymphocytic Leukemia (CLL) B cells opsonized with CD20 mAbs is due to influx of calcium into the cell.

Does calcium influx play a role in killing PNH erythrocytes? (Our blood does not contain Mg-EGTA, calcium is not chelated in vivo!)

2. C9 is **NOT** required to promote C-mediated killing of CD20 mAb-opsonized CLL cells.
C5b-8 forms lethal pores to allow for calcium poisoning.

Is C9 required to lyse PNH E?

3. Numerous lines of evidence indicate that the alternative pathway of complement (APC) plays a critical role in the destruction of E in:

PNH,

Sickle cell disease (SCD),

and malaria

Can the lessons learned from studying PNH be applied to gain insight into possible treatments for these two more prevalent hemolytic diseases?

COMPLEMENT

- Defined in 1894 by Jules Bordet
- Bacteriolytic activity of sheep serum against *V. cholerae* requires 2 components:
  - A heat-stable specific Ab
  - A heat-labile component required for lytic activity

How is the complement cascade manipulated to treat disease?

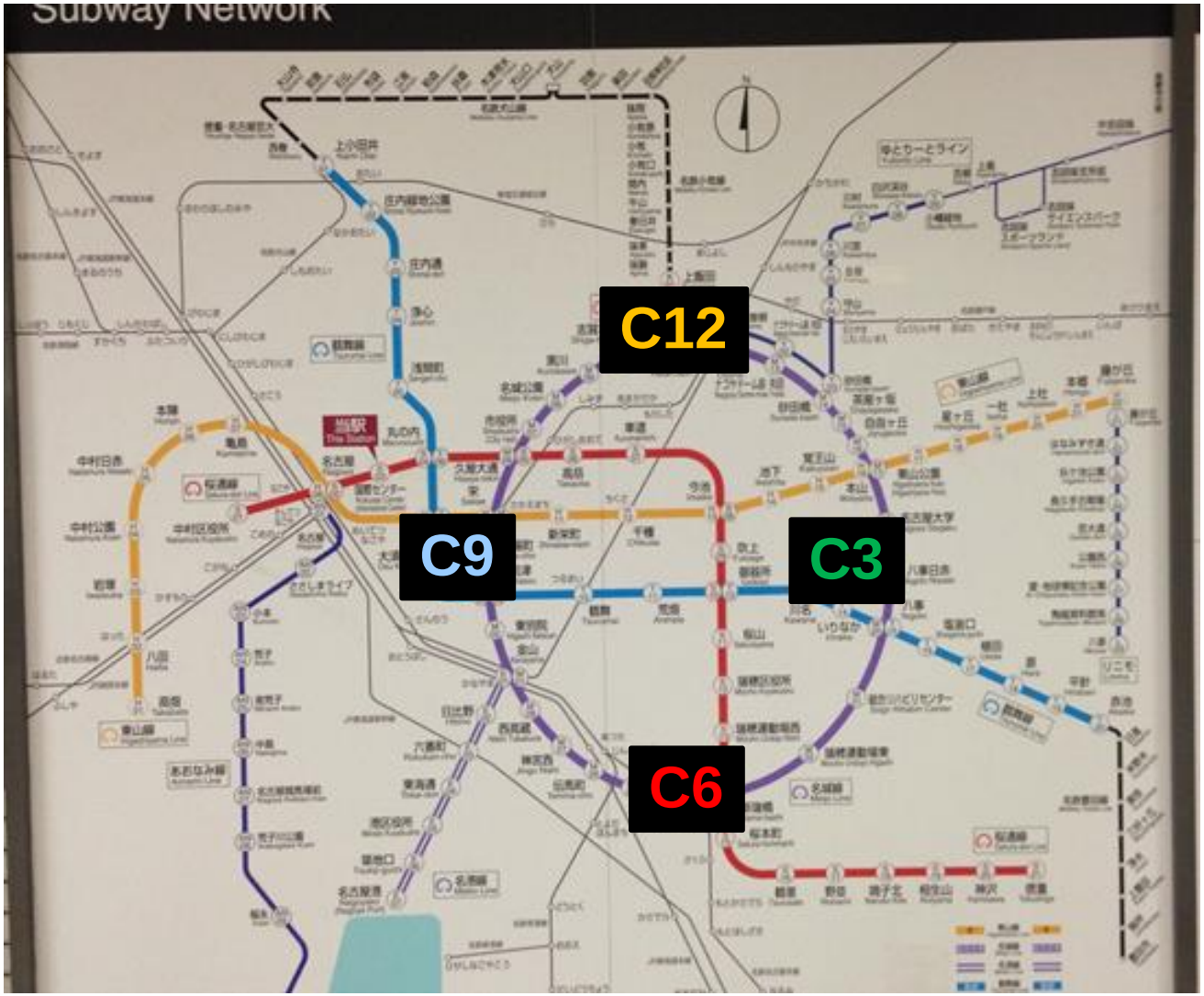
ACTIVATE complement

- Kill bacteria, viruses, cancer cells,
- For example, anti-CD20 mAbs targeted to B cells in CLL

BLOCK Complement to prevent complement-mediated damage to cells and tissues in:

- Paroxysmal nocturnal hemoglobinuria (PNH)
- atypical hemolytic uremic syndrome (aHUS)
- C3 glomerulopathy (C3G)
- IgA Nephropathy (IgAN)
- Idiopathic Immune Complex-mediated Glomerulonephritis (ICMPGN)
- Cold Agglutinin Disease (CAD)

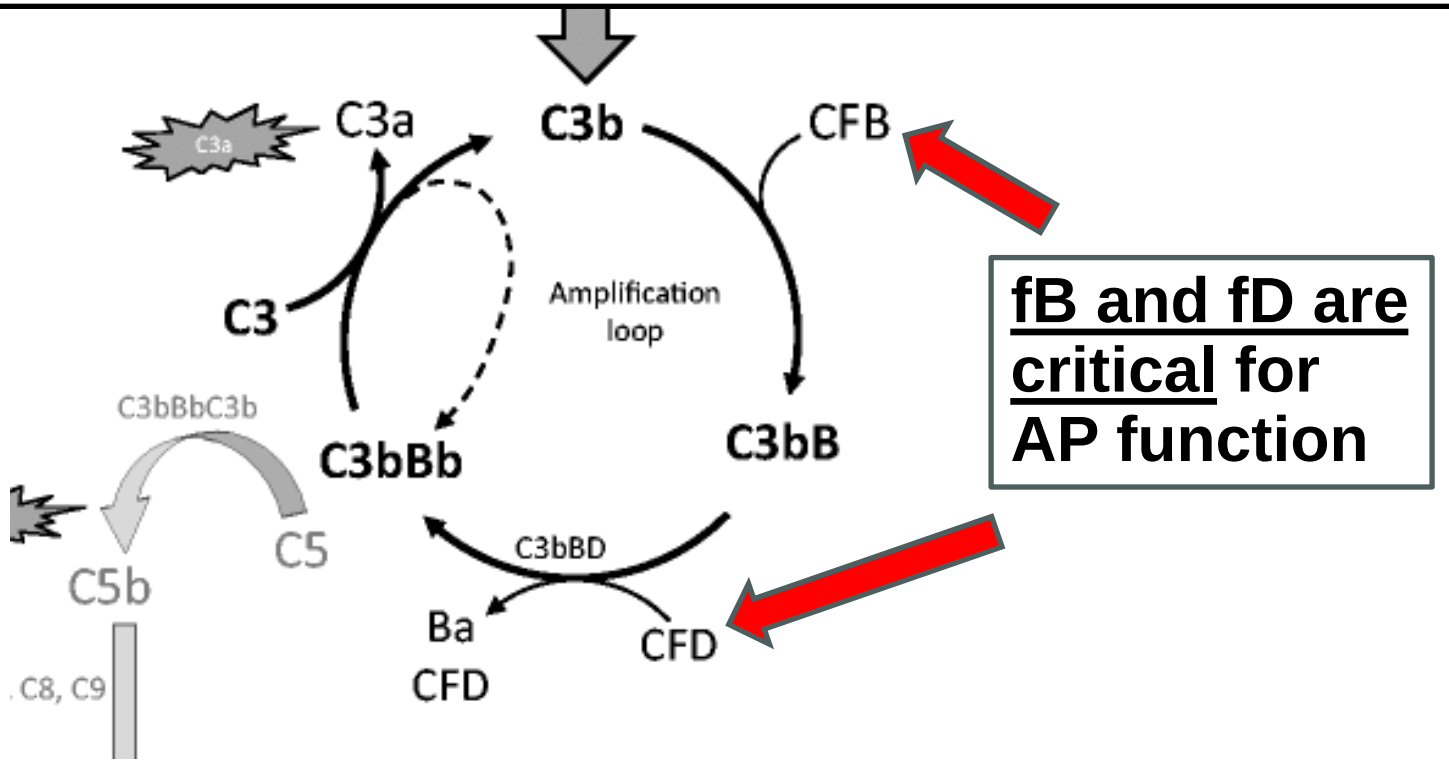
The three complement pathways are very easy to understand



You need only be confused by the alternative pathway!

In the presence of excess C3 and fB and fD, activated C3b combines with fB, forming C3bBb, which generates MORE activated C3b – in an AMPLIFICATION loop.

Activated C3b deposits covalently on substrates.



fB and fD are critical for AP function

*** M Pangburn, Immunolog Reviews, 2023; M. Pangburn, in Immunobiology of the complement System,1986.**

Is it safe to block complement in adults?

- Innate immunity (complement) is supplemented by antibody-mediated immunity.
- For example, antibodies can promote phagocytosis and neutralization of bacteria in the absence of complement.
- “Patients undergoing C3 inhibition therapy will likely need to be immunized against (certain) bacterial diseases. There is no evidence pointing to a vital role for C3 in adults who have a fully developed adaptive immunity.”[a]

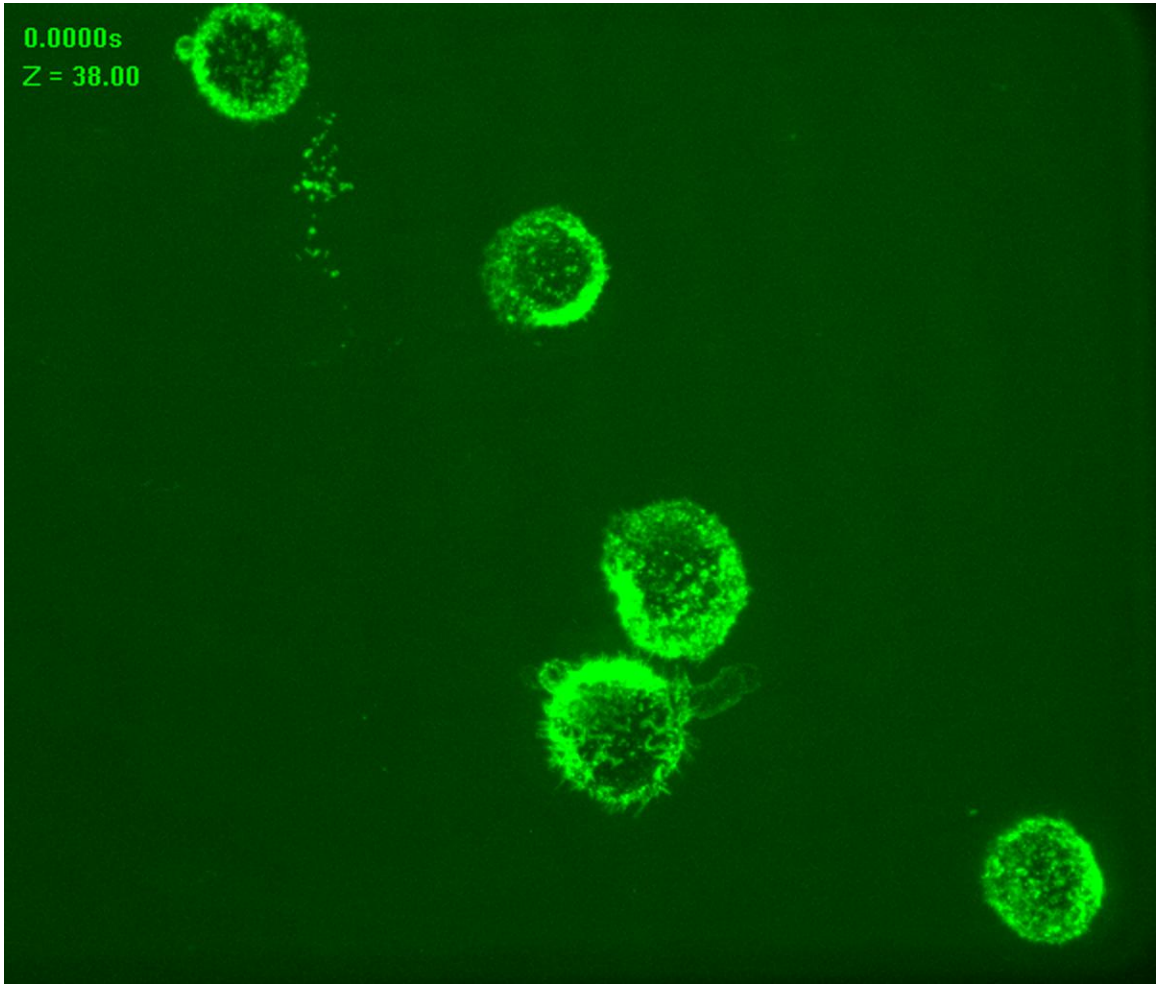
[a]**Lambris group, Immunol Rev 2016**

Questions

- During complement-dependent killing of CD20 mAb-opsonized B cells, can we image the cells and follow activated C3b deposition in real time?

mAb 3E7 is specific for covalently deposited C3b, and binds to deposited C3b even in the presence of the 1 mg/ml C3 in NHS.

Reaction of Al488 RTX-opsonized Daudi cells in NHS results in the TOTALLY UNEXPECTED: generation of streamers!!! (Tunneling nanotubules)

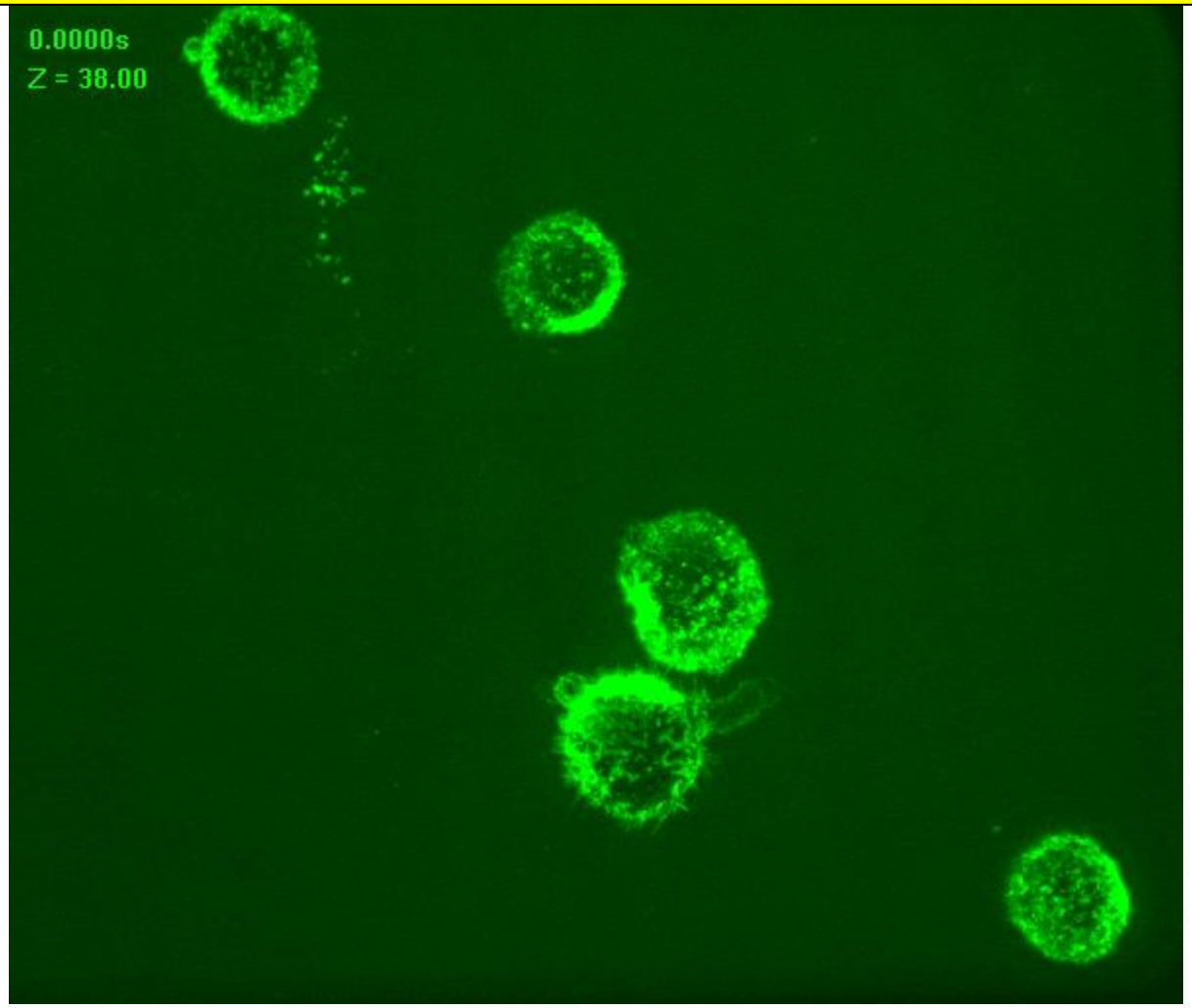


Green
Al488
RTX

Daudi cells opsonized with **Al488 RTX** and reacted with 50% NHS

Beum et al JI 2008

Reaction of Al488 RTX-opsonized Daudi cells in NHS results in the TOTALLY UNEXPECTED: generation of streamers!!! (Tunneling nanotubules)



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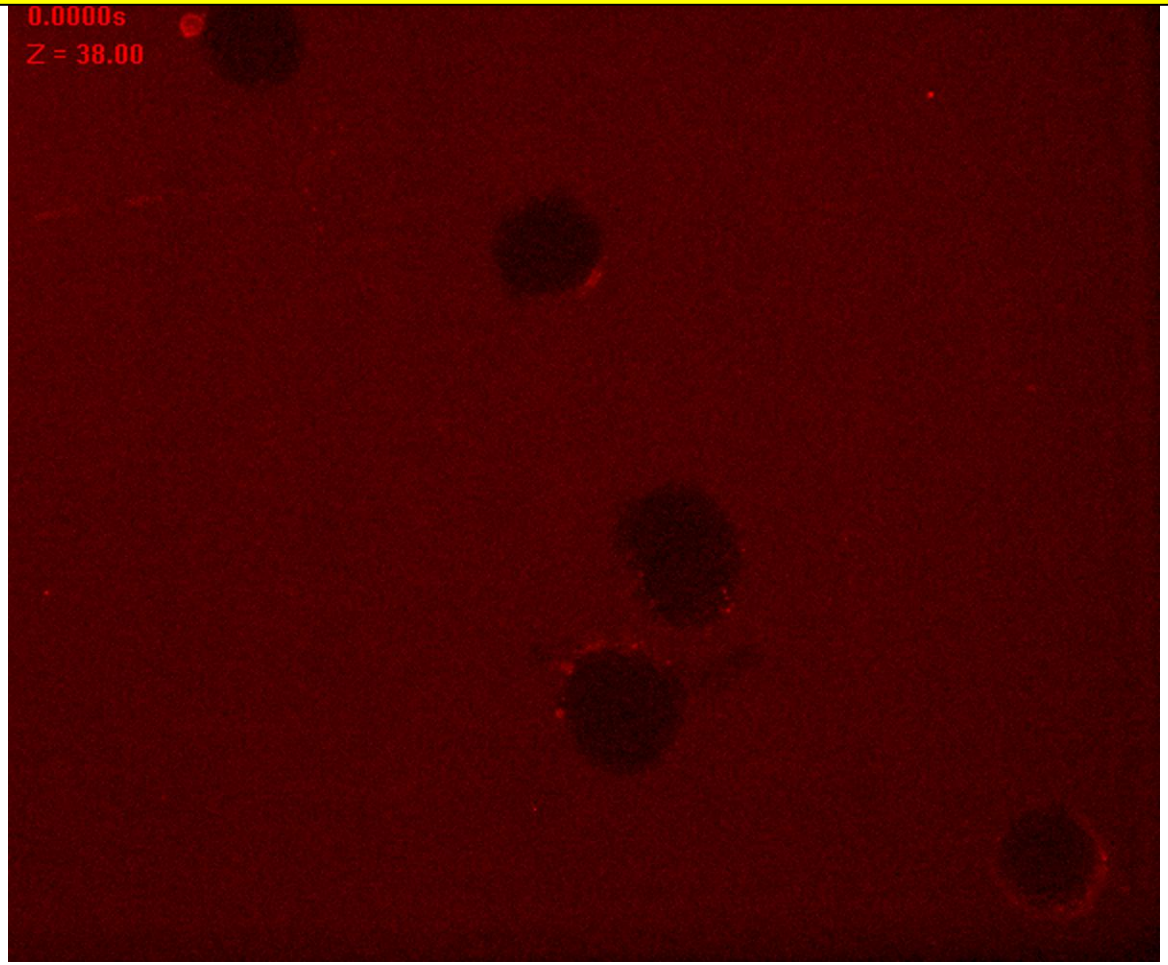
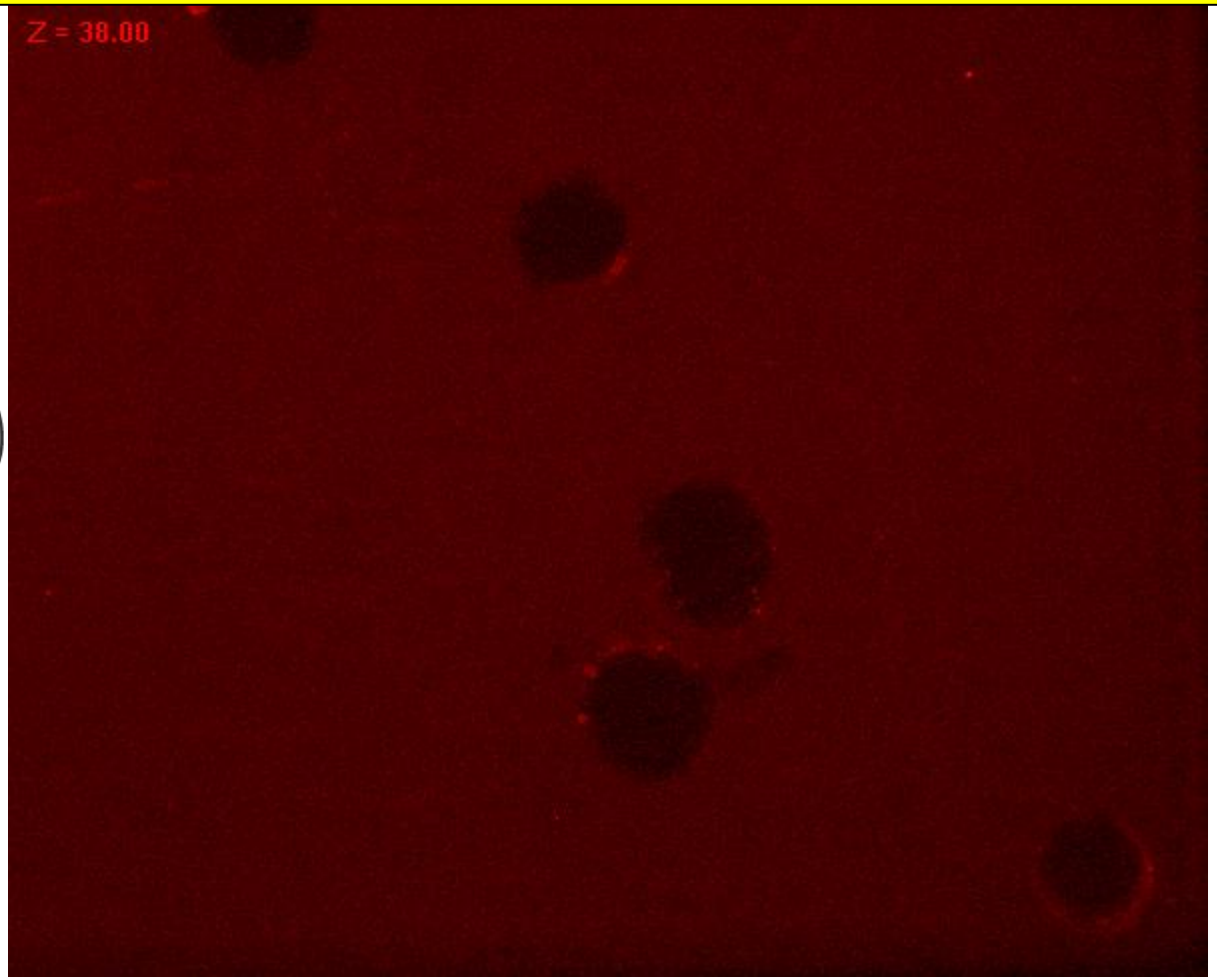


Red Al546 mAb 3E7 (C3b)

Daudi cells opsonized with **Al488 RTX** and reacted with 50% NHS in the presence of **Al546 mAb 3E7**

Beum et al JI 2008

Reaction of Al488 RTX-opsonized Daudi cells in NHS results in the TOTALLY UNEXPECTED: generation of streamers!!! (Tunneling nanotubules)



C3b deposition

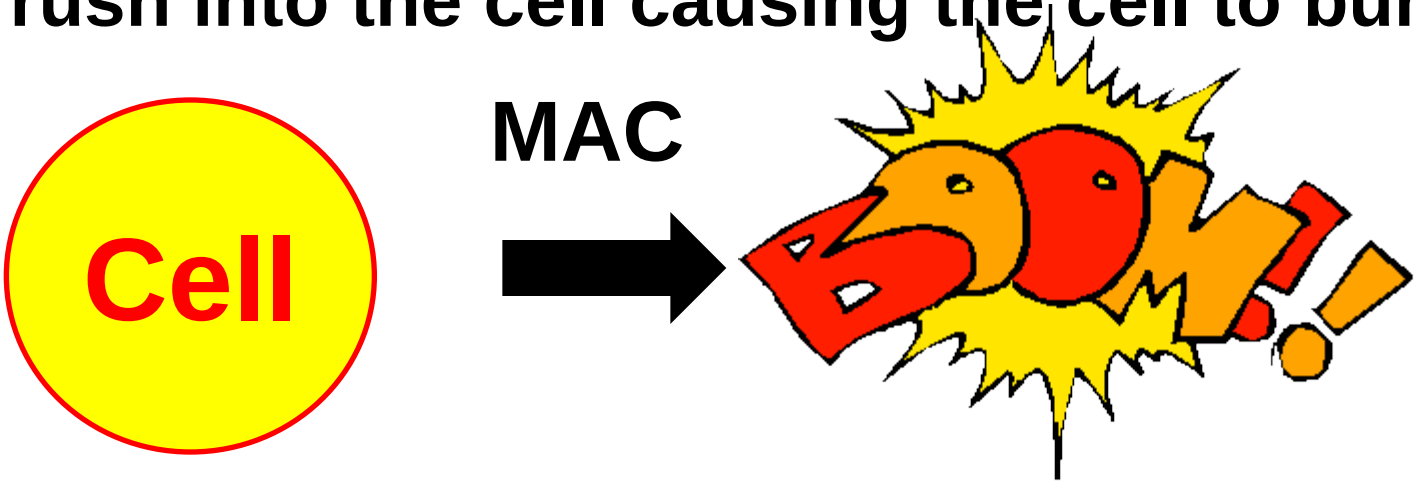
Daudi cells opsonized with **Al488 RTX** and reacted with 50% NHS in the presence of **Al546 mAb 3E7**

Beum et al JI 2008

What is the mechanism of streamer formation?

Dogma:

Formation of the MAC pore allows water to rush into the cell causing the cell to burst.



Calcium Dependence of Toxic Cell Death: A Final Common Pathway
Schanne et al., Science 206: 700-702, 1979

"Our data give new insight into the mechanisms of (**toxin**-induced) cell death"

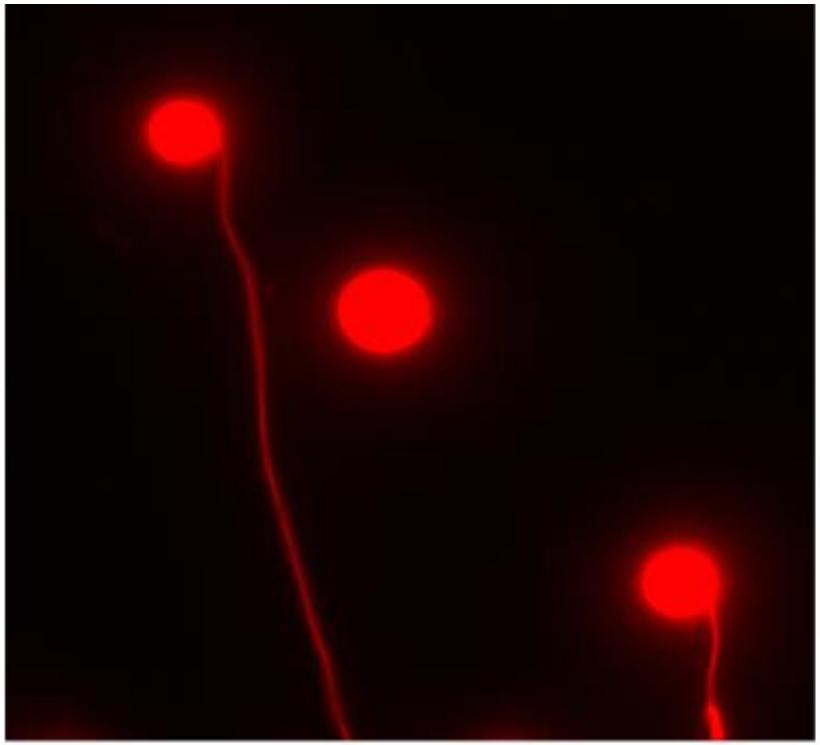
"The first step represents a disruption of the integrity of the plasma membrane and is independent of calcium."

"The second step … most likely represents an influx of Ca^{+2} across the damaged plasma membrane … and represents a final common pathway by which the cells are killed."

The membrane attack complex C5b-9 is certainly a toxin. Does it promote cell killing via calcium influx?

Generalization of the streaming reaction

Antibody-opsonized sheep E in NHS exhibit streaming.

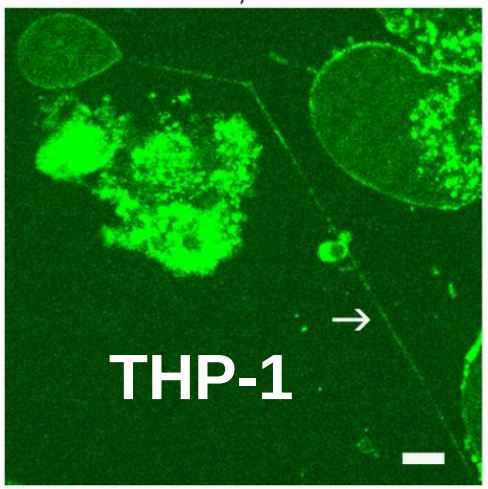


Red PKH26

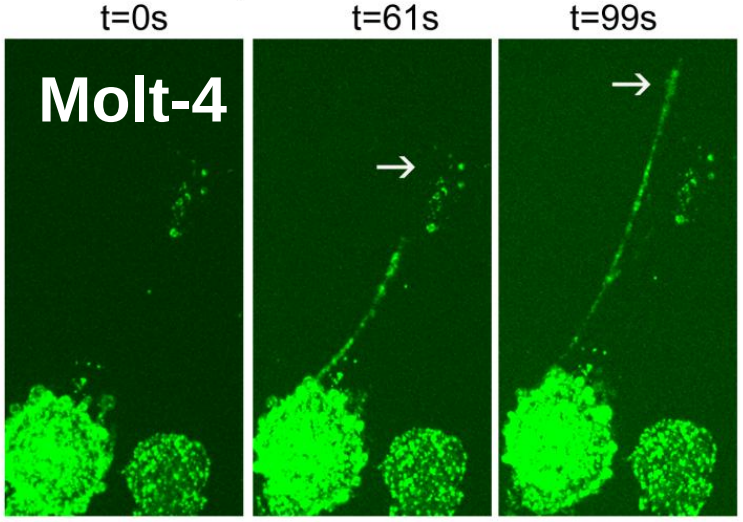
Beum et al JI 2008

In the presence of Ca2+, streamers can be generated in THP-1 monocytes, Molt-4 T cells and mAb-opsonized Daudi cells by addition of the detergent melittin.

A. THP-1 cells, melittin



B. Molt-4 cells, melittin

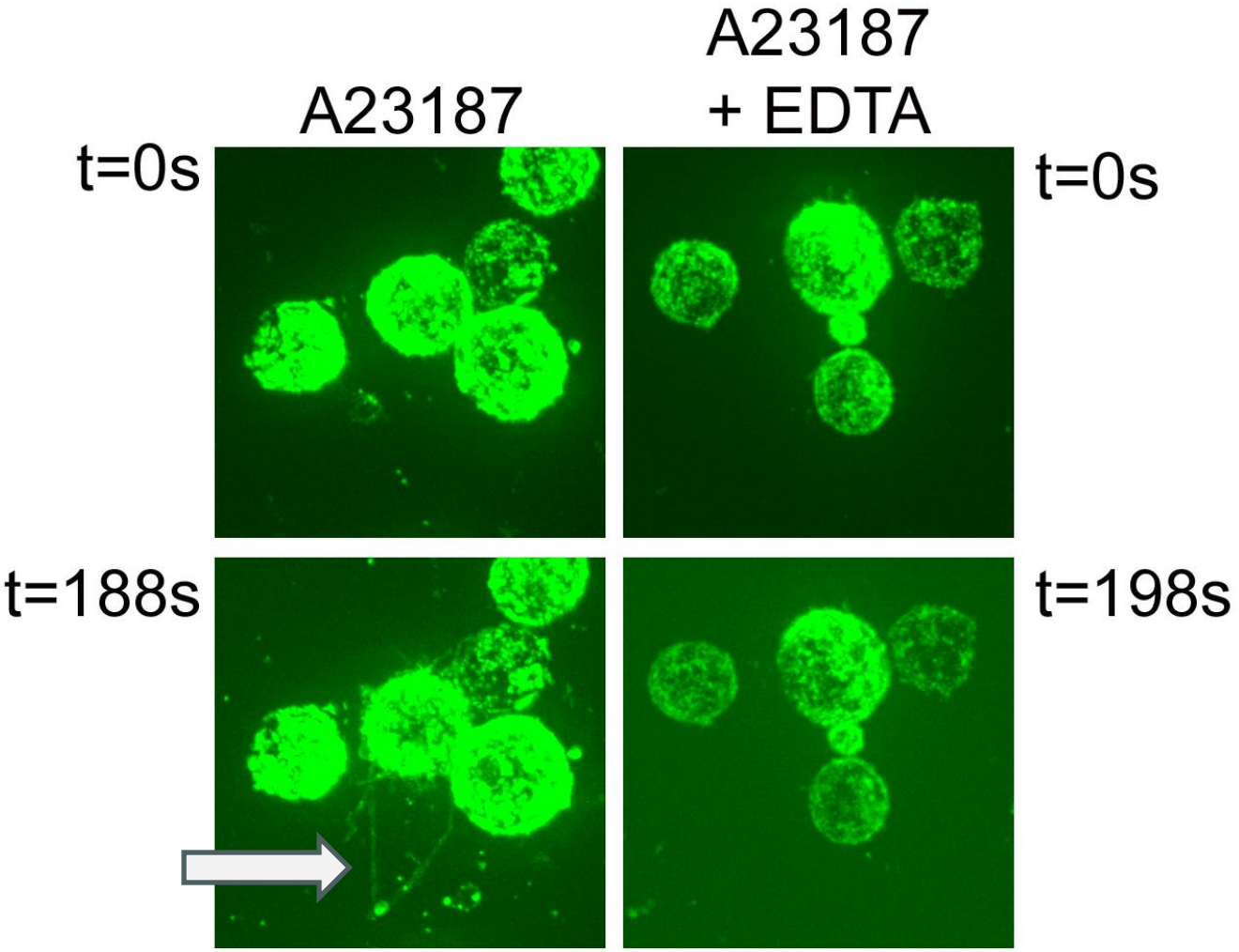


Most generally, influx of Ca2+ into cells produces streamers/TNT

B. Red, OFA (Daudi cells/melittin/media)

Beum et al EJI 2011; JI 2008

Cells treated with the Ca2+ ionophore A23187 also exhibit streaming. EDTA prevents streaming by chelating Ca2+.



Basic Science Experiments to Consider

- At what rate are normal or PNH E killed in serum-Mg-EGTA vs serum? Does influx of Ca2+ play a role?

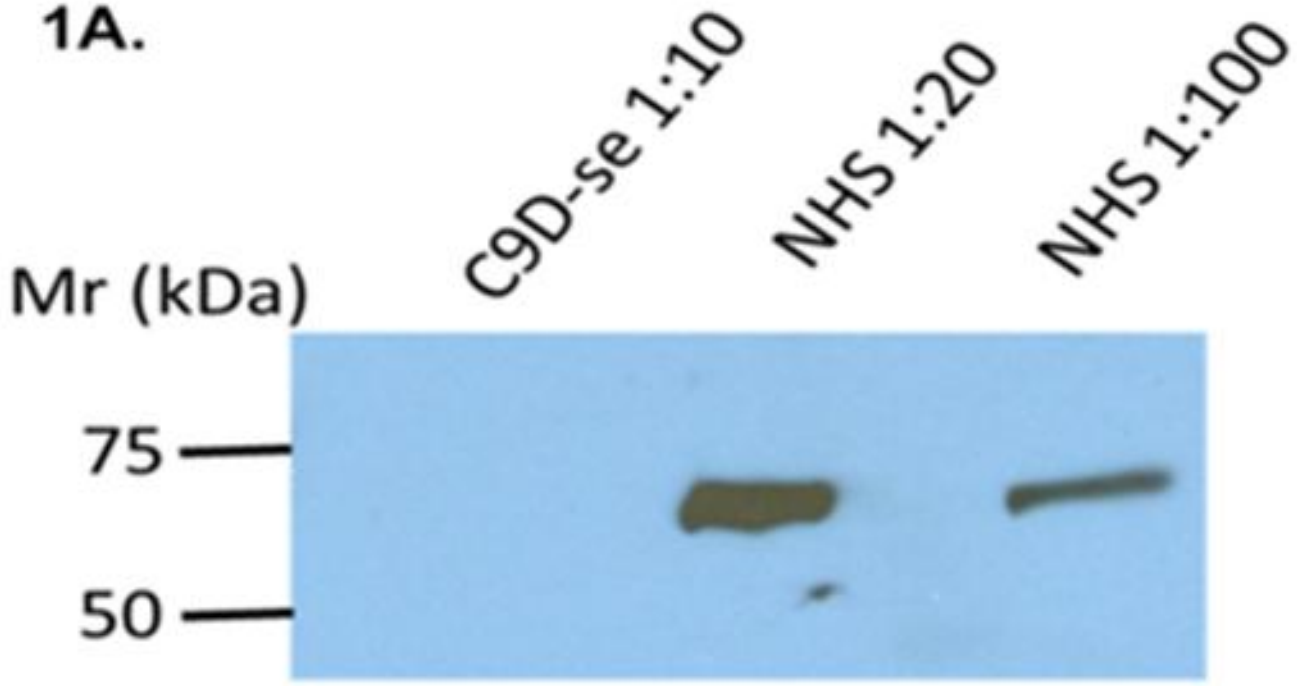
- Test naïve E, or E opsonized to the C3b stage, followed by serum, serum-EDTA, serum-Mg-EGTA +/- Ca2+.

- Will this information be useful in the future?

Is C9 ESSENTIAL to promote CDC of mAb-opsonized CLL cells?

There is a high incidence (~ 0.1%) of C9 deficiency among healthy blood donors in Osaka, Japan.
Int. Immunol. (1989) 1 (1): 85-89 Fukumori et al.

We addressed the question with serum from an individual with a genetic deficiency of C9.

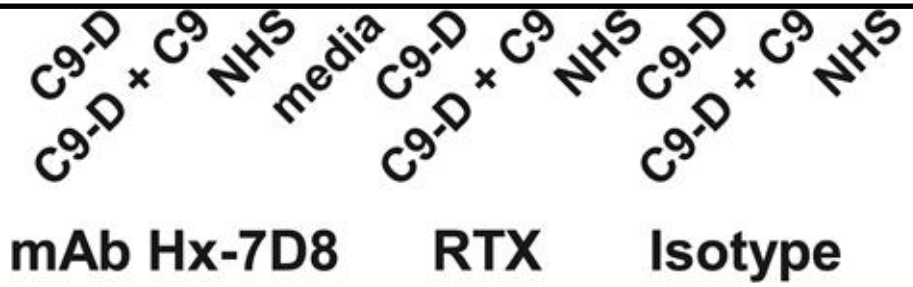


Taylor, RP, ….Morgan, BP, Clin Immunol, 2017

CLL cells opsonized with mAb Hx-7D8 are killed by CDC **in the complete absence of C9**

Can normal or PNH E be killed in C9-deficient serum?

These interesting basic science experiments should be done. If cells are not killed, then blocking C9 might be an acceptable adjunct treatment strategy.



6 patients,
10 µg/ml mAb

Taylor, RP, ….Morgan, BP, Clin Immunol, 2017

Other hemolytic diseases

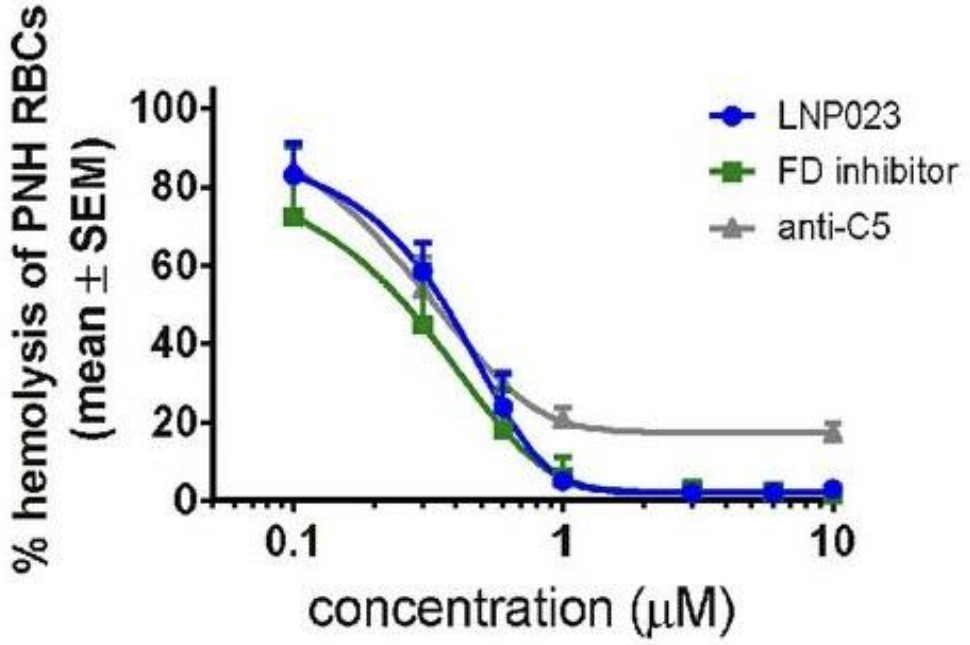
Numerous lines of evidence indicate that the alternative pathway of complement (APC) plays a critical role in the destruction of E in PNH, Sickle cell disease (SCD) and malaria.

Questions to Consider

- Can the lessons learned from studying PNH be applied to gain insight into possible treatments for two more prevalent hemolytic diseases: SCD and malaria?
- Can oral drug inhibitors of the APC, approved for PNH, be utilized?

Small-molecule factor B inhibitor (Novartis) for the treatment of complement-mediated diseases

- 250,000 small molecules screened for potential to block the proteolytic action of cobra venom factor-Bb complex
- Minor chemical modifications optimized binding of LNP023 to factor B



- LNP023 exhibited very high levels of activity in suppressing the APC, both in mouse models and with PNH RBCs.

Schubart, Risitano et al, PNAS 2019

Targeting fB for PNH:
LNP023 (aka Iptacopan, Fabhalta)

Approved by the FDA, December, 2023

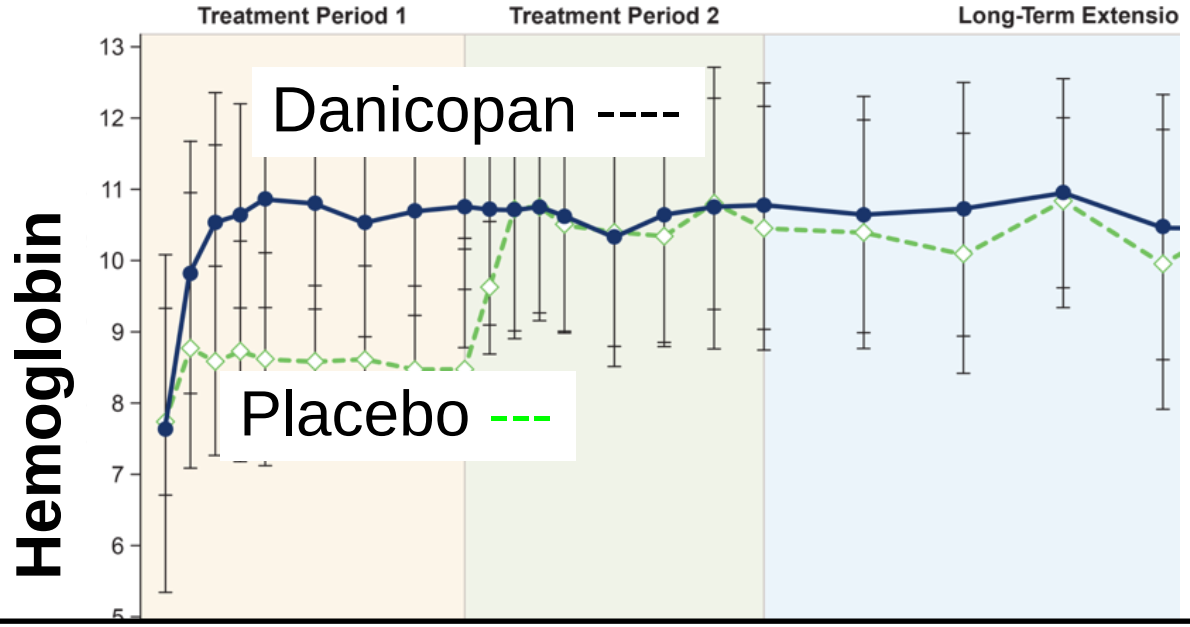
Based on the Apply-PNH phase 3 Trial

(Compared Anti-C5 to Fabhalta) and the

Appoint-PNH trial (Complement-inhibitor naïve patients)

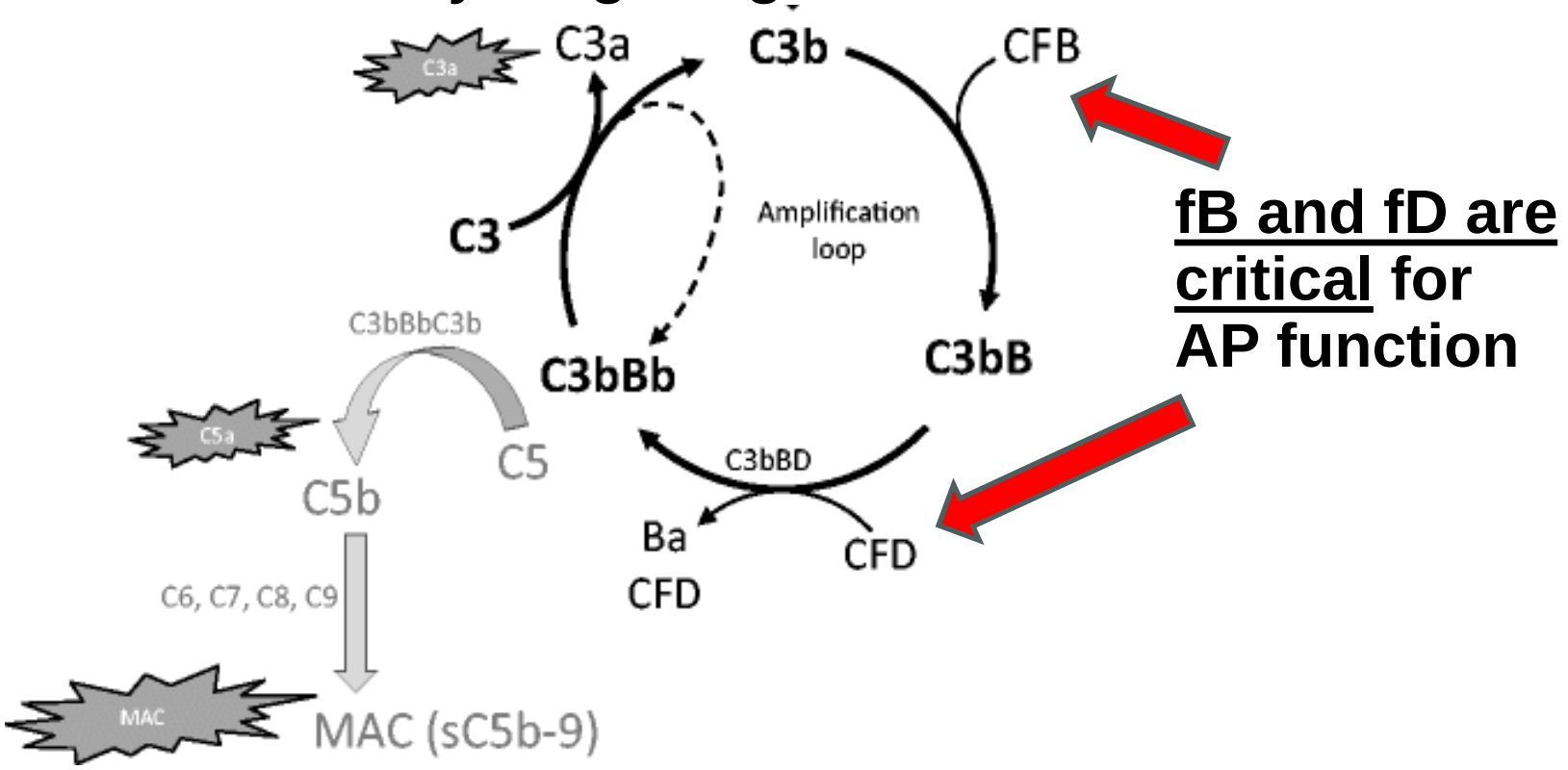
"In the anti-C5 group which was switched at 24 weeks to Fabhalta, mean hemoglobin levels increased to near normal: 9.1 g/dL at 24 weeks (anti-C5) to 12.1 g/dL at 48 weeks (24 weeks of Fabhalta)" *Novartis announcement*

Targeting Factor D in PNH: danicopan



On April 1, 2024, AstraZeneca announced the approval of danicopan (Voydeya) as an add-on therapy to eculizumab (Soliris) or ravulizumab (Ultomiris) in the treatment of PNH in the US. Danicopan is an oral first-in-class factor D inhibitor designed to treat adults experiencing extravascular hemolysis (EVH).

What is the advantage of blocking the AP by targeting fB or fD?

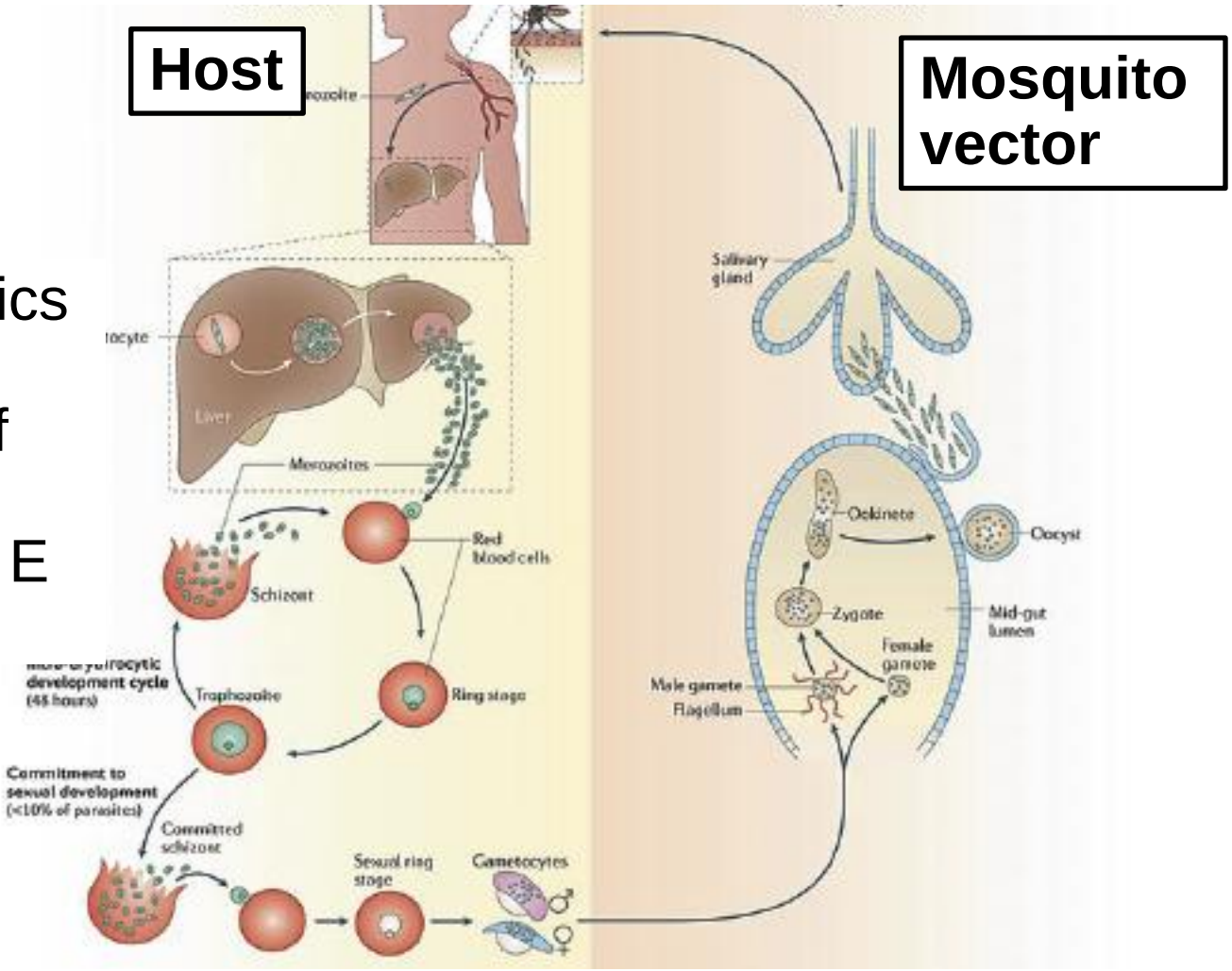


<u>fB and fD are critical</u> for AP function

Both the classical and MBL pathways will still function (especially relevant in vaccinated individuals), preserving an important element of protection against infection.

The “usual” introduction to Malaria: Life Cycle of Plasmodium

These schematics NEVER show the fate of uninfected, nonparasitized E



Taylor, Stoute, and Lindorfer, Mechanisms of Complement Activation in Malaria 2018
Springer International J.A. Stoute (ed.) Complement Activation in Malaria Immunity and Pathogenesis

The APC and Malaria

- Hemolysis of E releases hemoglobin, which is broken down to heme.
- “Heme promotes uncontrolled complement alternative pathway amplification by interfering with the regulatory capacity of factor I”[a]
- Hb and heme can be neutralized in the circulation by haptoglobin, and hemopexin, respectively.
- If there is substantial hemolysis, these factors can be overwhelmed, and then heme can actvate the APC and promote deposition of C3 fragments on uninfected E[b,c,d].
- In malaria, **at least 10-20 non-parasitized E** are removed for every infected E.

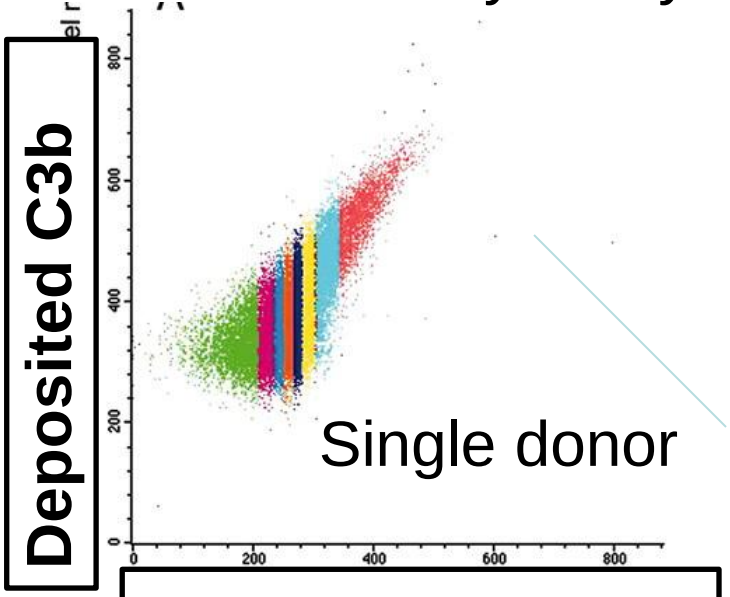
[a]Gerogianni, Frontiers Immunol 2022; [b]Pawkuczkowycz, Lindorfer, Taylor JI 2007;
[c]Lindorfer, Risitano, Lambris and Taylor, Clin Immunol 2016; [d] Merle et al., JCI Insight 2018

The APC and Malaria

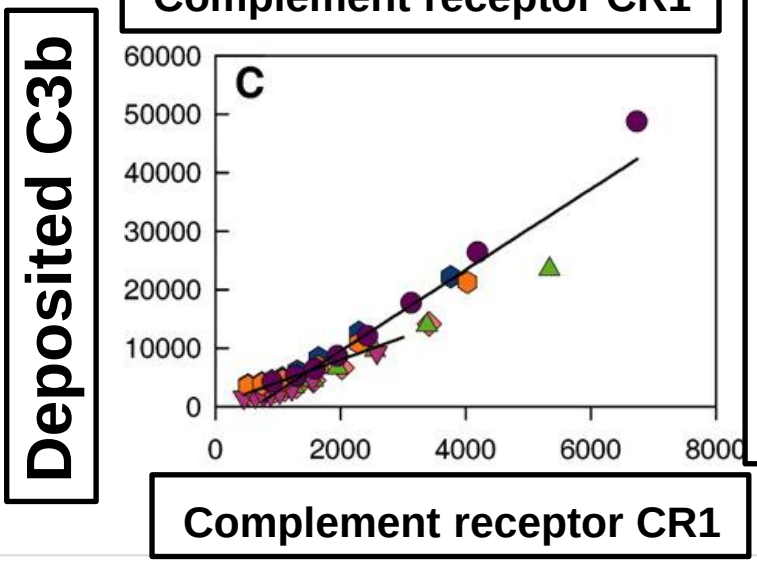
- In malaria, **at least 10-20 non-parasitized E** are removed for every infected E.
- “Phagocytosis apppears to be the predominate mechanism of removal of non-parasitized RBC” (in malaria)[e].
- “Phagocytosis (is due to) membrane opsonization through enhanced binding of complement factor C3b.”[e]
- **Can uninfected, but C3 fragment-opsonized E, be cleared by phagocytosis in the liver and spleen (Risitano mechanism)?**

[e]Gallo, Malaria Journal 2012

Hematin Promotes Alternative Pathway-Mediated Deposition of C3b on Human Erythrocytes via CR1



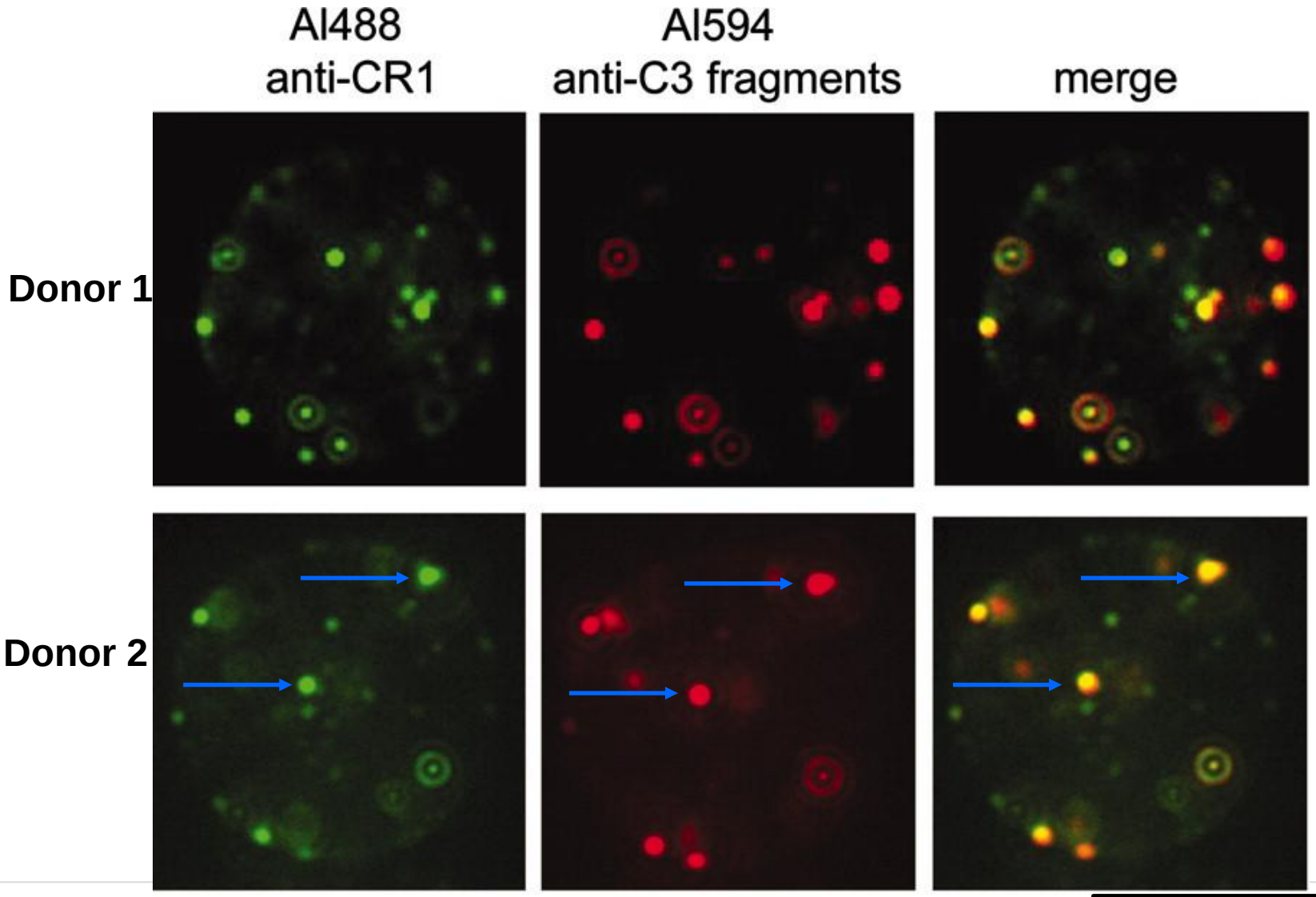
E were reacted with hematin in 40% NHS.
E with the highest levels of CR1 were opsonized with the most C3b fragments.



In malaria, if the youngest E (highest CR1 and most highly C3b opsonized) are then cleared by extravascular hemolysis, **this could lead to sudden and catastrophic anemia.**

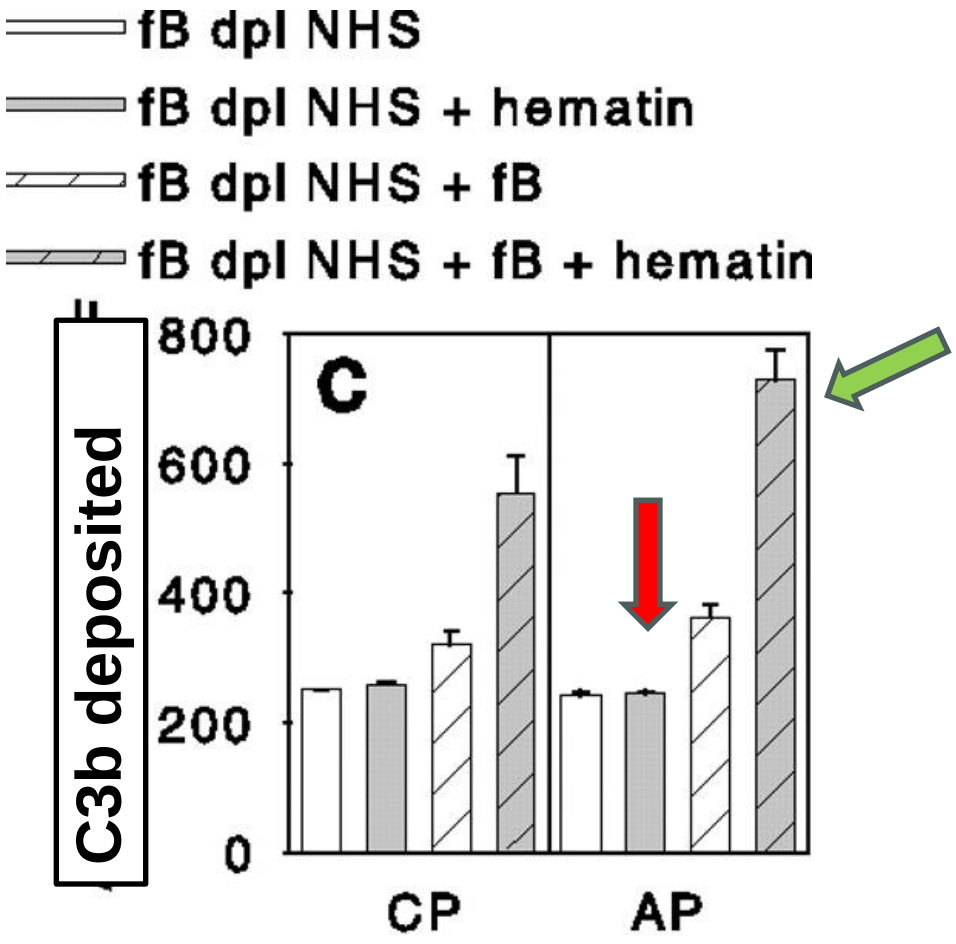
Taylor, Lindorfer et al. J Immunol. 2007

CR1 plays an active role:
deposited C3 fragments are co-localized with CR1



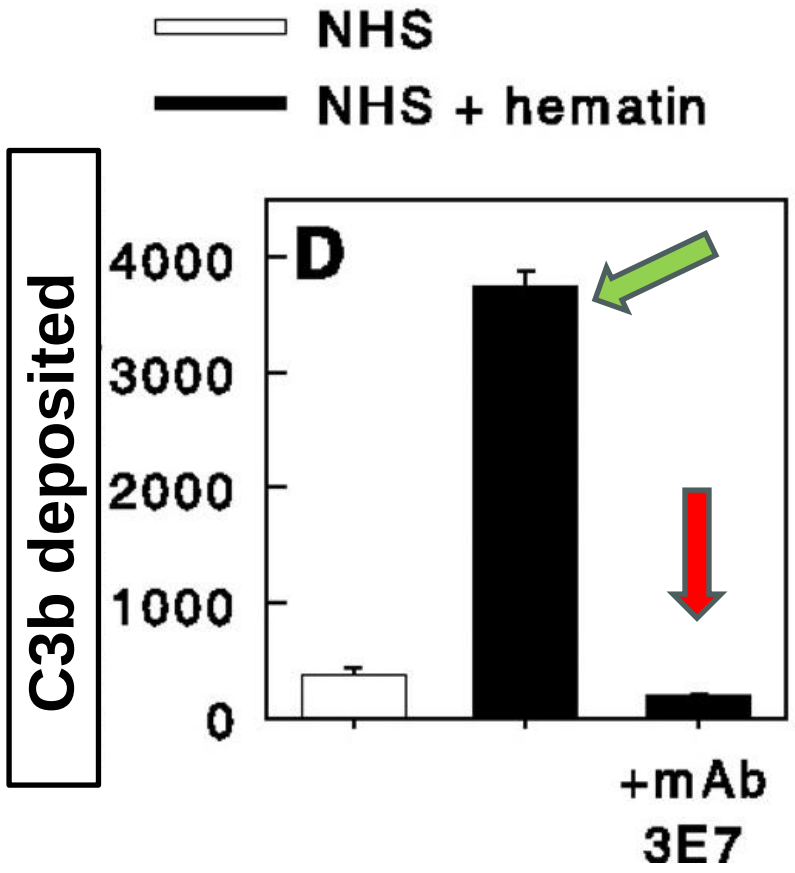
Taylor, Lindorfer et al. J Immunol. 2007

C3b deposition on E mediated by hematin is abrogated in fB-depleted serum



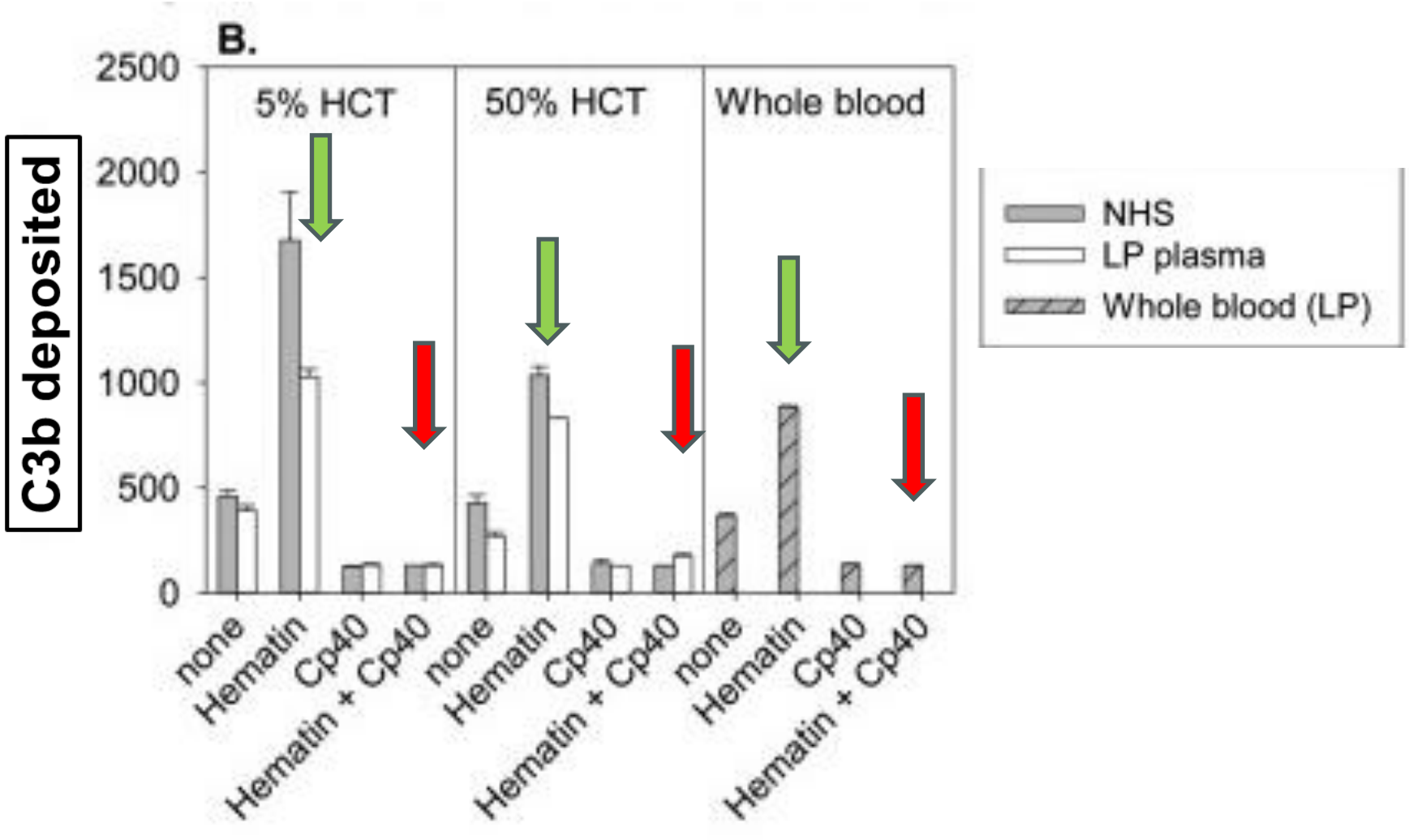
Taylor, Lindorfer et al. J Immunol. 2007

C3b deposition on E mediated by hematin is also abrogated in serum containing mAb 3E7 (blocks APC).



Taylor, Lindorfer et al. J Immunol. 2007

Compstatin Cp40 blocks hematin-mediated deposition of C3 fragments on E



Taylor, Lindorfer, Risitano, Lambris et al Clin Immunol, 2016

Malaria Summary

- Substantial evidence implicates E breakdown products (especially heme) in C3b-opsonization and subsequent removal of non-parasitized E in malaria.

- In vitro experiments demonstrate that agents that block/prevent activation of the APC (mAb 3E7, Factor B depleted serum) prevent C3b-opsonization of E.

- Can FDA-approved oral Factor B or Factor D inhibitors of the APC be examined “off-label” for the treatment/prevention of severe anemia in childhood malaria?

Sickle Cell Disease and the APC

Complement activation during painful crisis in Sickle Cell anemia[1]
“Alternative pathway activation is increased during painful crisis. Membrane phospholipid changes in deoxygenated sickle erythrocytes lead to alternative pathway activation.” (1995)

Complement Component C5 …Mediates Heme-Induced Thromboinflammation in Human blood[2]
"Plasma heme and complement activation was markedly increased in a SCD patient accompanied by depleted hemopexin and haptoglobulin.

[1]Mold et al. Clin Immunol Imunopathol 1995; [2]Thomas et al, JI 2019

Sickle Cell Disease and the APC

Cold exposure induces vaso-occlusion and pain in sickle mice that depend on complement activation[3]

“Complement activation occurs through the assembly of alternative pathway C3 and C5 convertases on the phosphatidylserine-rich outer membrane of sickle red blood cells and the microparticles they release.

Can FDA-approved oral Factor B or Factor D inhibitors be examined “off-label” for the treatment/prevention of pathologies associated with SCD?

[3]Ivy et al, Blood 2023

- **How difficult (and expensive) will it be to explore the science of blocking complement in malaria and SCD?**
- **Can complement inhibitors be used successfully in these diseases at reasonable costs?**
- **Addressing these questions will be like running a marathon, but more difficult.**
- **However, there is precedent for a complementologist running a marathon.**

Addressing the C3 opsonization-extravascular hemolysis problem in PNH was like running a marathon, but Dr. Risitano persisted

Thank you for your attention

- I hope you have lots of questions.