The Complement Cascade – When Activation Mediates Toxicity

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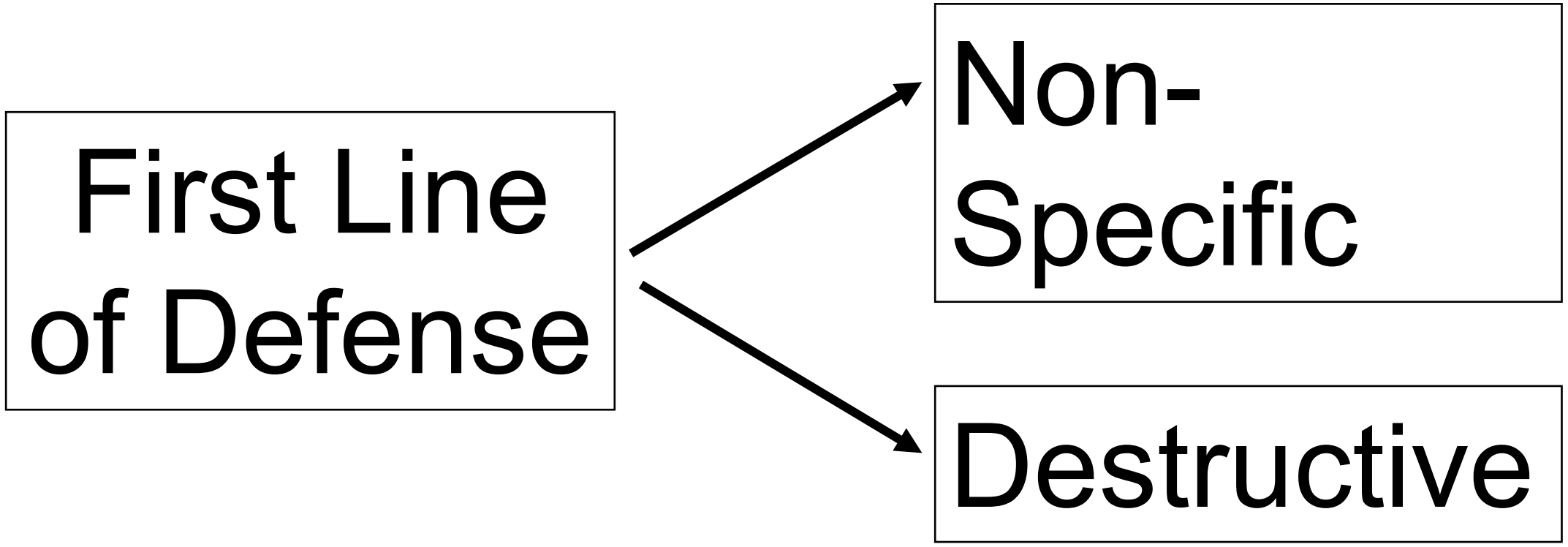
What I Hope to Cover in this Webinar

- Introduction to the complement cascade
  - The basic functions and physiological effects
  - The components
- Biomarkers of complement
  - Matching markers to a pathway
  - Considerations when measuring complement
- Complement activation as mediator of AEs
  - What we have learned for ASO off target complement activation
  - Emerging information of complement and AAVs

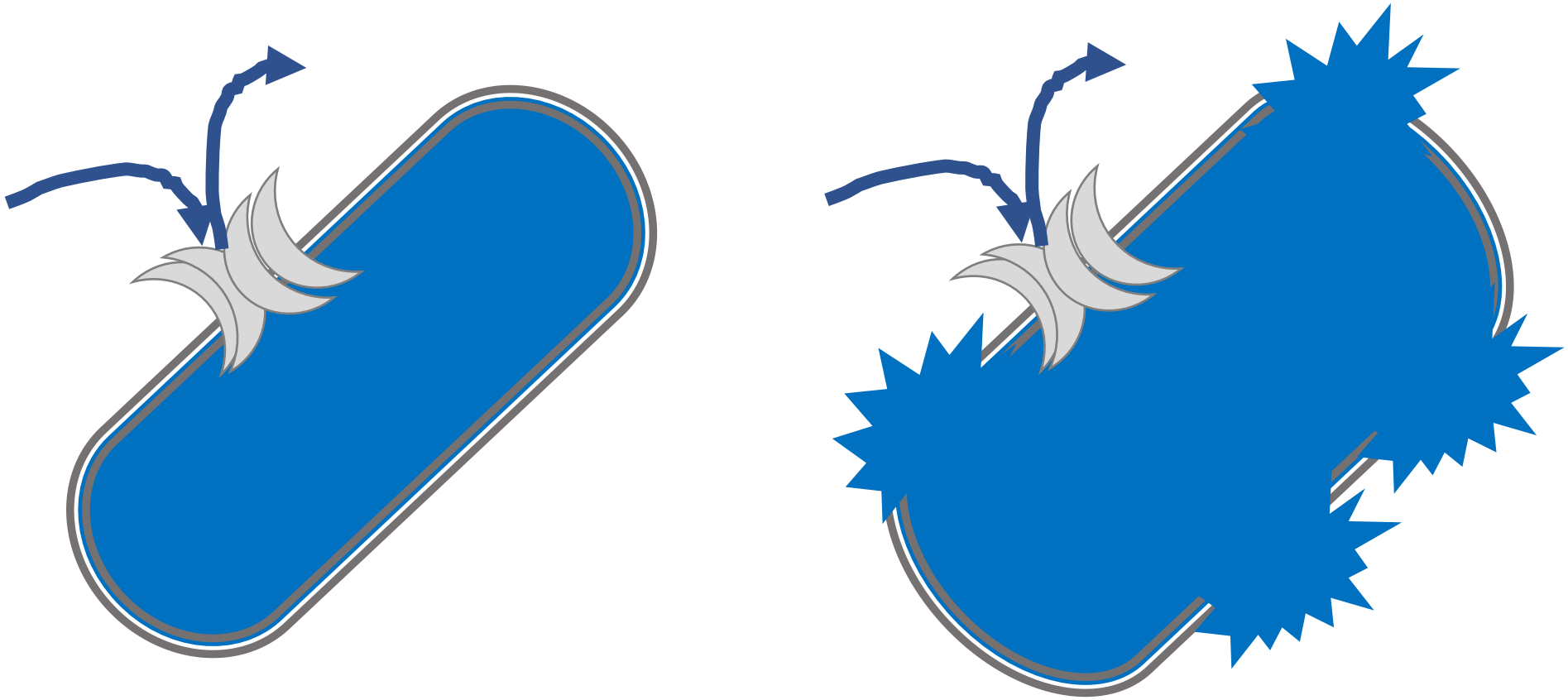
The Basics of Complement Function

Starting simple so you can develop a foundation to understand when things get complicated

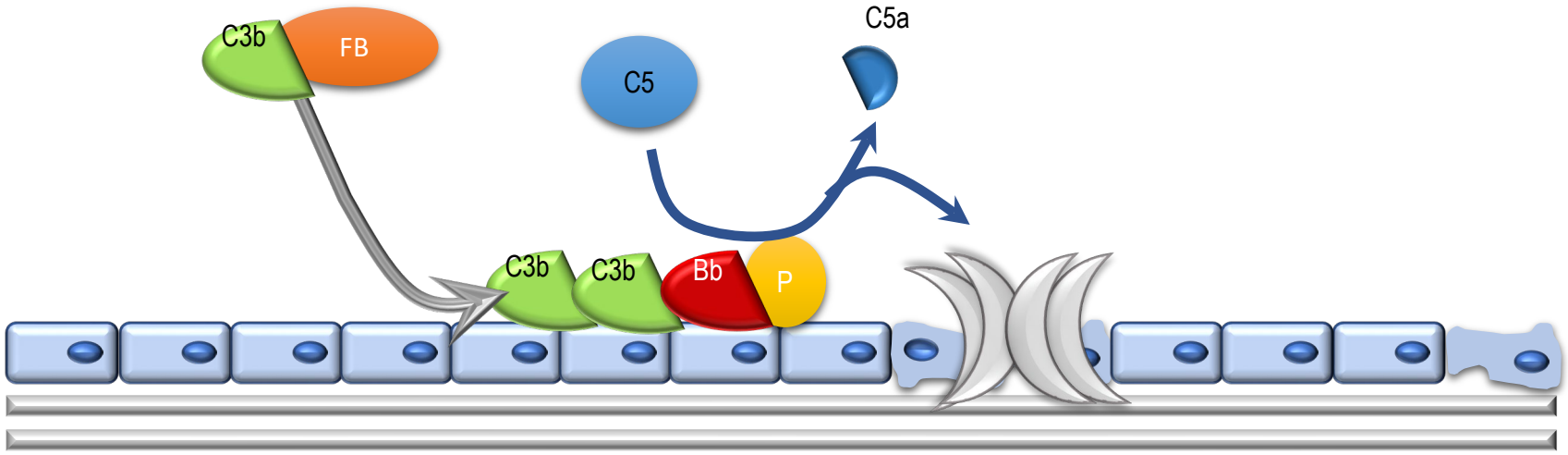
Complement: Innate Humoral Immunity



1. Membrane Attack Complex Fighting Infection

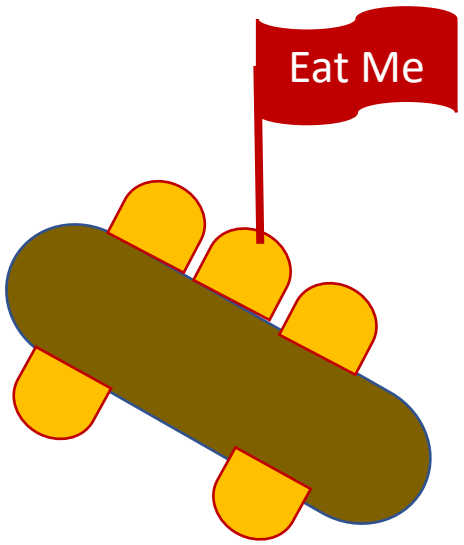


Membrane Attack Complex Turning on the Host

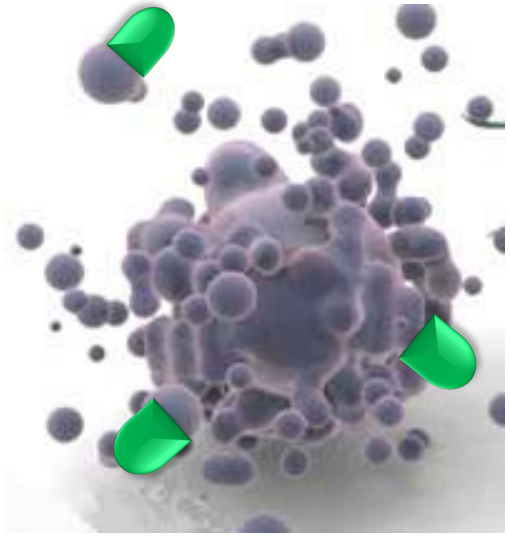


Resulting in Deposition and Tissue Damage

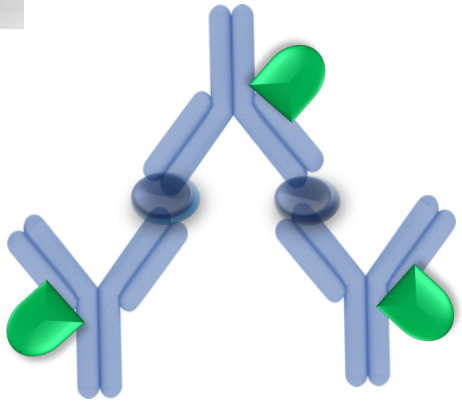
“Opsonization” Tagging Invaders and Debris for Removal



Clear Bacteria

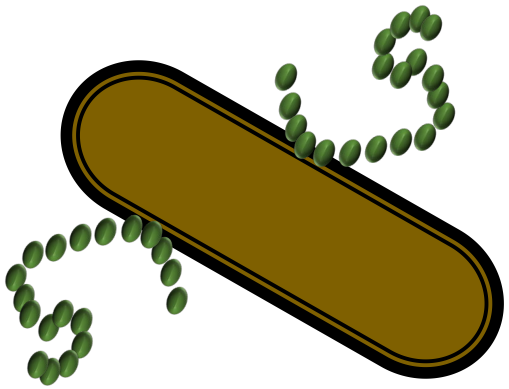


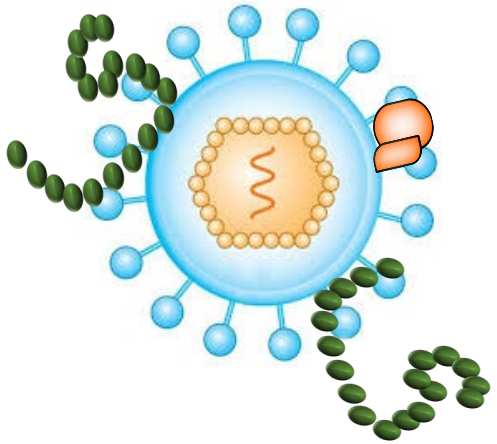
Clear Cellular Debris



Clear Immune Complexes

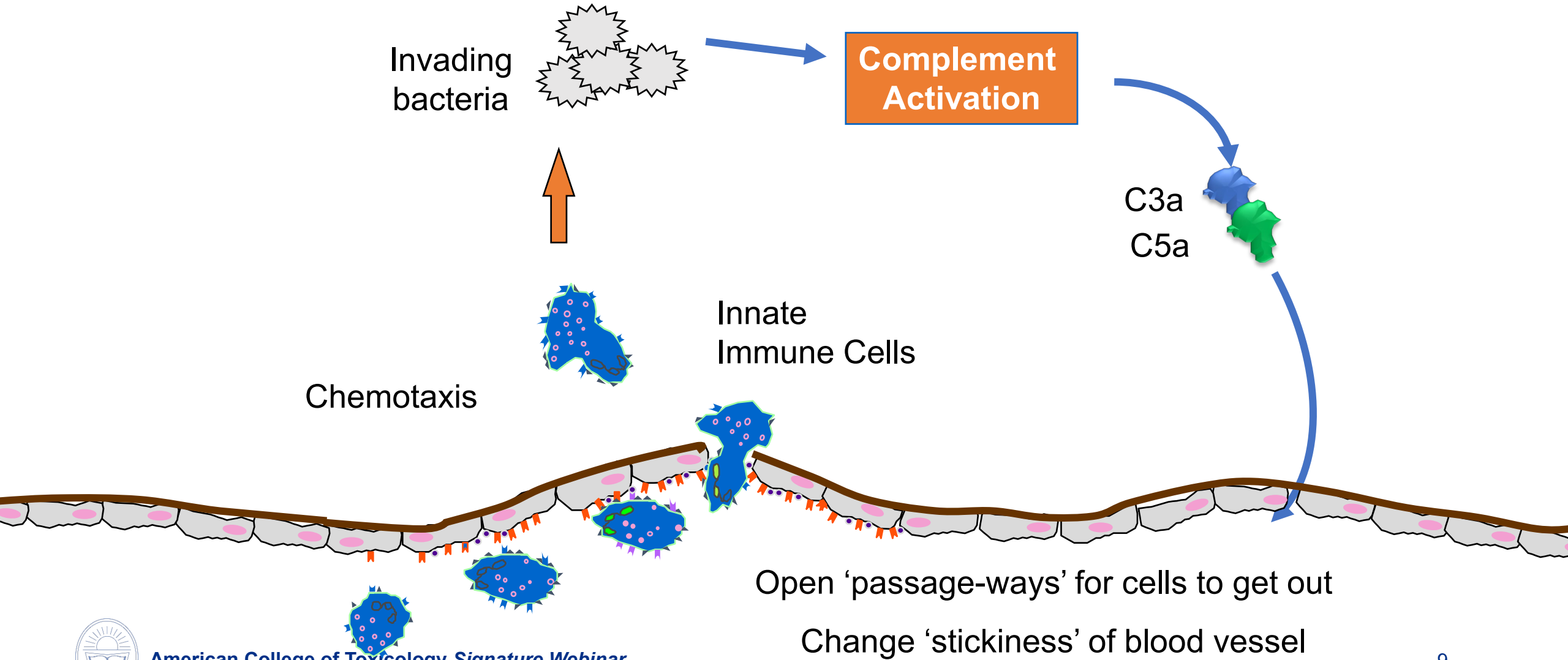
“Opsonization” Tagging Invaders and Debris for Removal



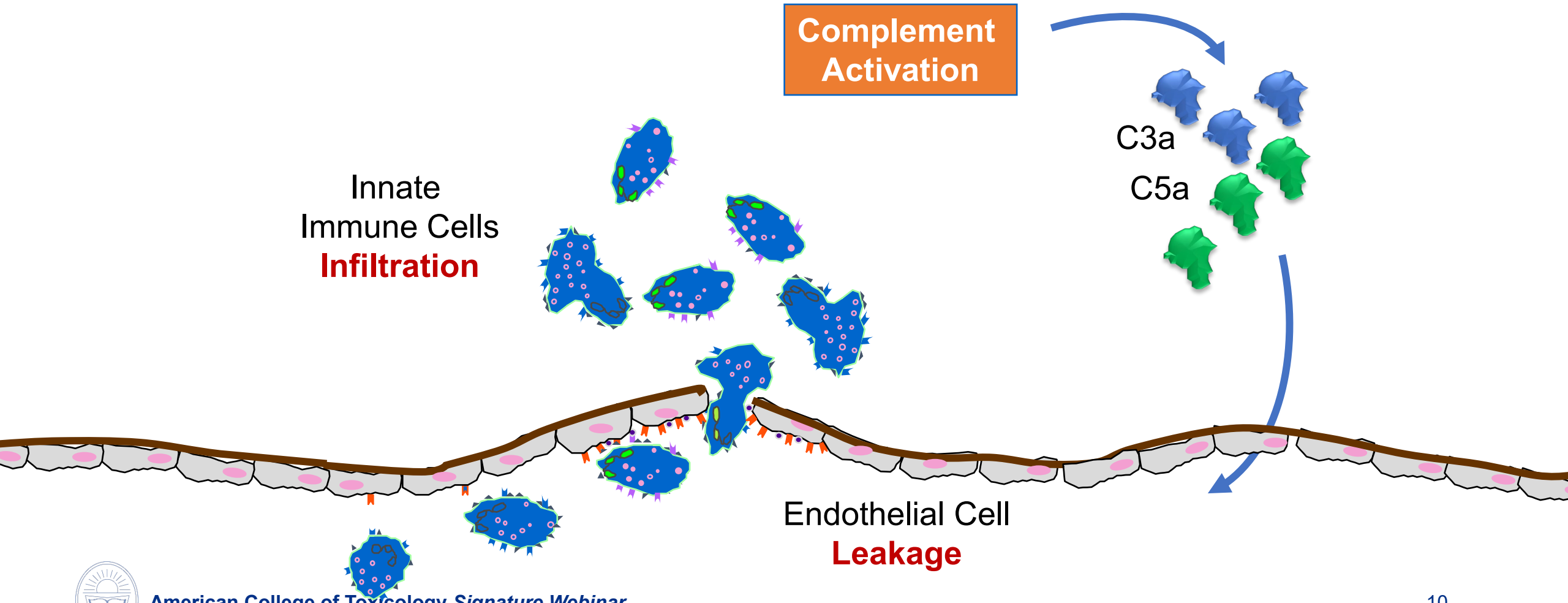


Number of bacteria and viruses coat themselves in complement regulators to thwart complement and even facilitate cell entry

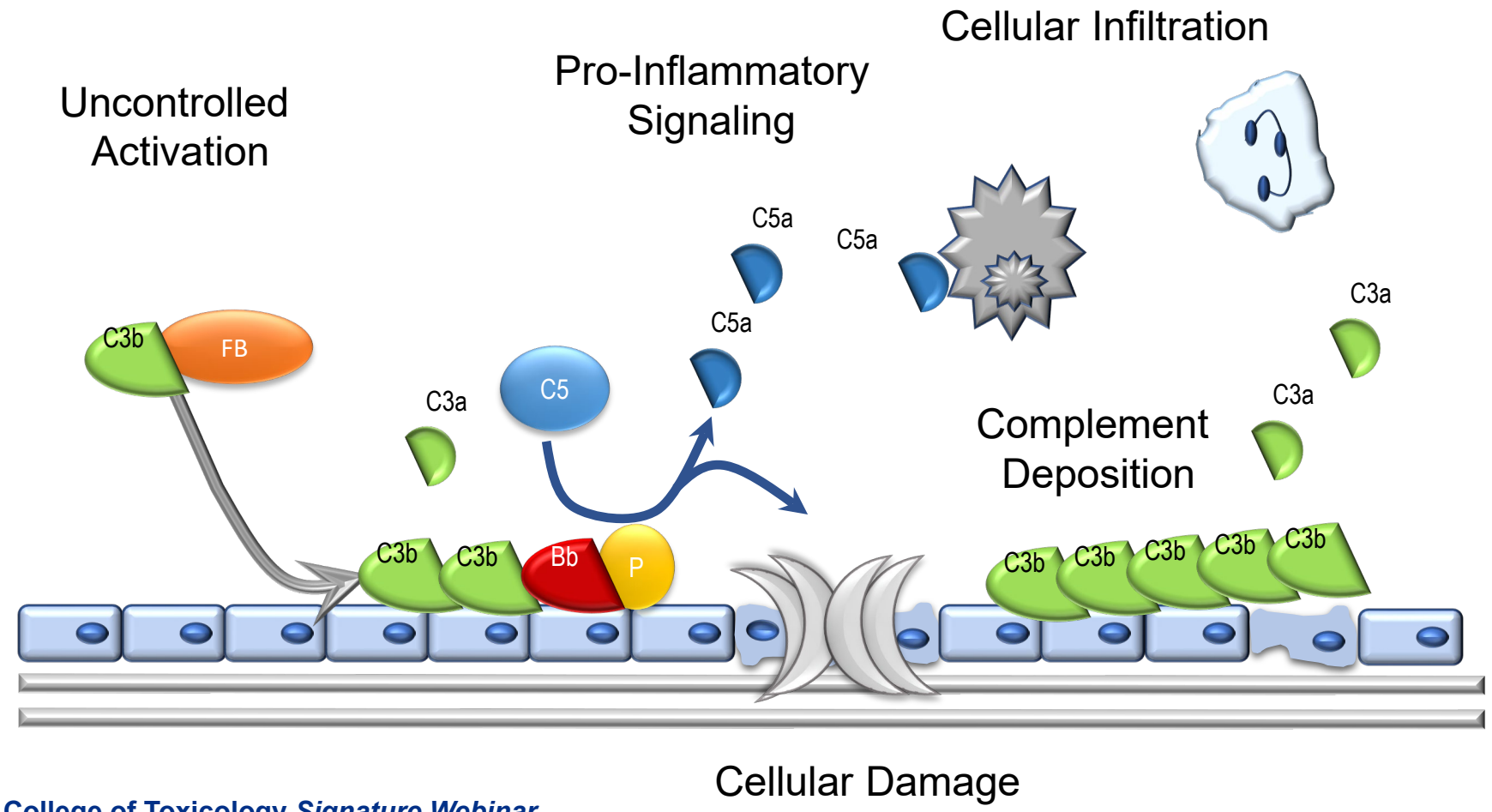
Direct Inflammation & Larger Immune Response



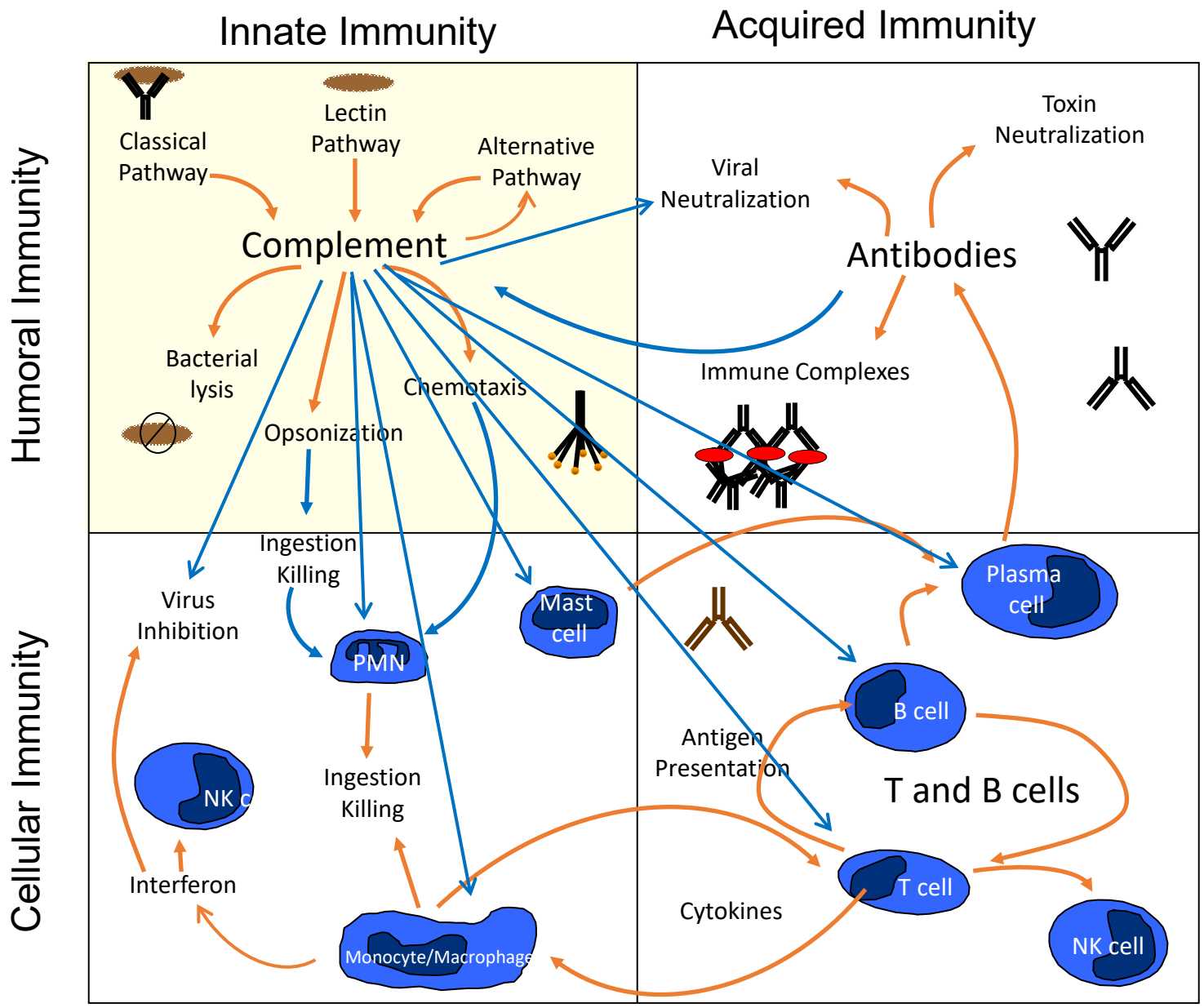
Uncontrolled Inflammation and Immune Response



Complement Dysregulation Adverse Outcome



Complement does not work alone



The Complement Components

- Protein cascade similar to coagulation
- >50 proteins
- Enzymes, proenzymes
- Co-factors
- Regulators
  - Circulating
  - Membrane bound
- Receptors

The Complement Nomenclature

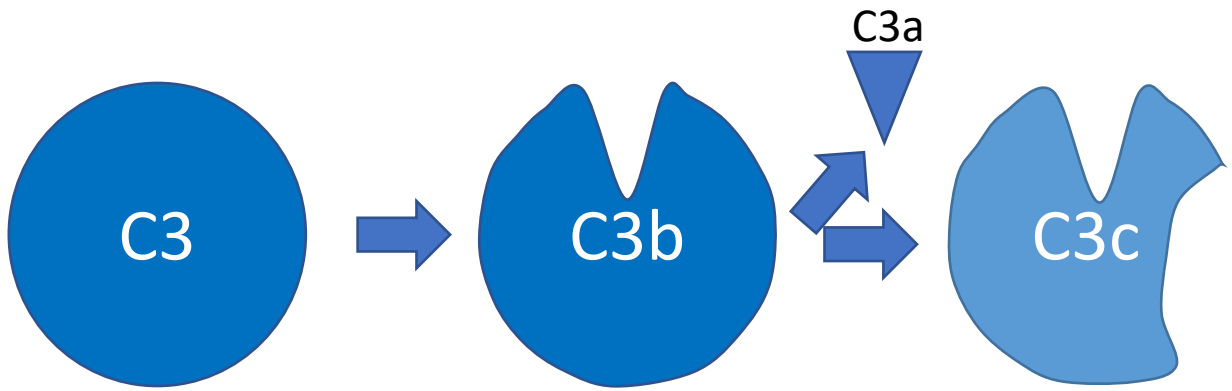
- "C" = classical & terminal pathway

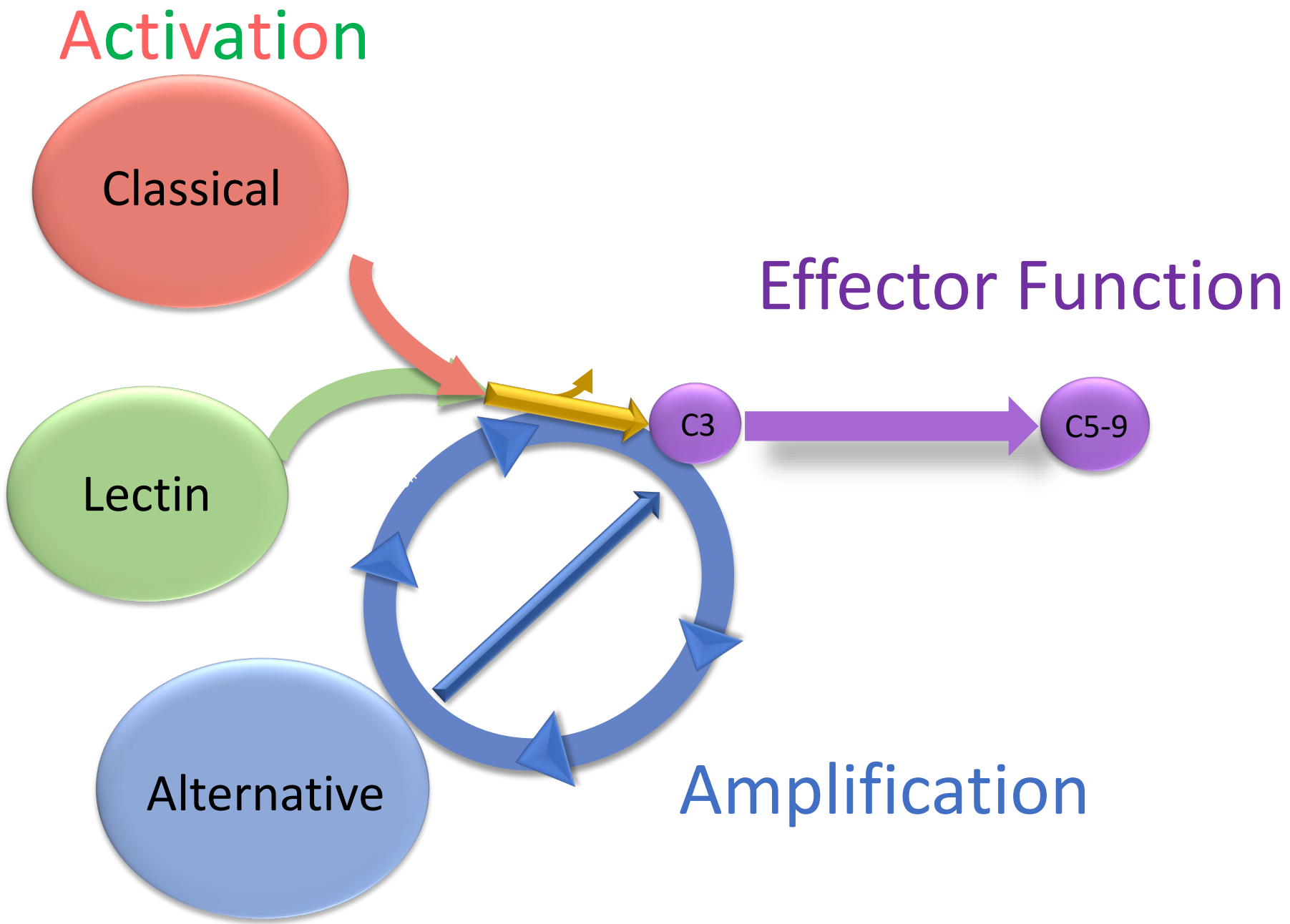
  Numbering:

  C1, C4, C2, C3, C5, C6, C7, C8, C9
- MBL, MASP = lectin pathway
- "Factor" = alternative pathway
- No "Factor C"

The Complement Nomenclature

- Lower case letter indicates cleavage “activation fragments”
  - C3a, C3b, iC3b, C3dg
  - Bb, Ba
- True for all pathways

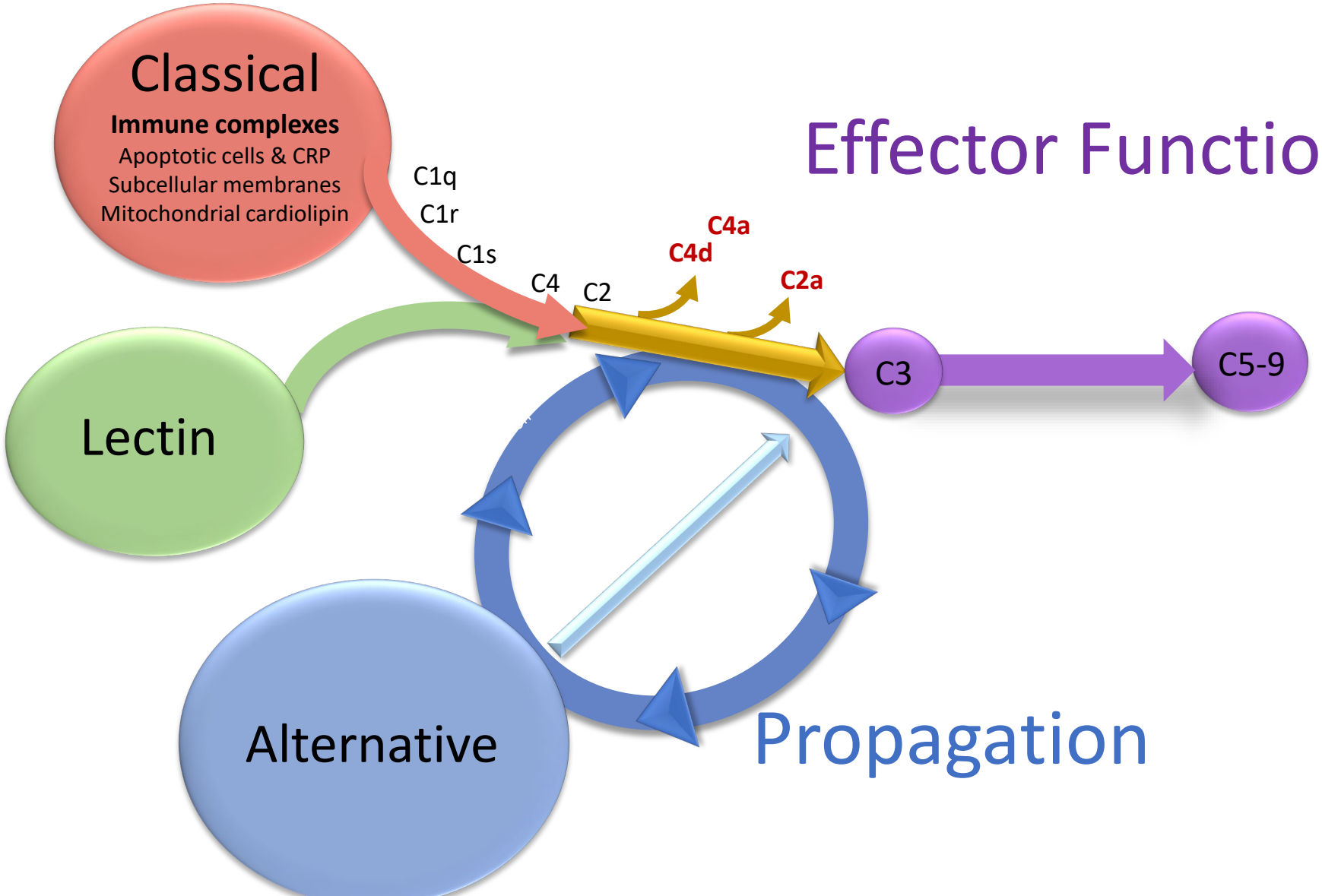



Activation
Classical
Lectin
Alternative
Effector Function
C3
C5-9
Amplification

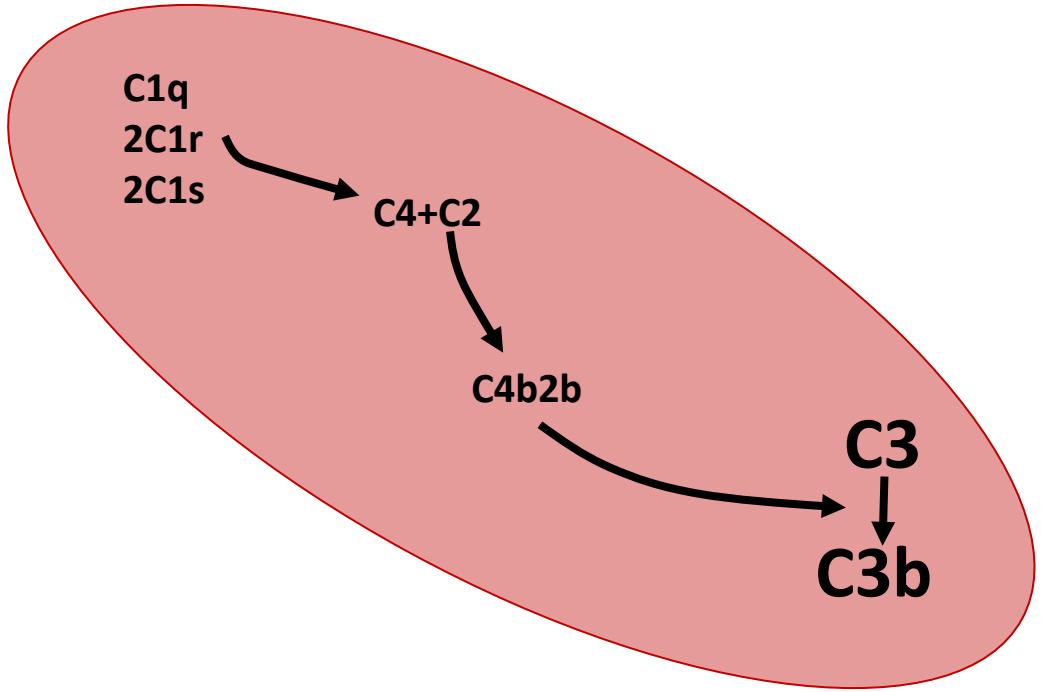
Classical Pathway Activation

- Immune Complexes (IgM, IgG)
- C-reactive proteins
- Apoptotic bodies
- Beta-amyloid fibrils
- Serum amyloid P
- Mitochondrial products
- C4 nephritic factor
- Lipid nanoparticles

Activation


Classical
Immune complexes
Apoptotic cells & CRP
Subcellular membranes
Mitochondrial cardiolipin
C1q
C1r
C1s
C4
C2
C4a
C4d
C2a
Effector Function
C3
C5-9
Lectin
Alternative
Propagation

Classical Pathway



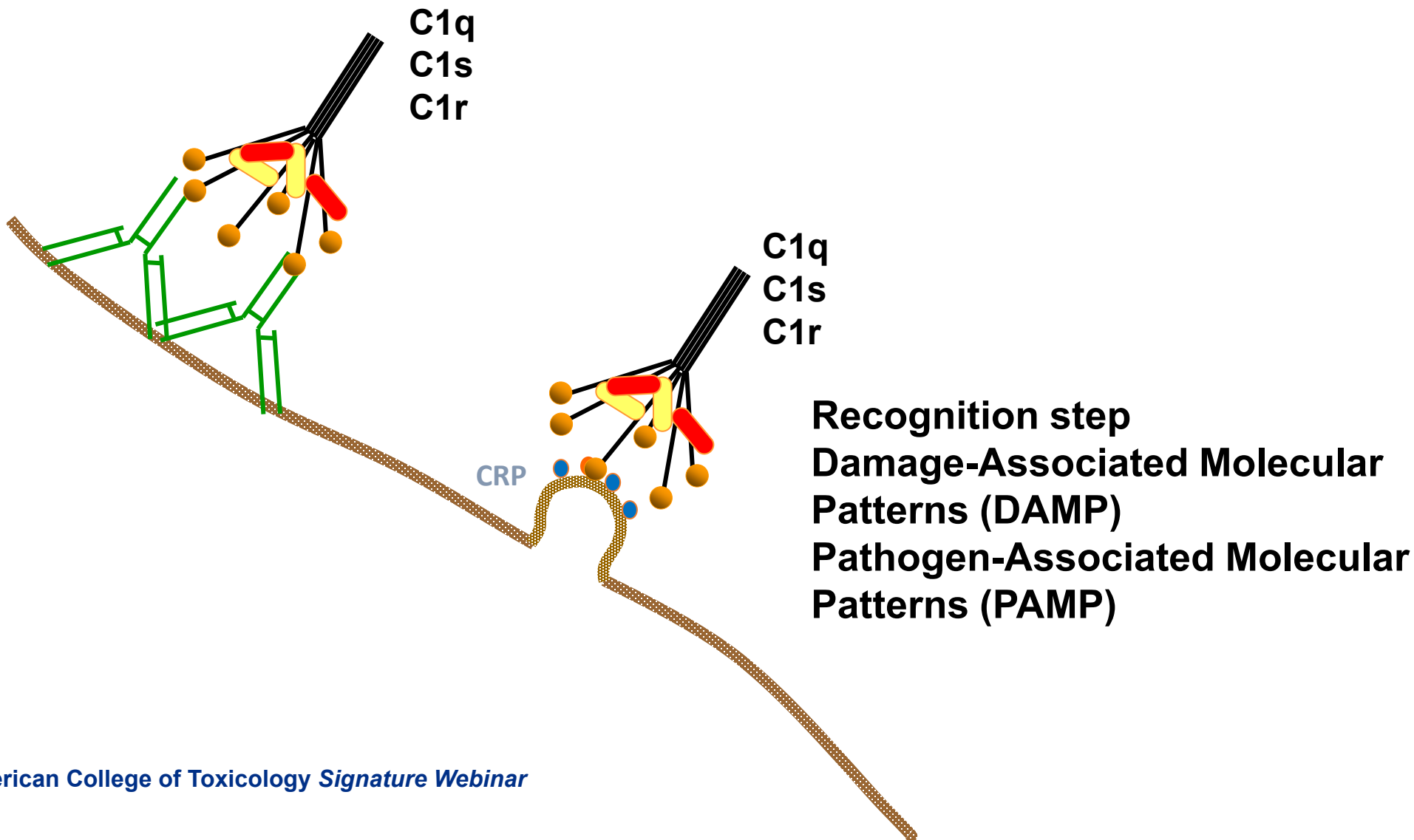
Ability of Imunoglobulin Isotypes to Activate Complement

IgM > IgG3 > IgG2 > IgG2 > > IgG4

IgA activates the alternative pathway

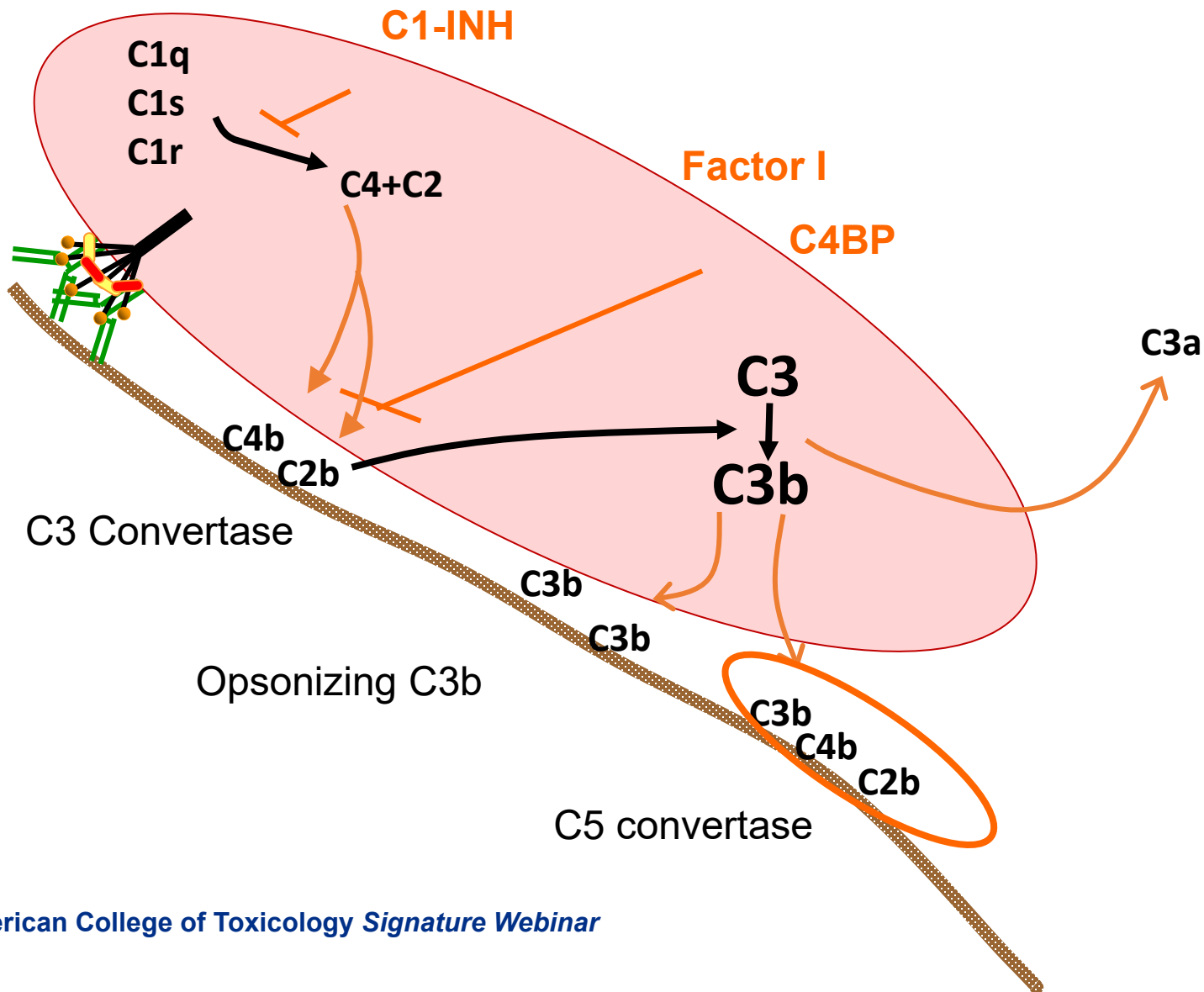
IgE not effective

Classical Pathway



Recognition step
Damage-Associated Molecular Patterns (DAMP)
Pathogen-Associated Molecular Patterns (PAMP)

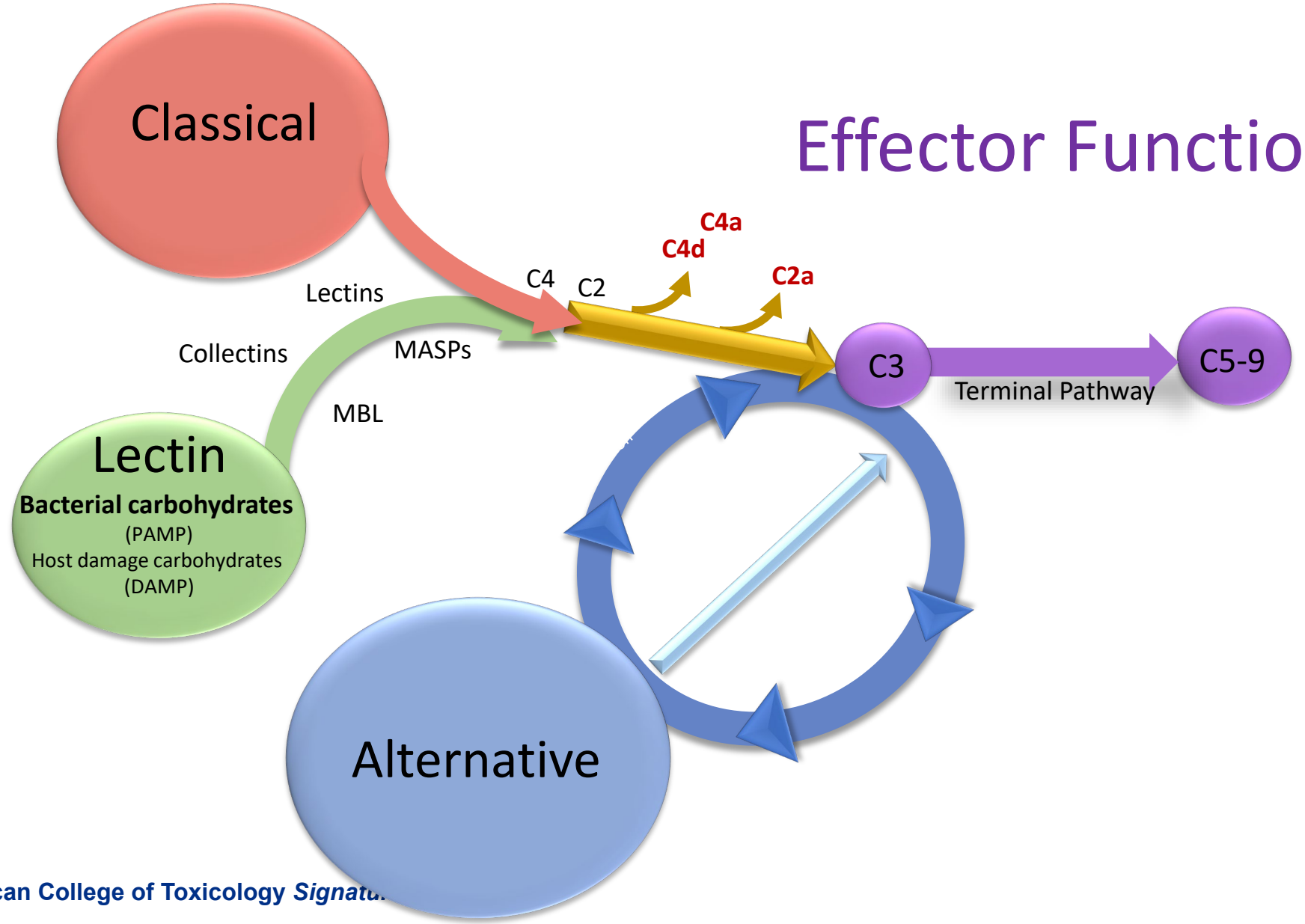
Classical Pathway



Lectin Pathway Activation

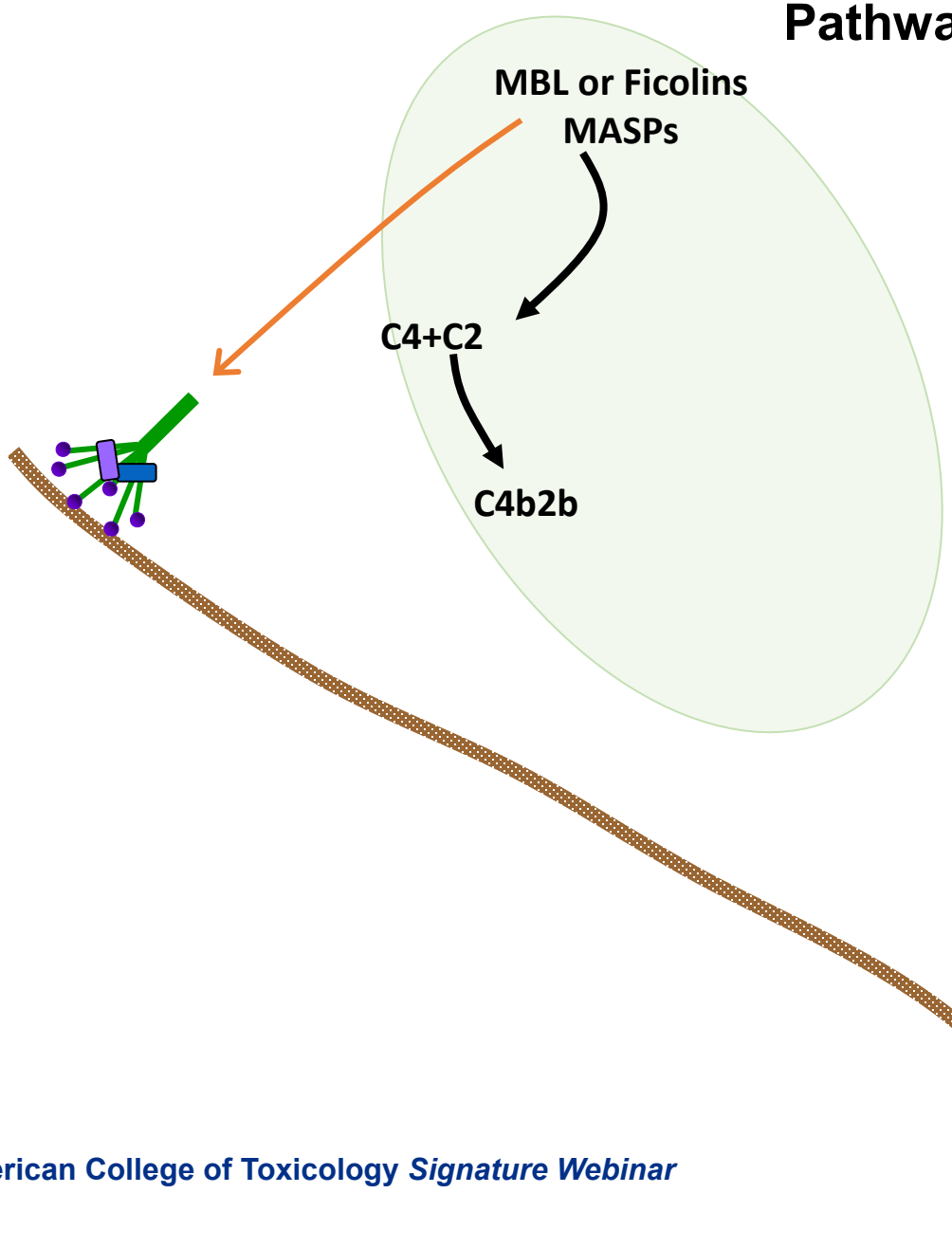
- Repeated simple sugars (e.g., mannose)
- G0 carbohydrate glycoforms
- Cytokeratin-1
- Lipid nanoparticles

Activation



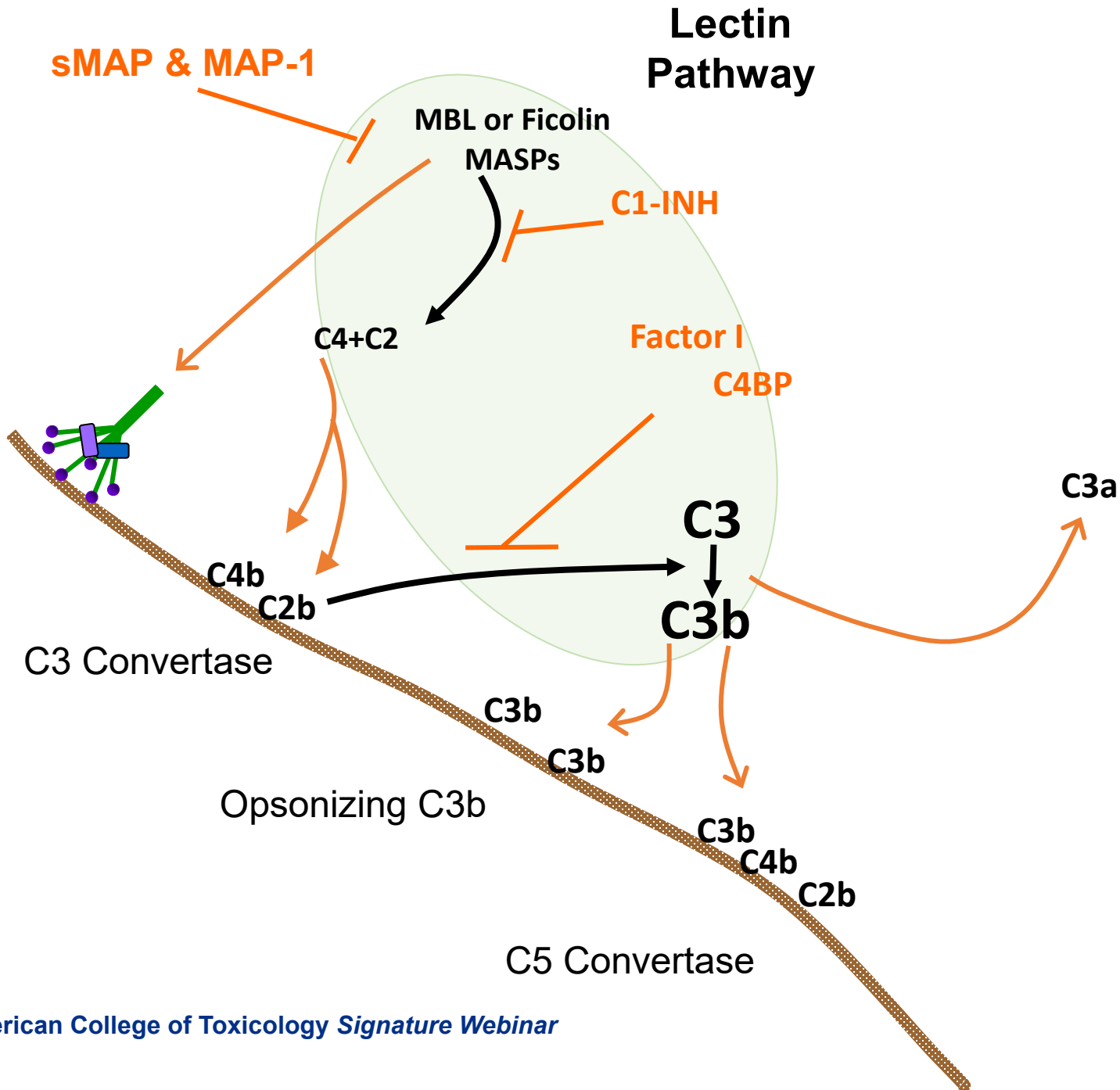
Effector Function

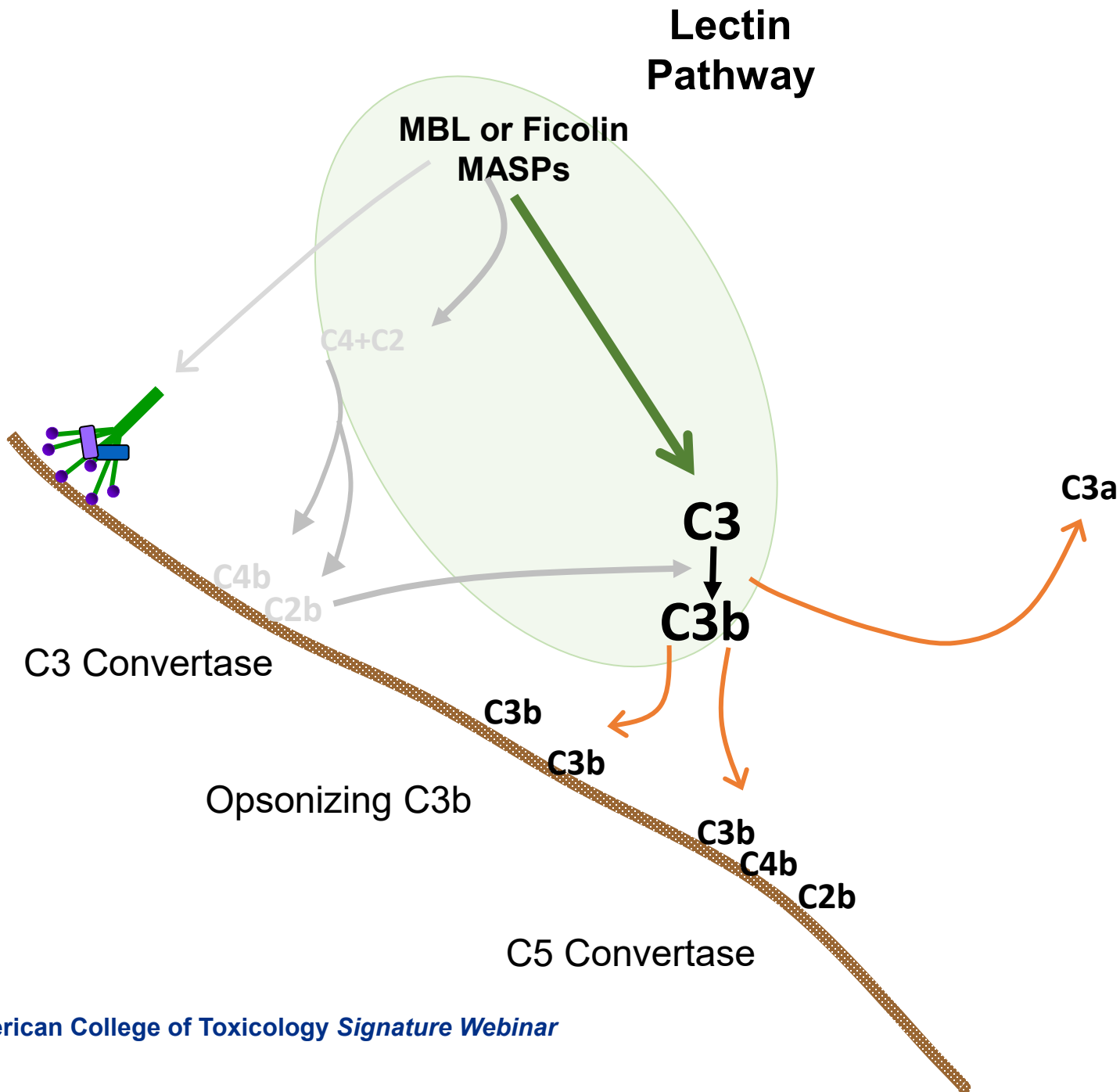
Lectin Pathway



MASPs =
MBL Associated
Serine Proteases

Act similar to C1r and C1s starting the cleave of C4 and C2

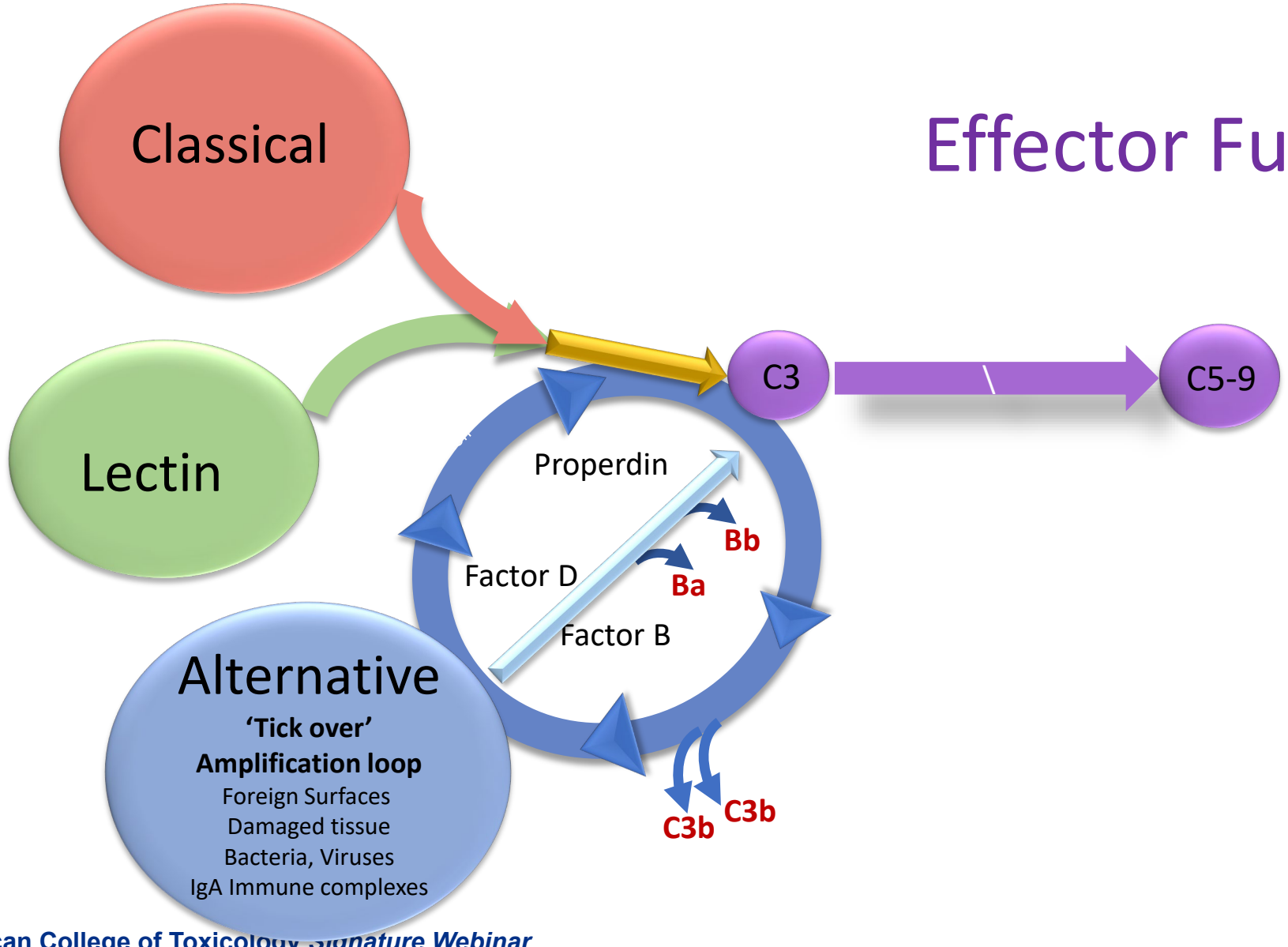

Lectin
Pathway
sMAP & MAP-1
MBL or Ficolin
MASPs
C1-INH
C4+C2
Factor I
C4BP
C3a
C3
C4b
C2b
C3b
C3 Convertase
C3b
C3b
Opsonizing C3b
C3b
C4b
C2b
C5 Convertase


Lectin
Pathway
MBL or Ficolin
MASPs
C4+C2
C4b
C2b
C3 Convertase
C3
C3b
C3a
C3b
C3b
Opsonizing C3b
C3b
C4b
C2b
C5 Convertase

Alternative Pathway Activation

- **“Tick over”**
- Amplification of activation for classical or lectin
- Endotoxin
- IgA immune complexes
- Polysaccharides
- C3 nephritic factor

Activation

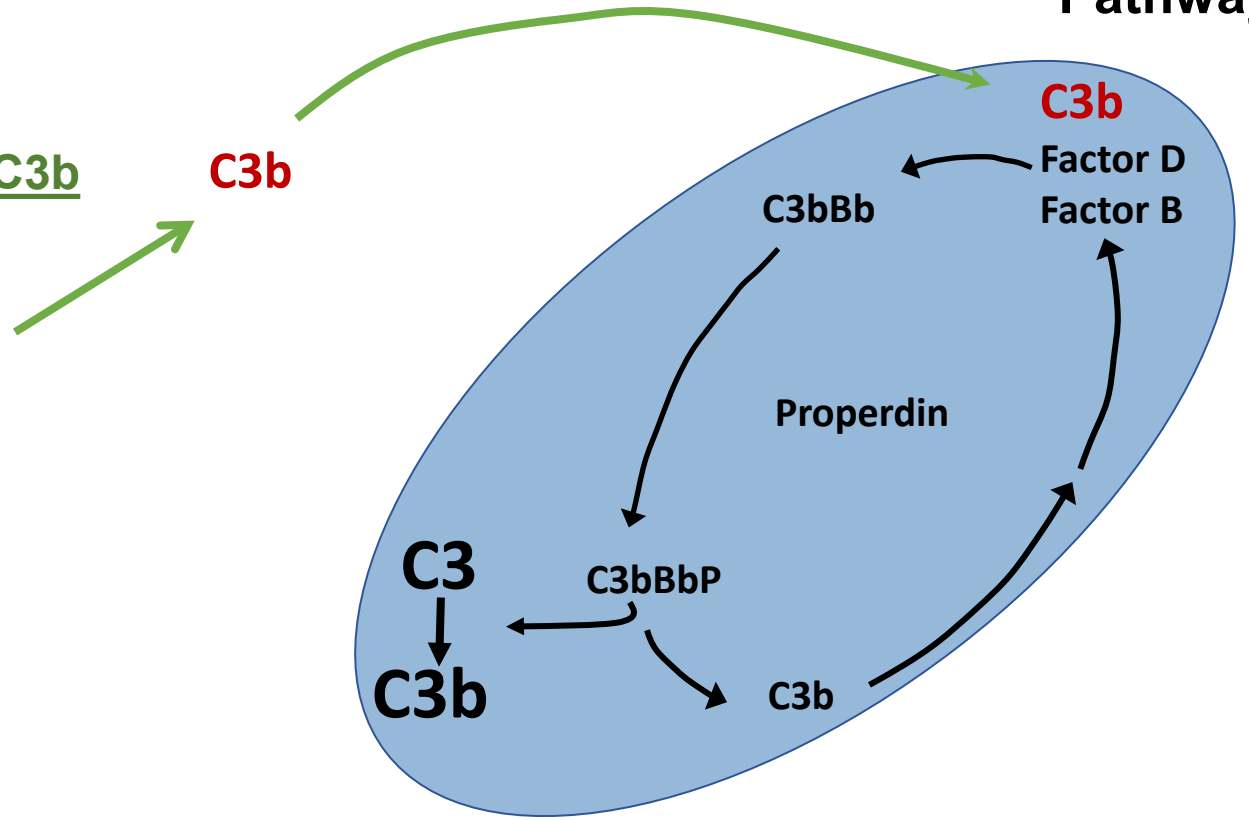


Effector Function

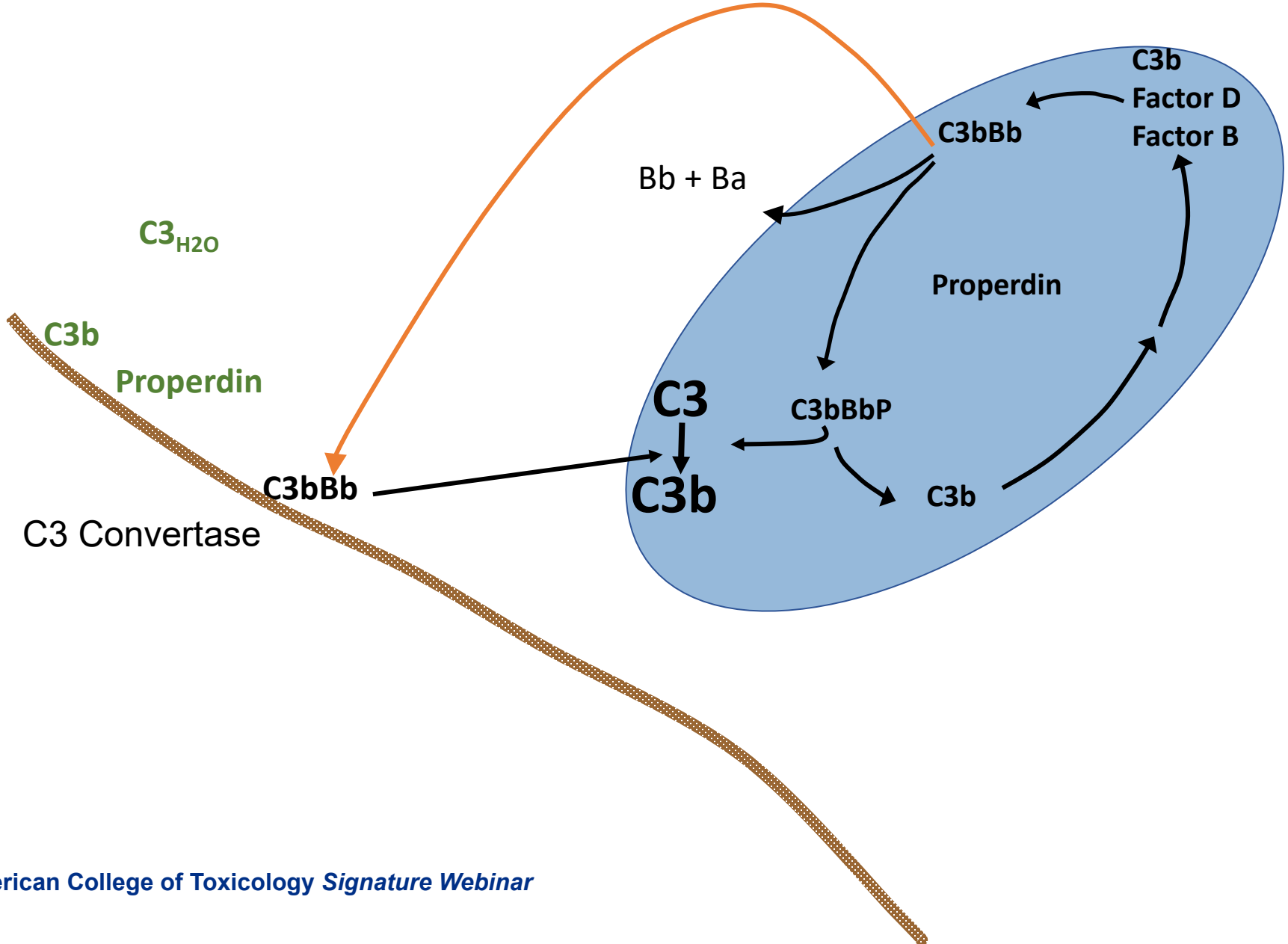
Alternative Pathway

Three Ways to get C3b

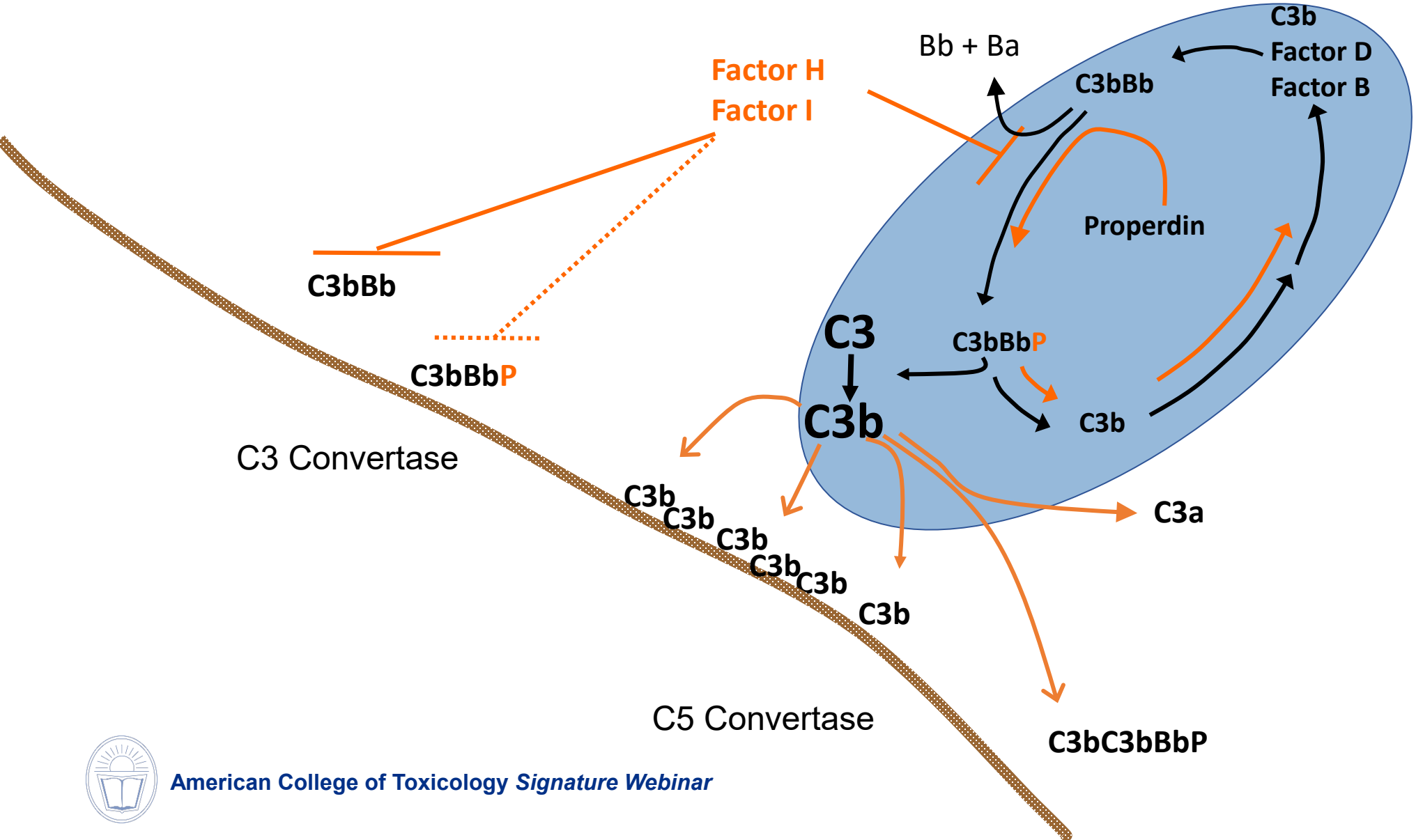
1. Tick over
2. Properdin
3. C3b on a surface



Alternative Pathway



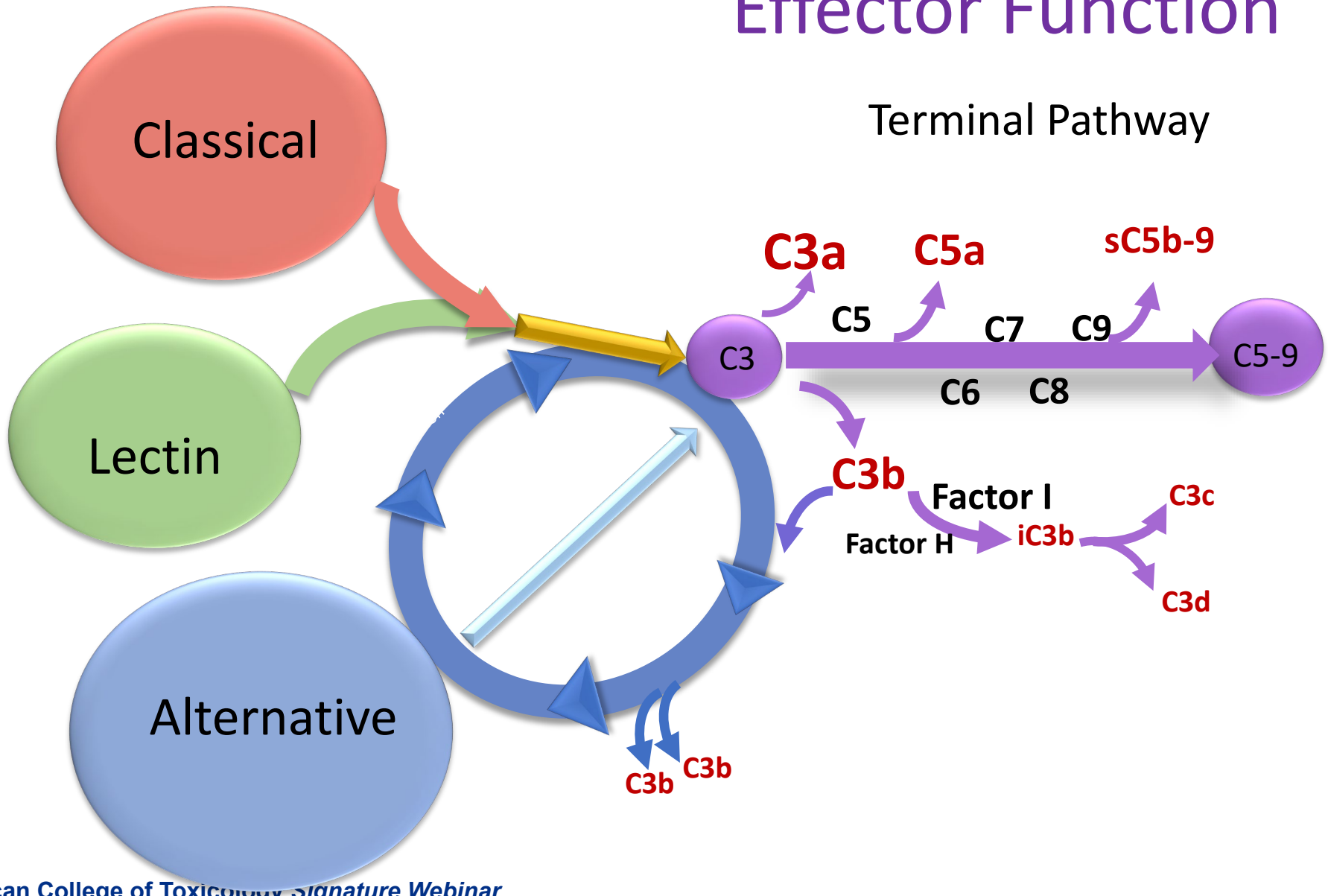
Alternative Pathway

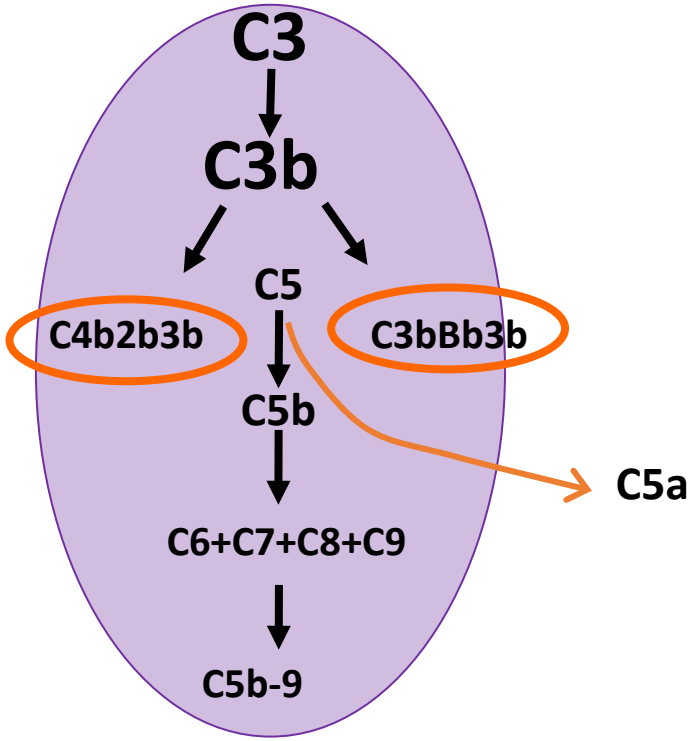


Terminal Pathway

- aka Shared Pathway or Effector Pathway
- Starts at C3
- Holds most of the proinflammatory functions

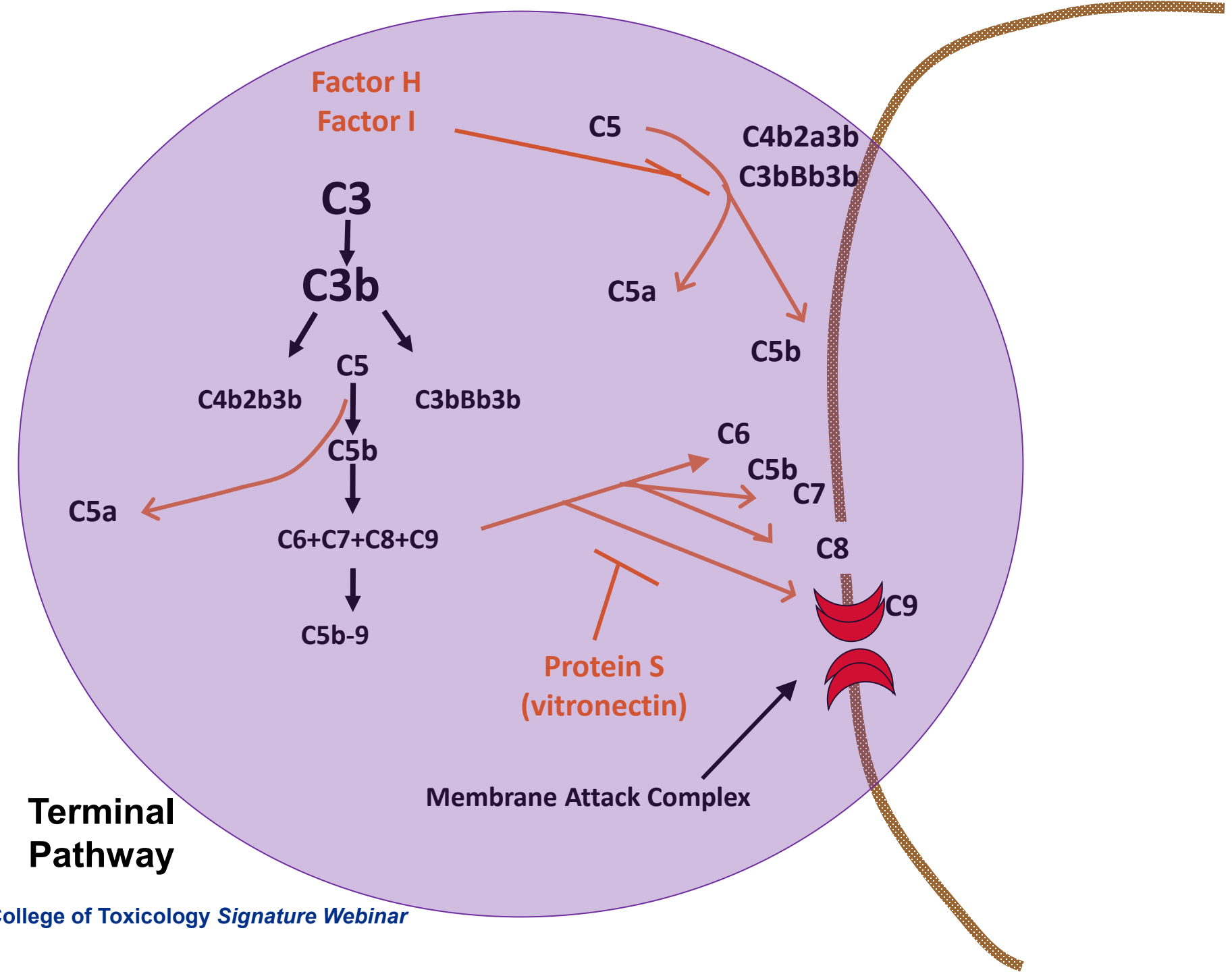
Effector Function





Terminal Pathway

Membrane Attack Complex



Terminal Pathway

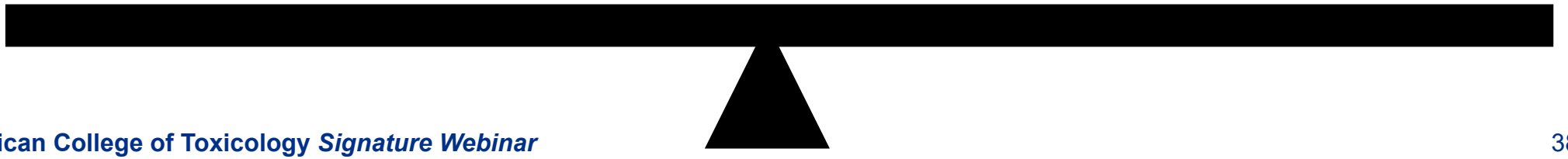
Complement Control

With Great Power Comes the Need for Great Control

Complement Cascade is a Balancing Act

<u>Too Much Control</u>:
Infection
Poor Housekeeping

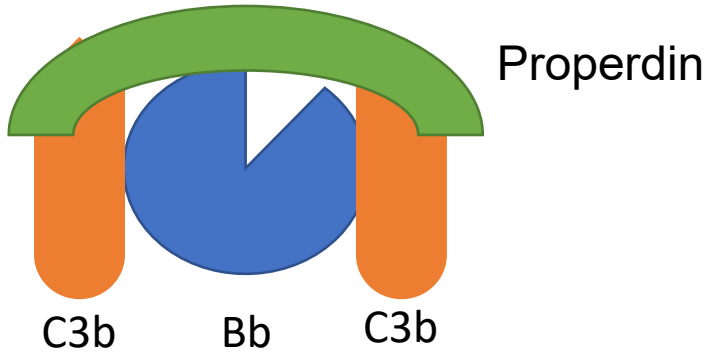
<u>Too Little Control</u>:
Inflammation
Attack Self

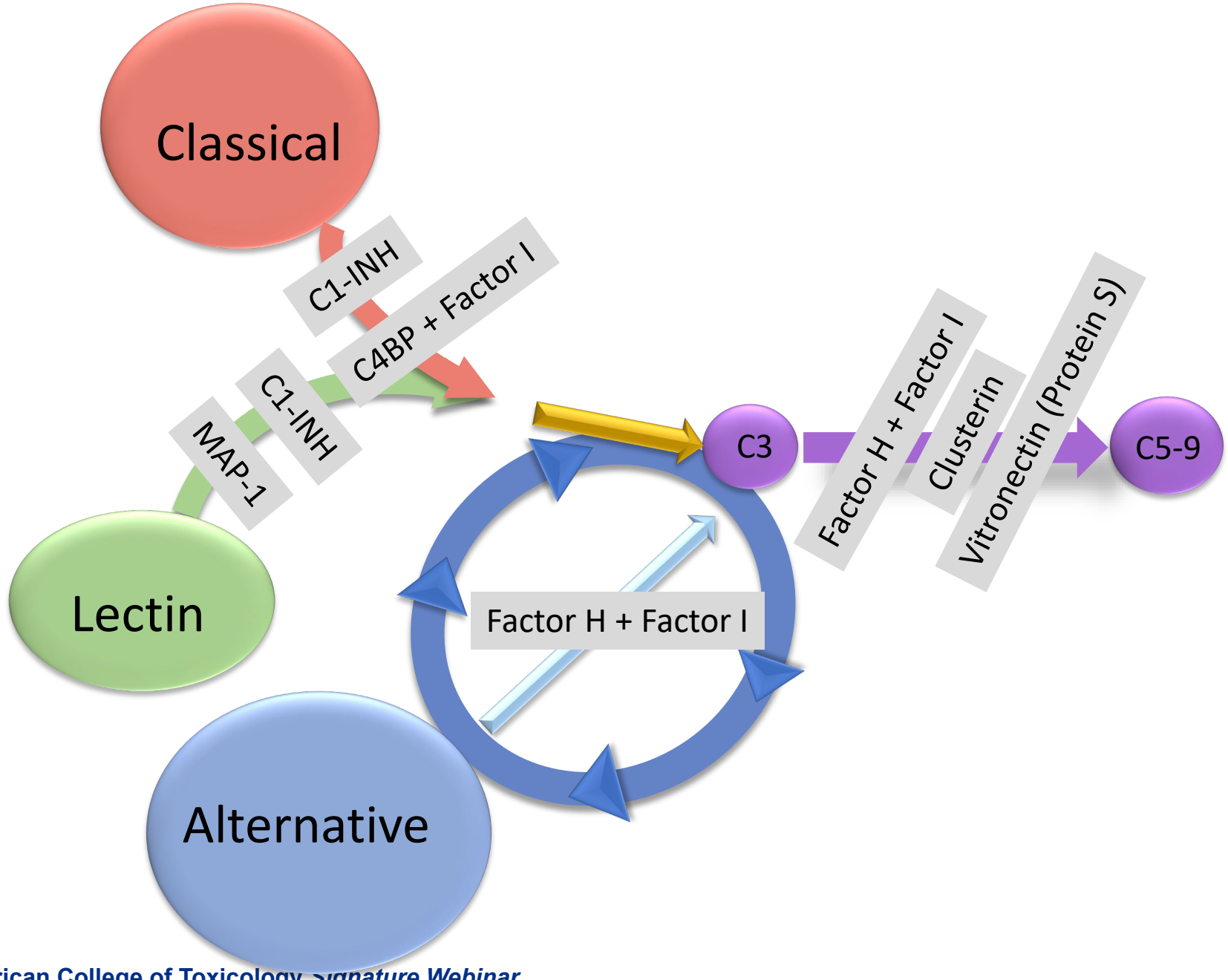


Control of Complement

- Convertase (enzymes) are <u>inherently unstable</u>
- C3 Convertase:
  - C3bBbP
  - C4bC2a
- C5 Convertase
  - C3bBbC3bP
  - C4bC2aC3b
- Properdin Stabilized –

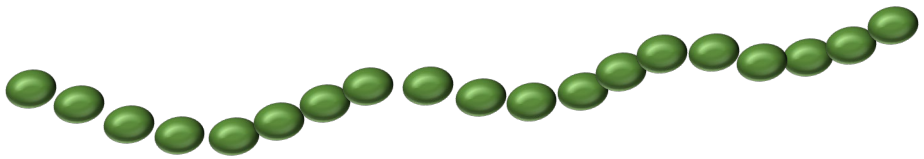
  alternative pathway C3 and C5 convertases




Classical
C1-INH
C4BP + Factor I
C1-INH
MAP-1
Lectin
Alternative
Factor H + Factor I
C3
Factor H + Factor I
Clusterin
Vitronectin (Protein S)
C5-9

Complement Control Proteins

- Repeating Domains in Complement Regulators
- Many of the regulators has multiple SCR, Short Complement Regulators
- These repeated structure “SCR”– beads on string
- Mutate one SCR get different diseases



e.g., Factor H

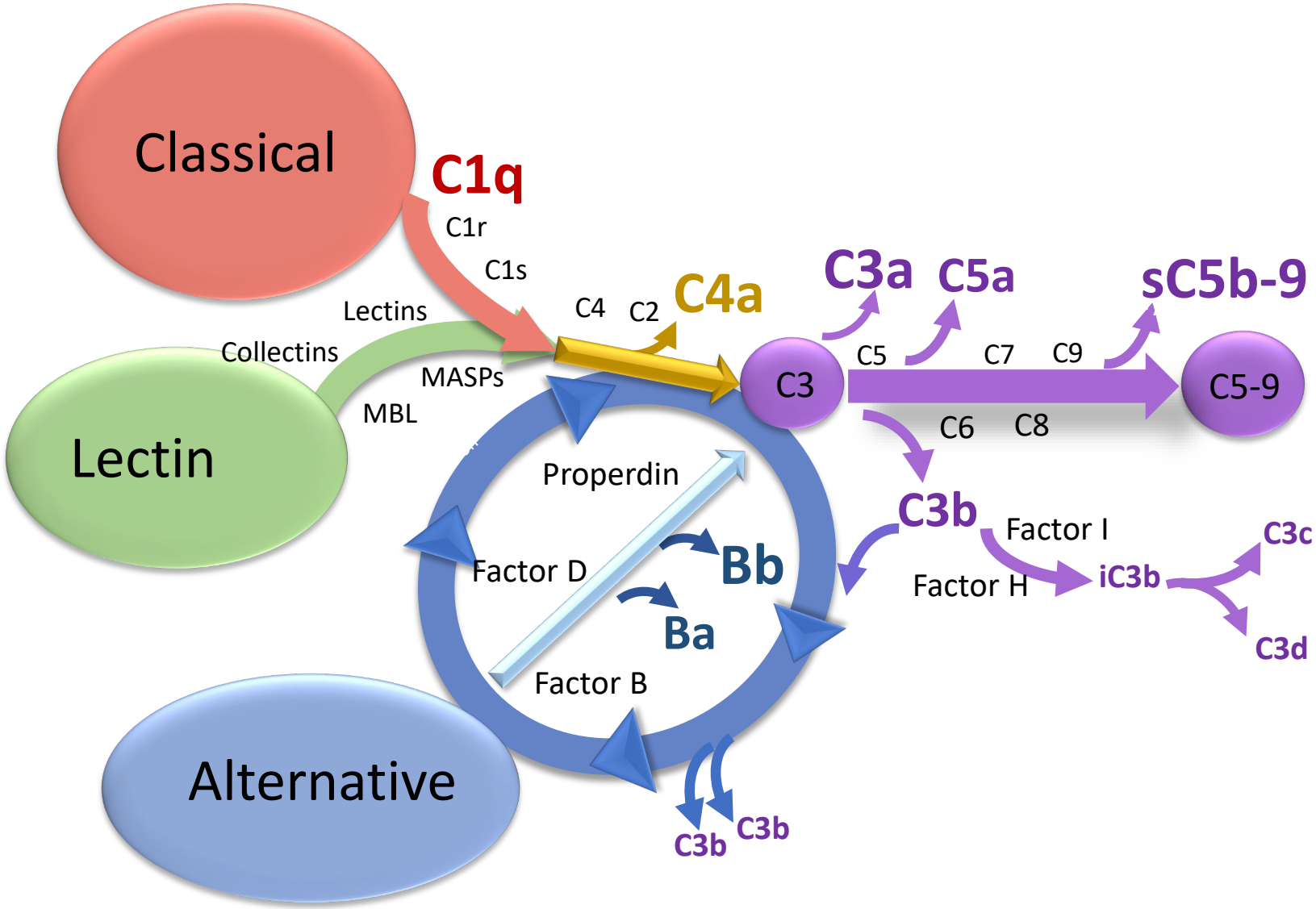
Factor H in Disease

- Key regulator of complement
- Cofactor for Factor I
- Control Alternative and Terminal
- So control amplification and effector functions
- Loss of Factor H Function linked to a number of diseases (aHUS, AMD, TMA)

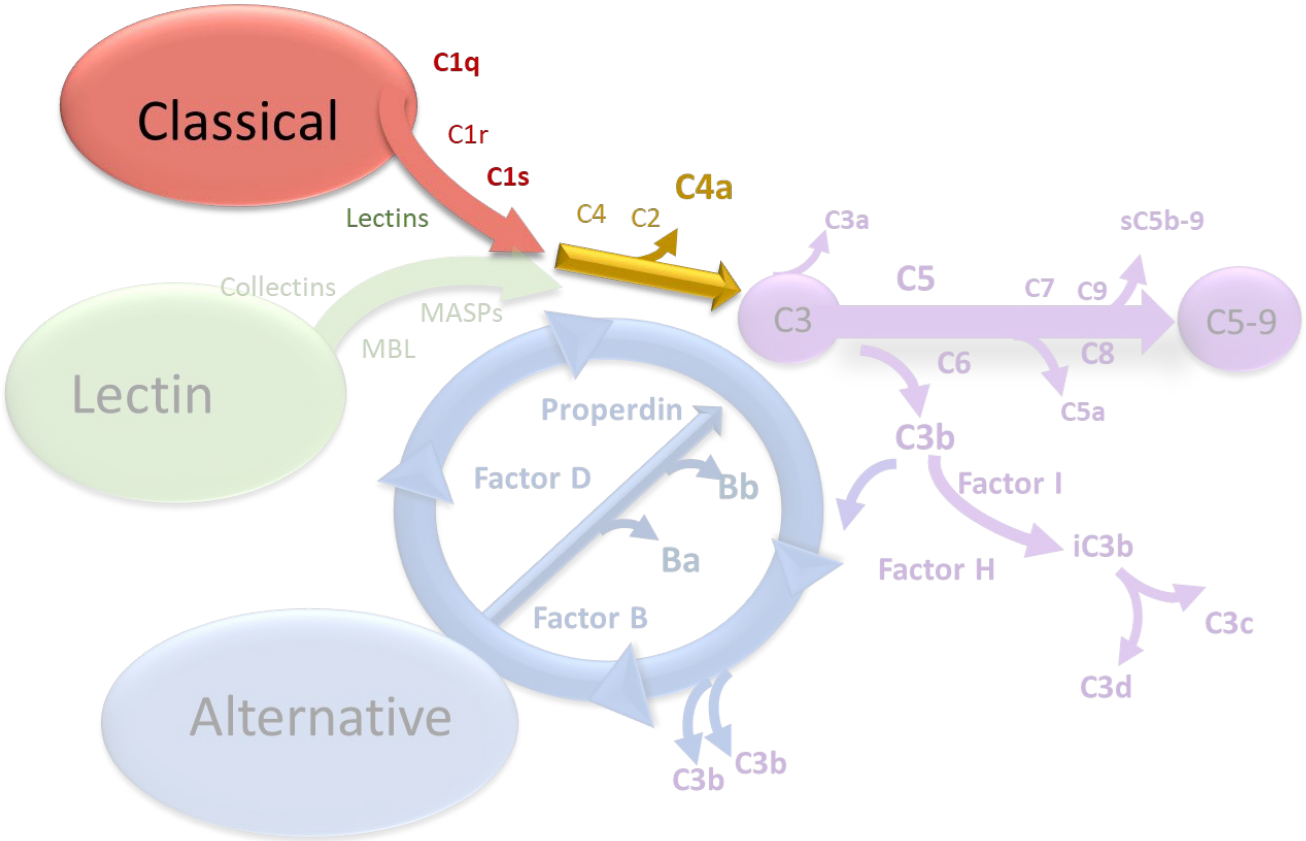
Measuring Complement

Biomarkers of Complement Activation

- Explore which tests for which pathway and which question
- Things to consider when picking a test
- What has been seen with AAV and complement

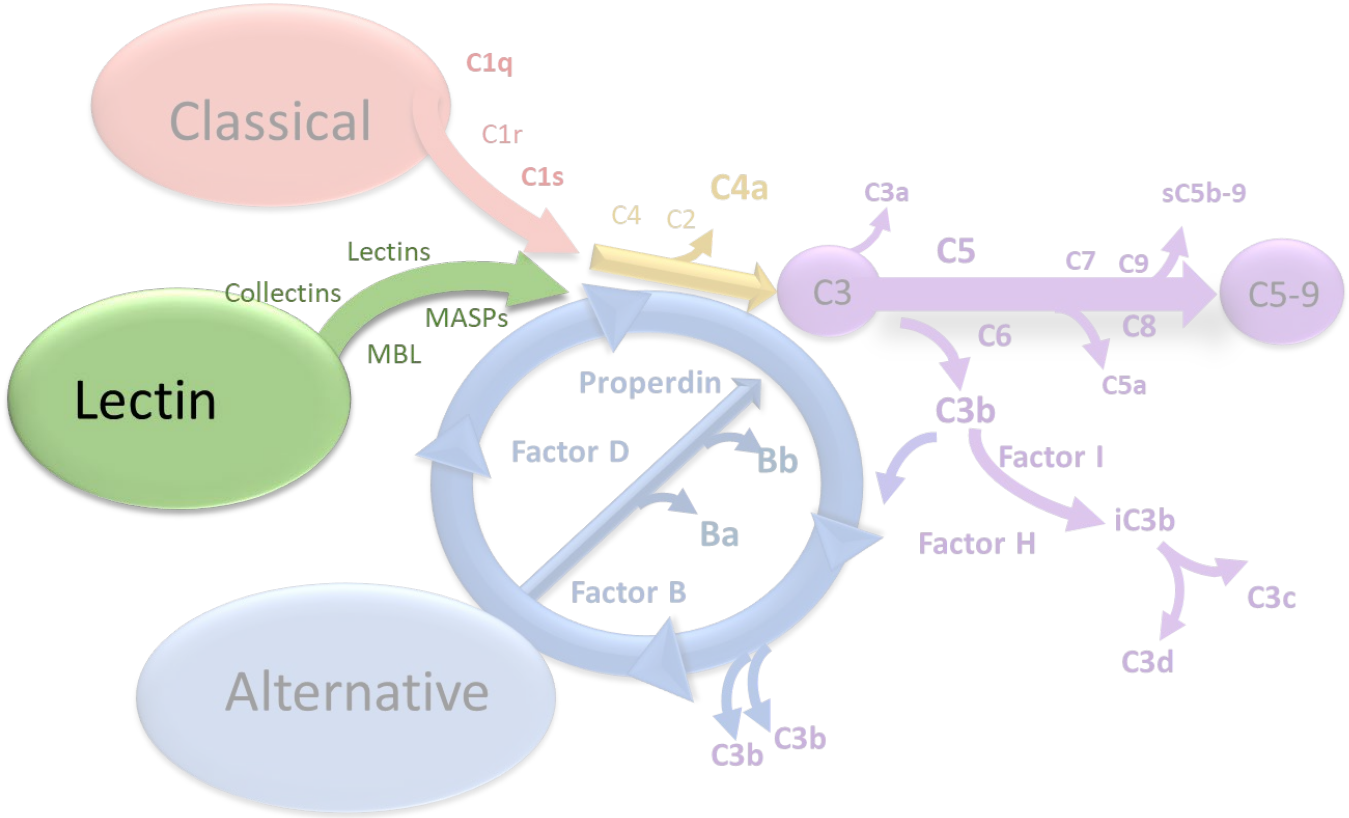

Classical
C1q
C1r
C1s
Lectins
Collectins
MASPs
MBL
Lectin
C4
C2
C4a
C3a
C5a
sC5b-9
C3
C5
C7
C9
C5-9
C6
C8
Properdin
C3b
Factor I
Factor D
Bb
Factor H
iC3b
C3c
C3d
Ba
Factor B
Alternative
C3b
C3b

Complement Classical Pathway Biomarkers:



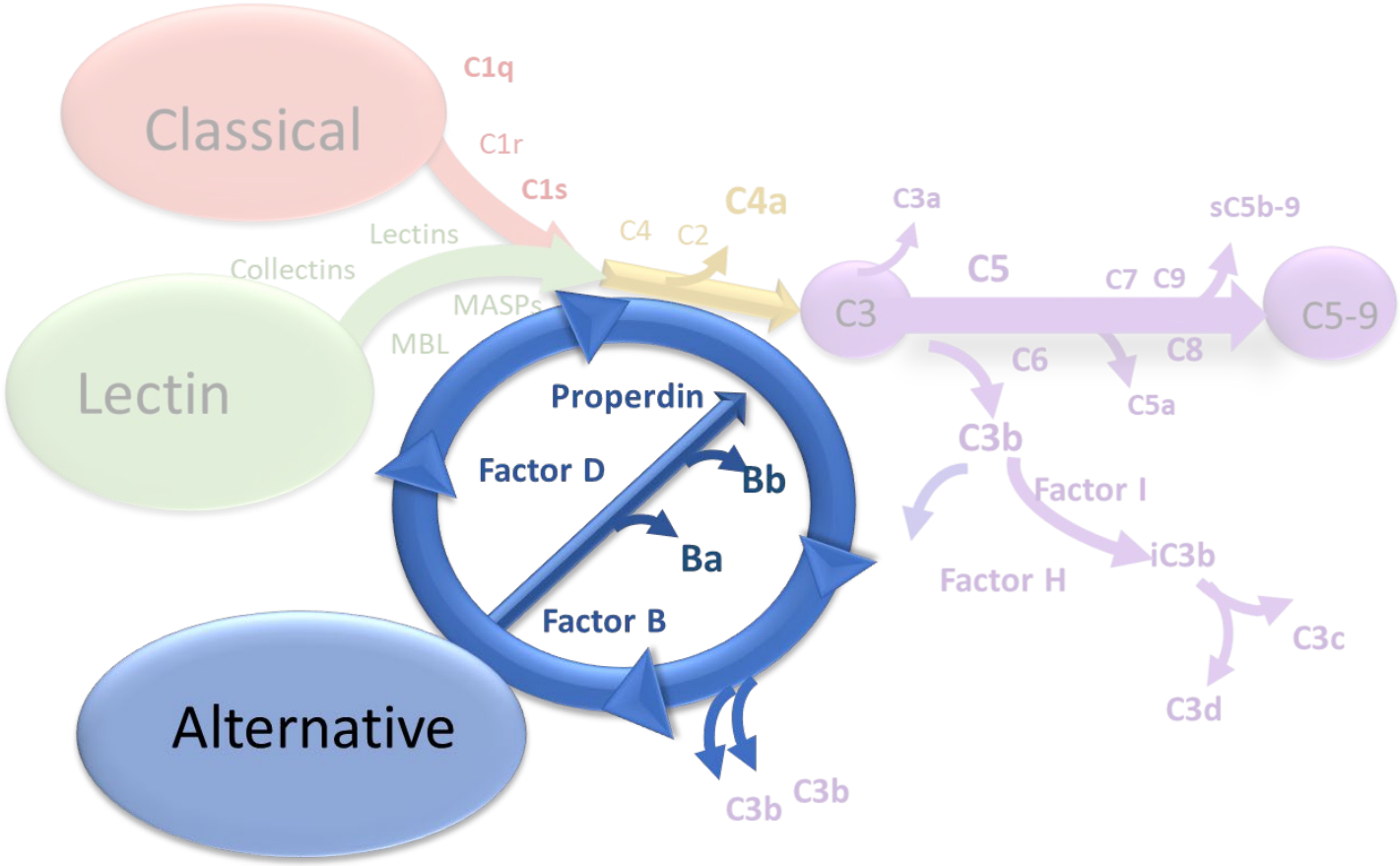
- CH50
  - Decrease with activation
- C4a
  - Increase with activation
  - Requires careful pre-analytic handling
- C1q
  - Decreases with immune complex levels

Complement Lectin Classical Pathway Biomarkers:



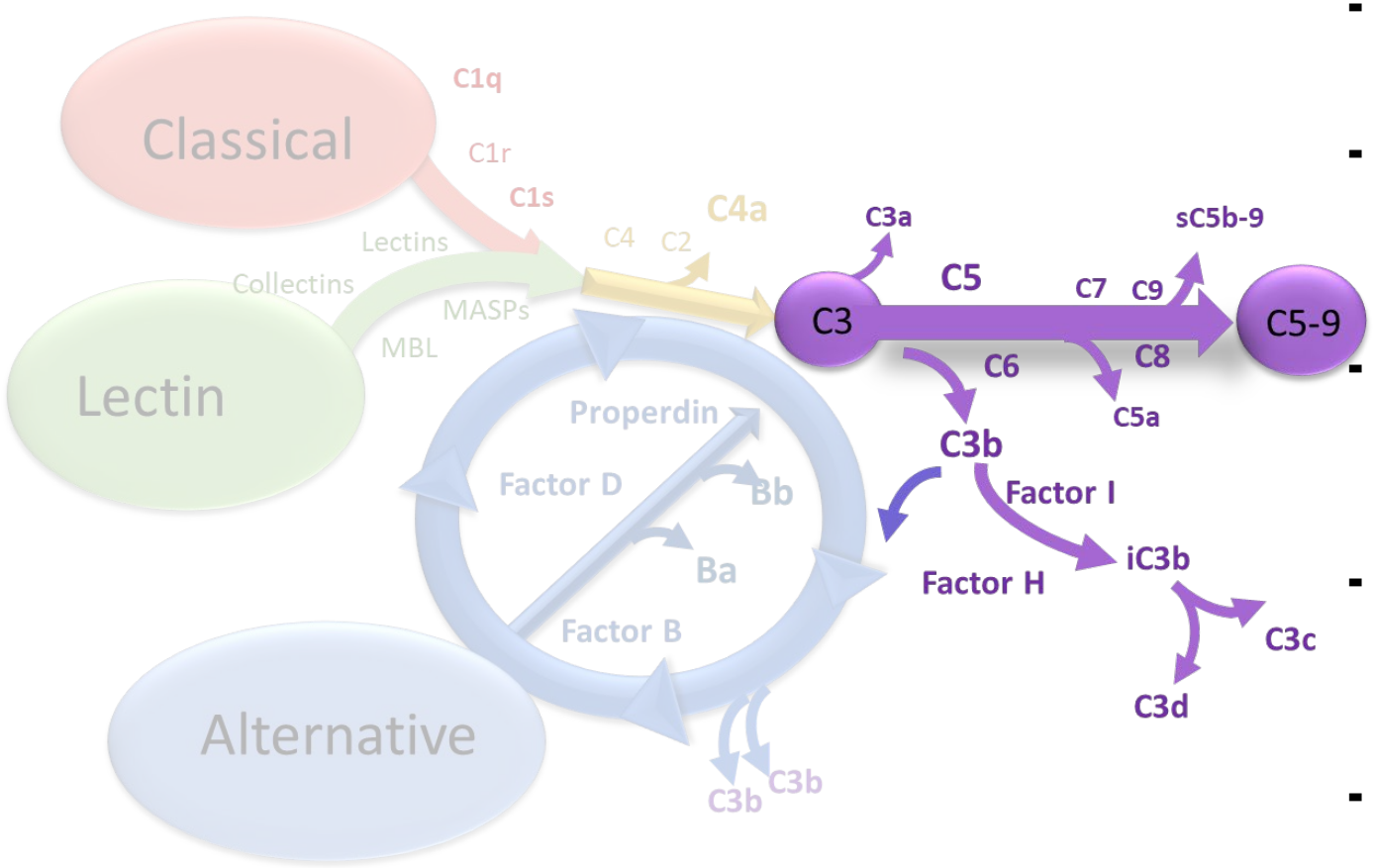
- Wieslab LP
  - Decrease with activation
- C4a
  - Increase with activation
  - Requires careful pre-analytic handling
- Hard to differentiate from Classical Pathway Activation

Complement Alternative Pathway Biomarkers:



- AH50
  - Decrease with activation
- Bb
  - Increase with activation
  - Accumulates so easier to catch the peak increase
  - Body of data with ASO
- Ba
  - Increase with activation
  - More dynamic so assays at greater sensitivity
- Factor B and Factor D
  - Not as dynamic or sensitive

Complement Terminal Pathway Biomarkers:



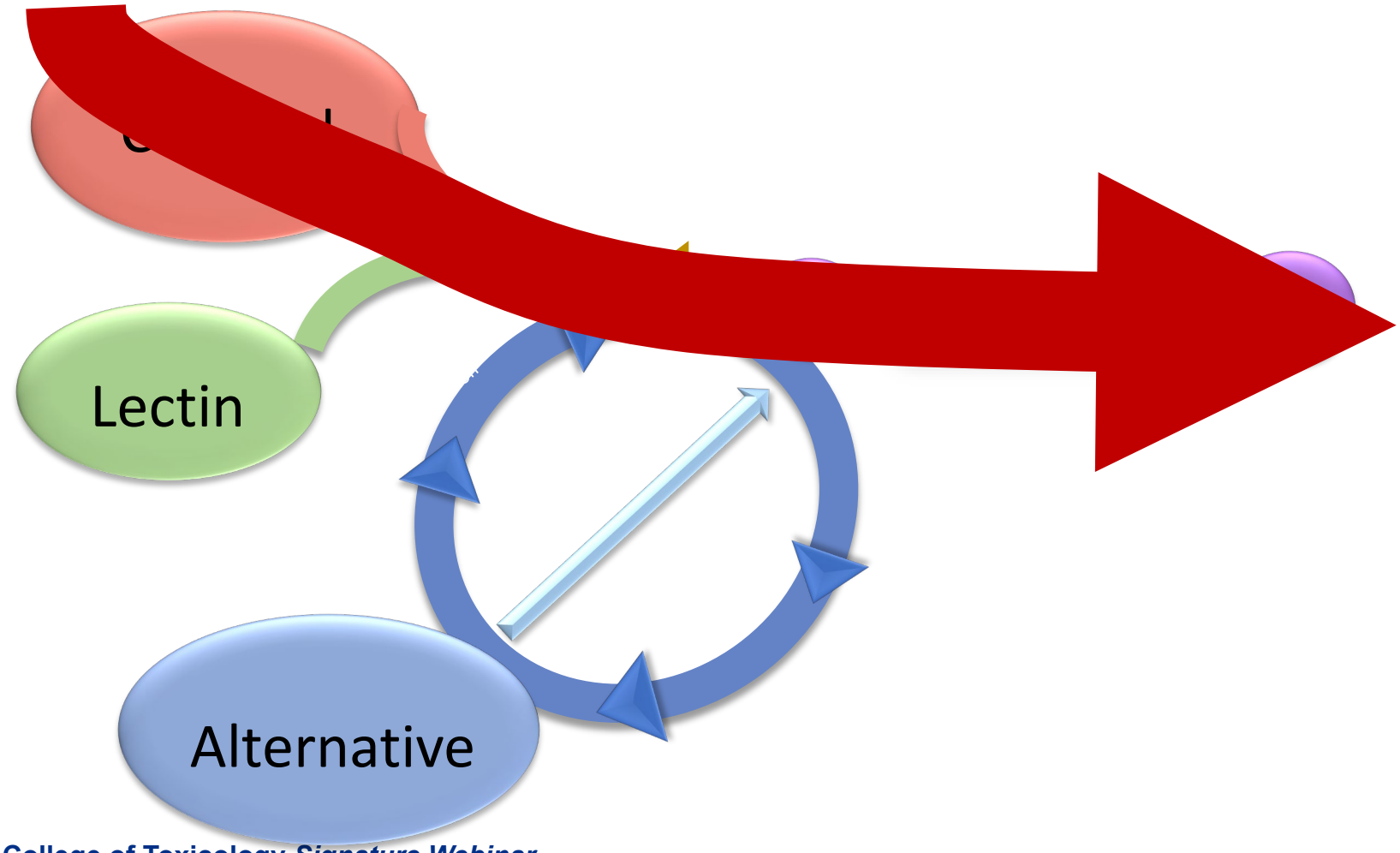
- CH50 and AH50
  - Both decrease with activation
- C3a
  - Increase with activation
  - Cleared in < 30 minutes
- iC3b and C3d
  - Increase with activation
  - Not cleared as quickly
- C5a
  - Increase with activation
  - Cleared in < 1 min
- sC5b-9
  - Accumulate so easier to measure
  - Gaining traction in diagnostics and following eculizumab

What About Complement C3 and C4?

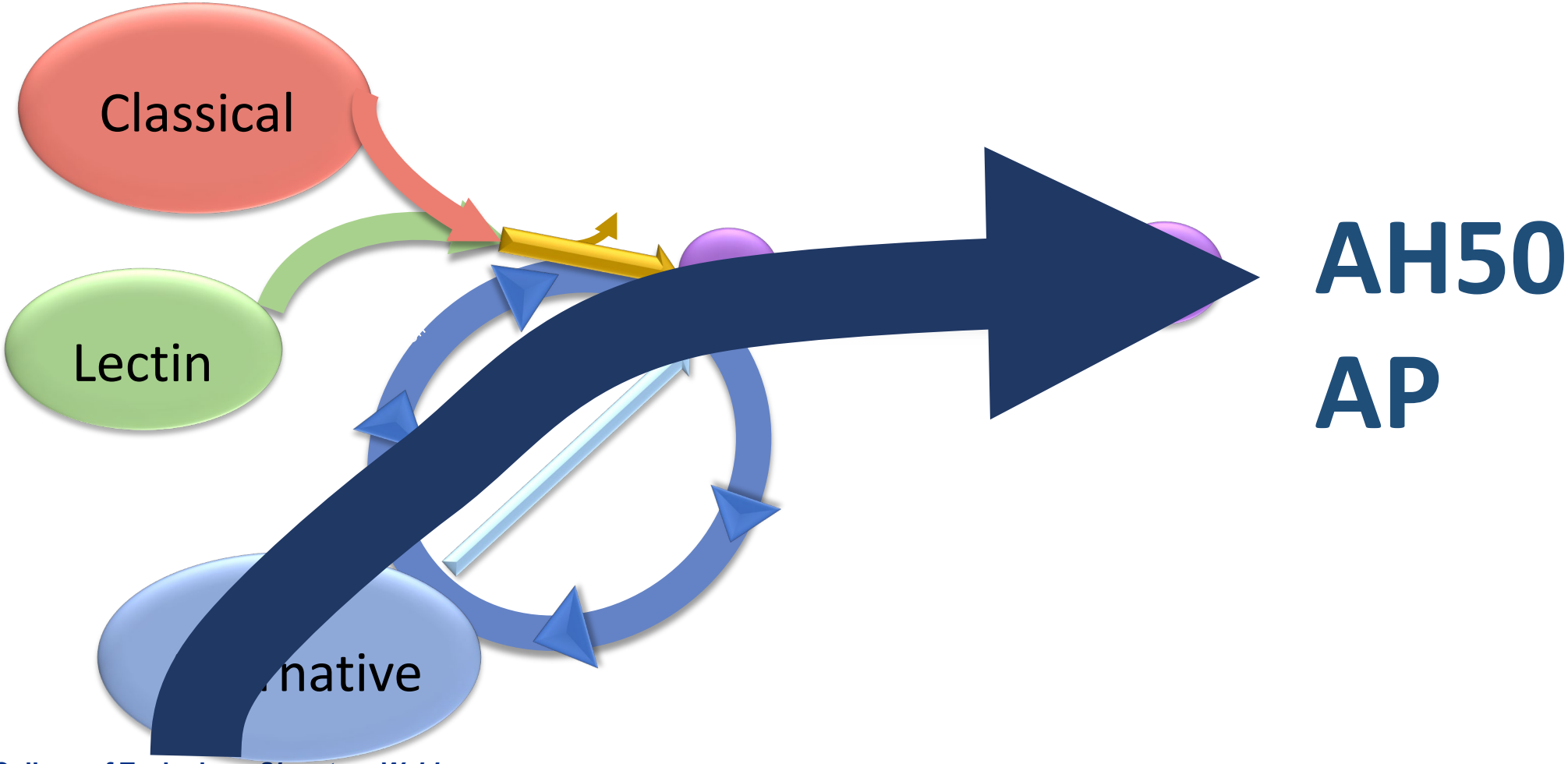
- Designed by not be temperature sensitive
- Extensive history in clinical testing
- If they are low that means something
- Are acute phase reactant
- That will increase the production
- Measuring balance activation and consumption

- Accessible first screen but maybe not as sensitive as needed

Measuring Complement Function



Measuring Complement Function



Measuring Complement Function

Hemolytic

- Very physiologic
- MAC lysis of RBCs
- Very sensitive
- Measures fluid phase and surface inhibition
- Requires RBCs
- All LDTs

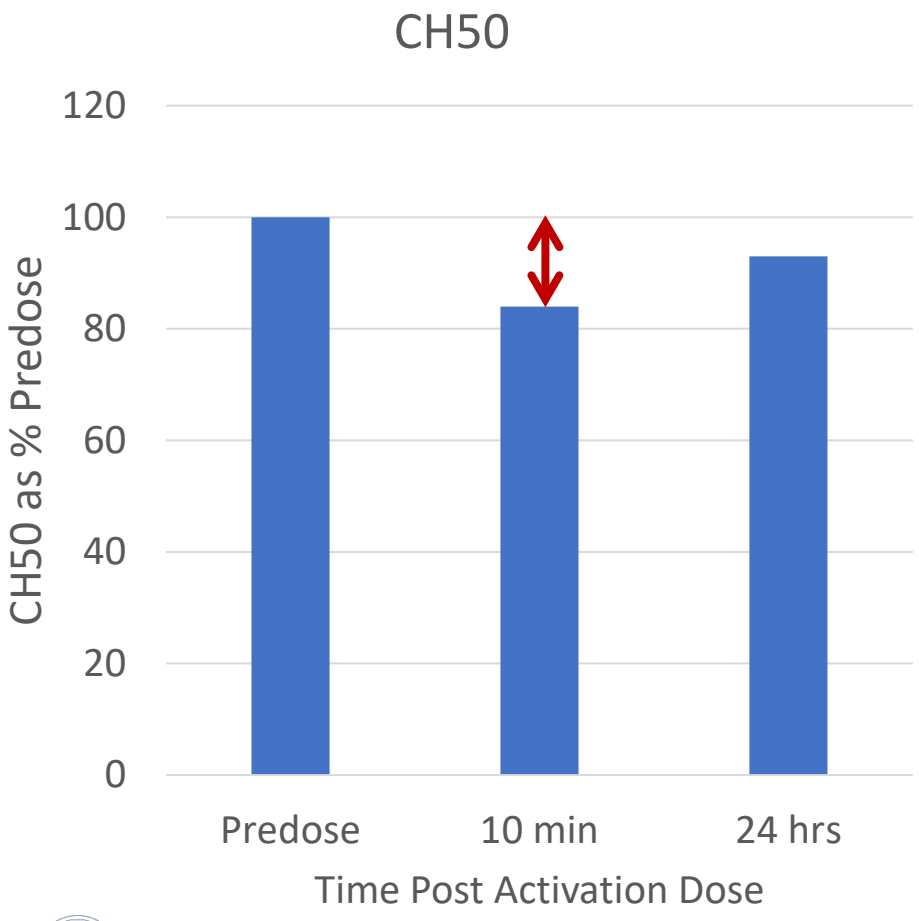
ELISA Style

- Essentially an ELISA
- Are 510K and CE marked
- More supply chain control
- AP method 2 points for calculation

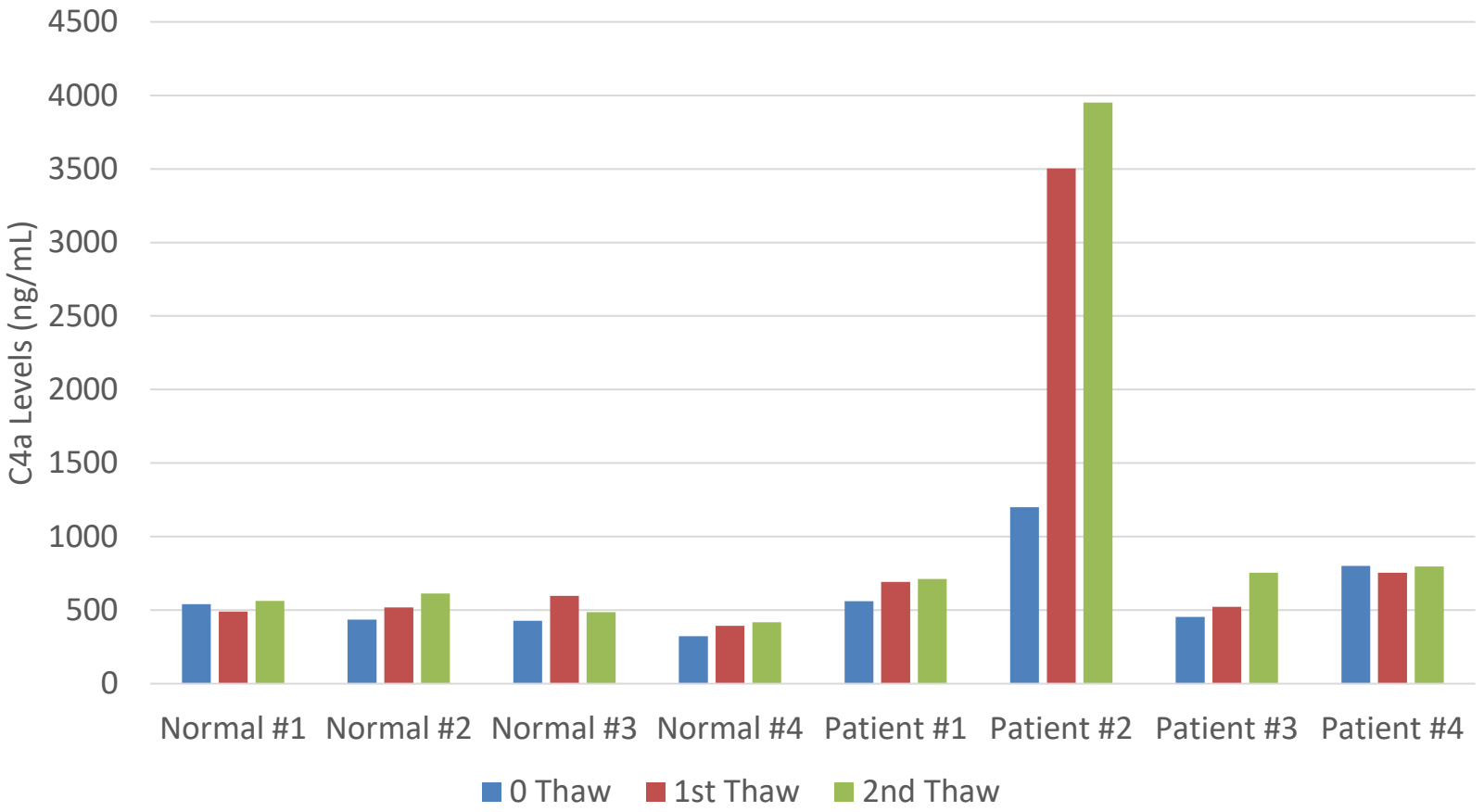
Pre-Analytic Considerations

- Mishandling will lead to ‘abnormal’ result not ‘normal’ result
- Storage at -80C is required
- Shipping on dry ice is required
- Once at -80C stable over one year

Relative Sensitivity: Functions vs. Fragments



Freeze/Thaw Stability



Problems is, we don't know which is Patient #2

Representation of data from patients with suspected chronic infections

Review of Basic Components

	Classical Pathway	Lectin Pathway	Alternative Pathway	Terminal Pathway
Initiation	C1q	MBL Ficolin 1,2,3 Collectins	$C3_{H2O}$ Properdin	
	C1r, C1s	MAPS1, MASP1, MASP3	Factor D	C3
	C4	C4	Factor B	C5
	C2	C2		C6,C7,C8,C9
Convertases	C4bC2a, C4bC2aC3b	C4bC2a, C4bC2aC3b	C3bBb, C3bBbC3b	
Control	C1-INH	C1-INH	Factor H + Factor I	Factor H + actor I
	C4BP + Factor I	MAP-1		FHR's
Produced on Activation	C4a, C4b	C4a, C4b	Bb, Ba	C3a, C3b, iC3b, C3dg
	C2b, C2a	C2b, C2a		C5a, C5b
				sC5b-9, C5b-9

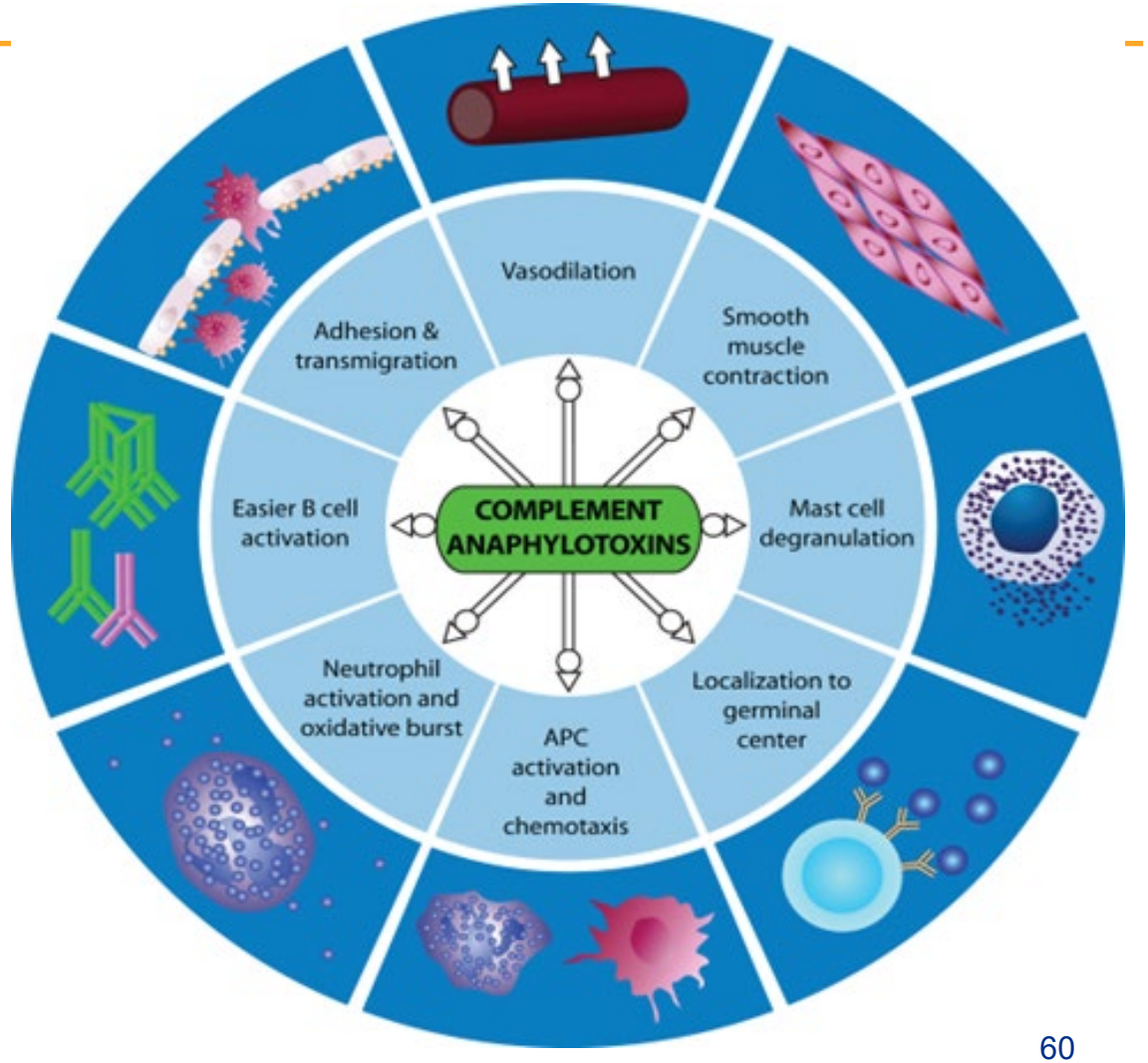
What we know about complement mediated adverse events

CARPA (Complement Activation Related Pseudo Allergy)

- Originally described in response to:
  - Radiocontrast agents
  - Liposomes
  - Micellar carries of intravenous drug
- Occurs at first exposure
- Rapid hypersensitivity reaction

Complement Anaphylatoxin (C3a & C5a) Effects

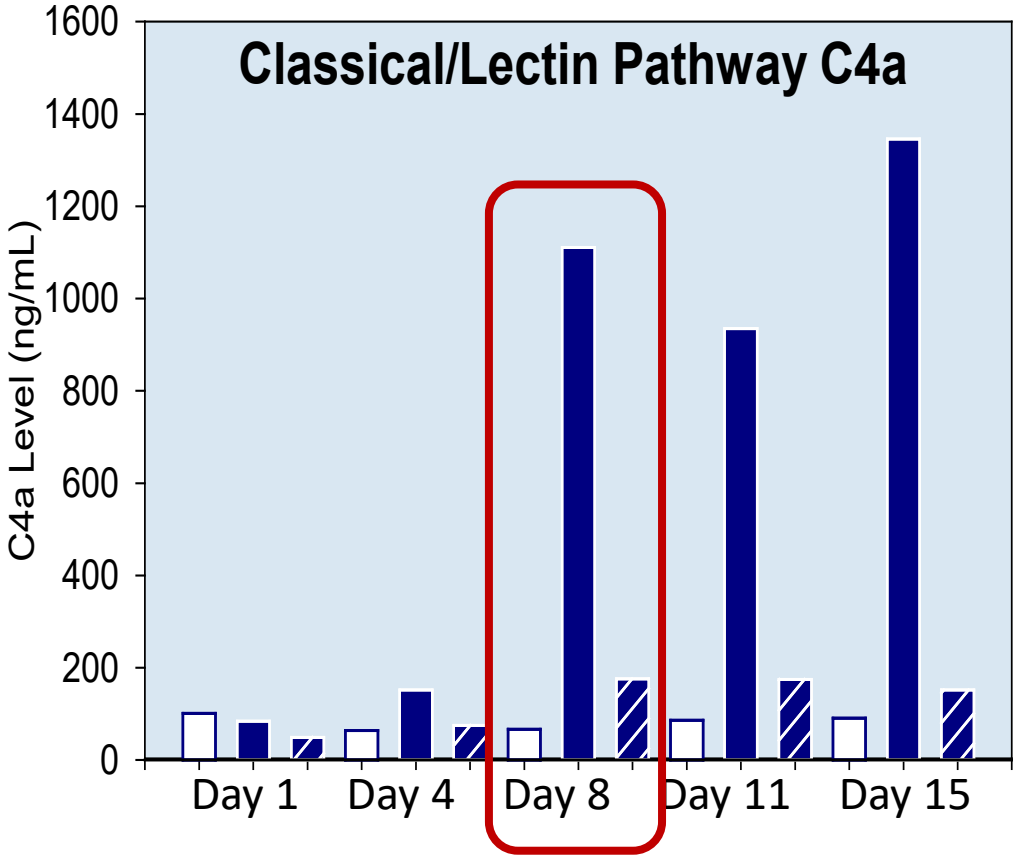
- Histamine release
- Initiate vasodilatation
- Induce smooth muscle contraction
- Direct inflammatory cell migration
- Oxidative burst
- TLR activation & cytokine production

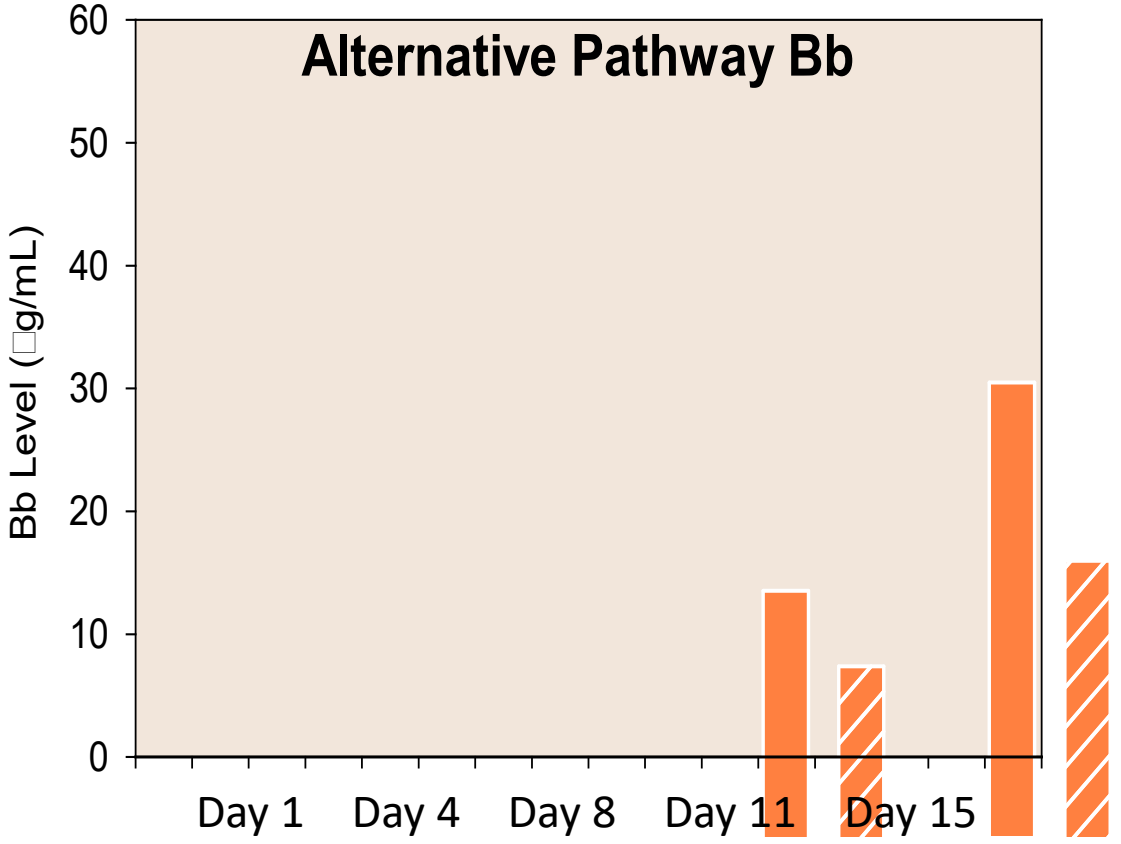


Complement Activation Secondary to Antidrug Antibodies (ADA)

- May start to see complement activation at Day 7
- IgM is a very good activator of complement
- IgM is produced as early as Day 7
- You can also see class-switch reflected in complement
- Note IgM is the mostly likely antibody for endogenous cross-reactivity

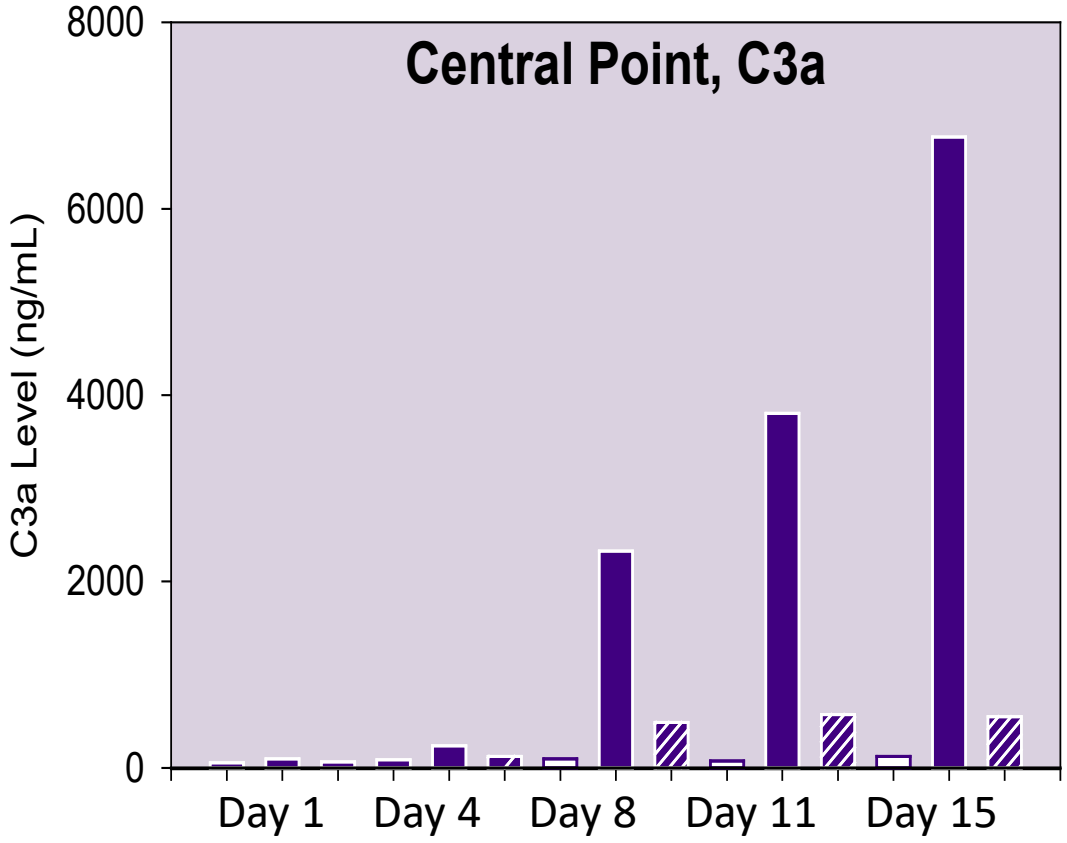
Complement Activation Secondary to Antidrug Antibodies (ADA)

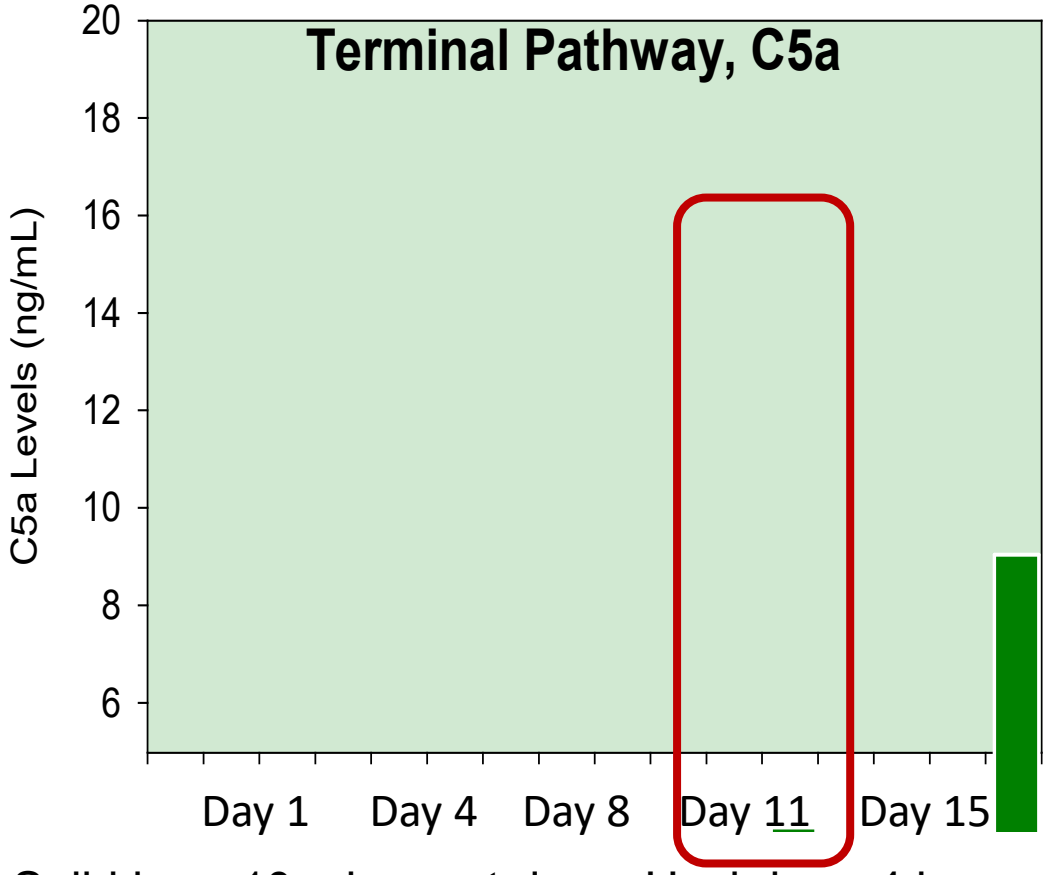




Open bars pre dose; Solid bars 10 min. post dose; Hash bars 4 hr. post dose

Complement Activation Secondary to Antidrug Antibodies (ADA)

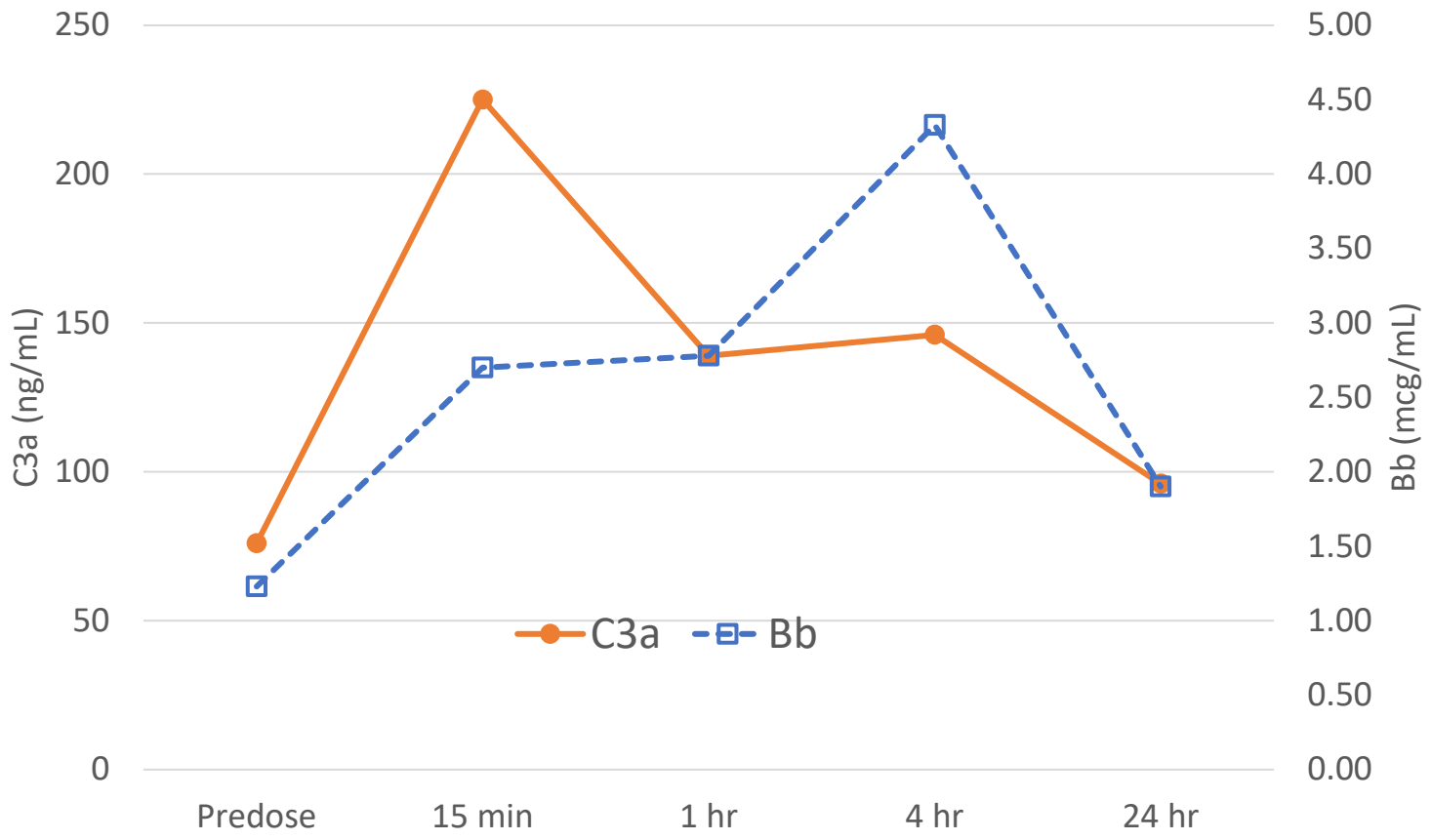




Open bars pre dose; Solid bars 10 min. post dose; Hash bars 4 hr. post dose

Kinetics and Draw Time is Important

- Matters when you sample
- Complement fragments are cleared
- Chronic activation can deplete complement



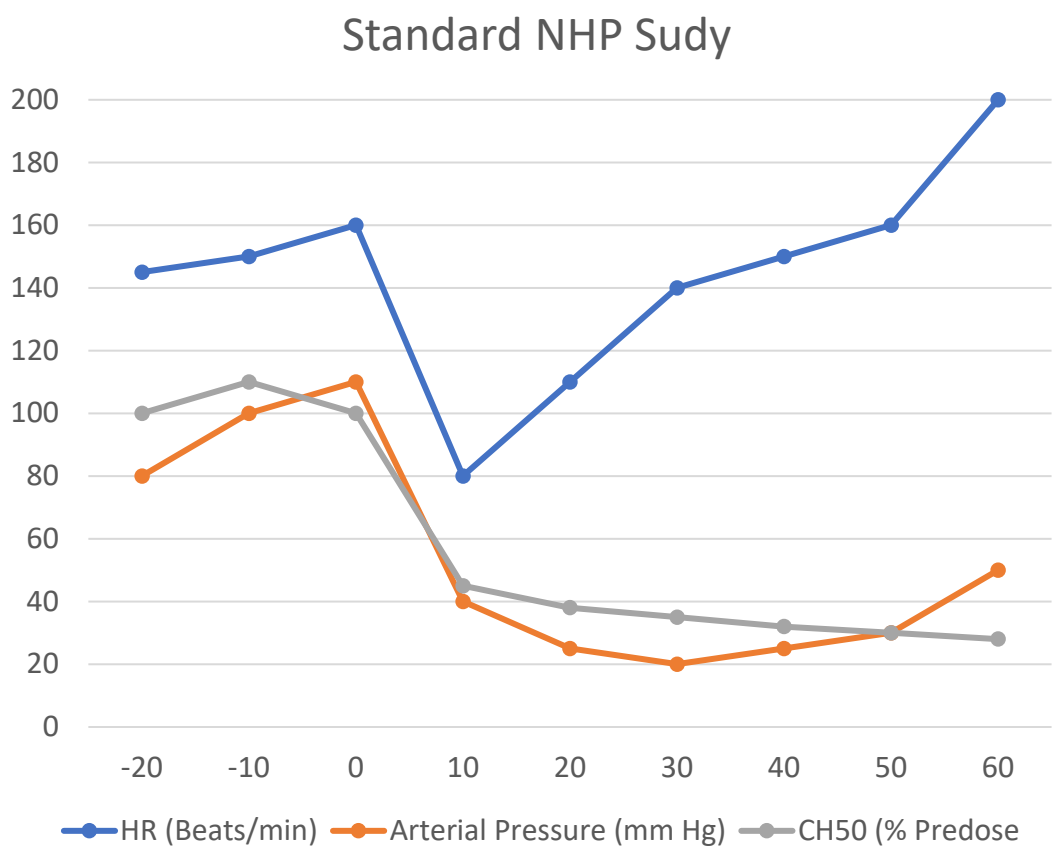
Complement Activation Phosphonothioate Oligonucleotides

(class of antisense oligonucleotides, ASO)

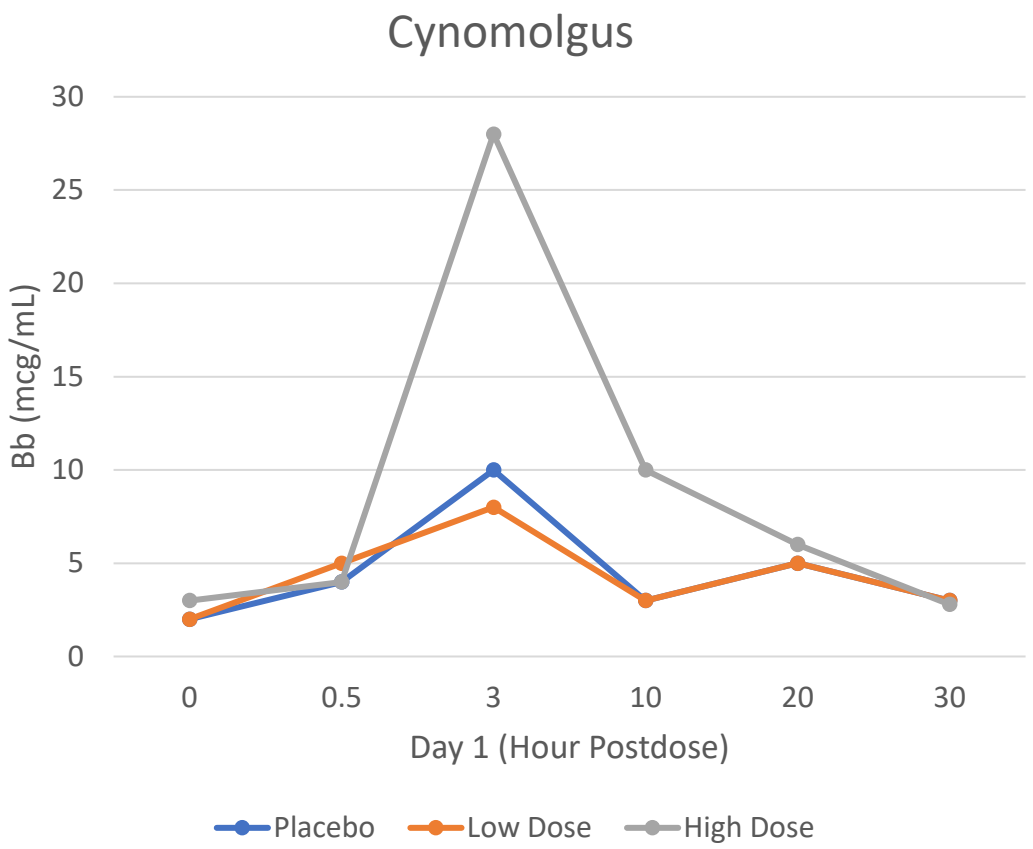
A lot of this work was by Ionis Pharmaceuticals

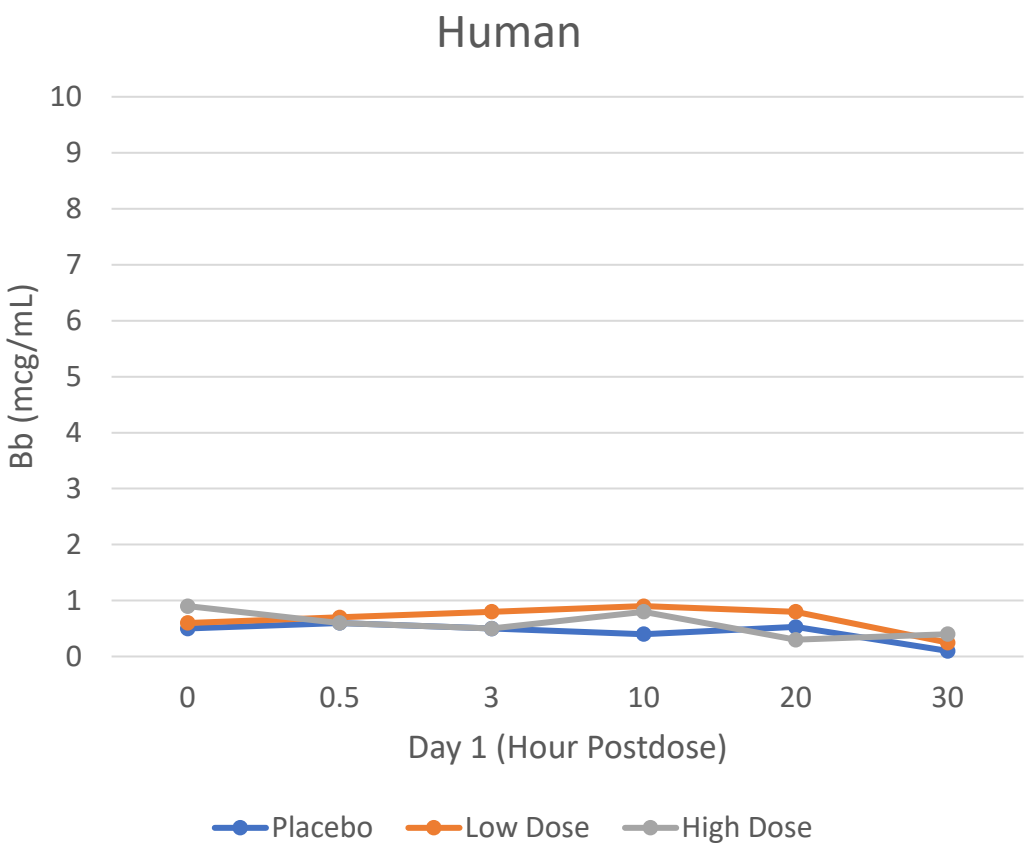
Early ASO Complement Activation in Non-Human Primates

- Work in 1990s demonstrated peek activation of complement coincided with drop-in heart rate and arterial pressure
- Shock like presentation

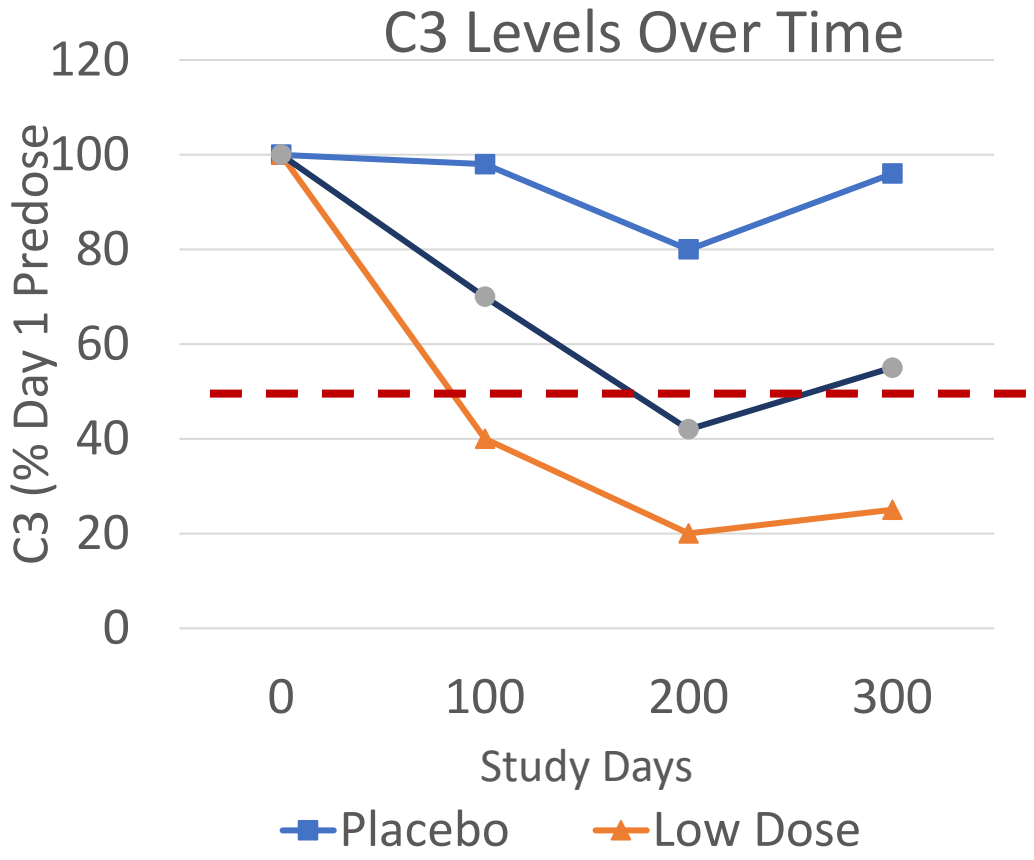


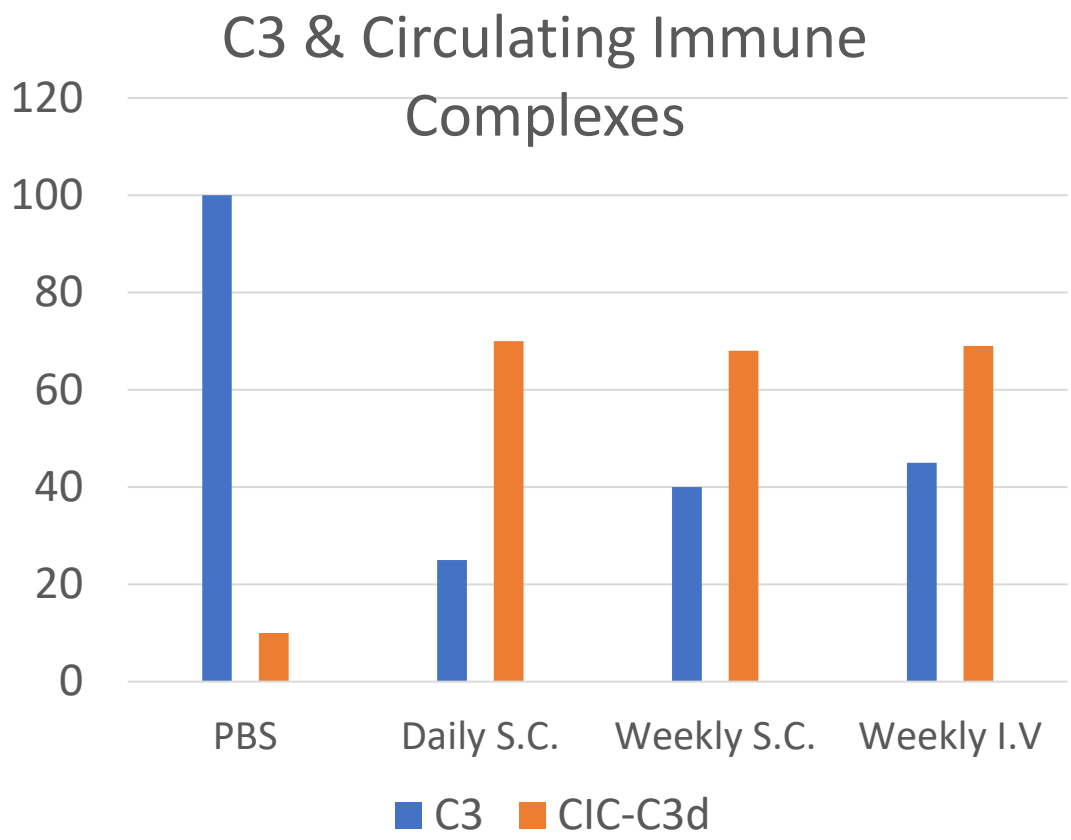
Different Effect Cynomolgus versus Humans





Chronic Low Activation in Humans Does Have Effect on C3





Other Effects of ASOs that may have a Complement Role

- Platelet hypersensitivity
- Thrombocytopenia
- Hypertension
- Coagulation issues

Complement and Adeno-Associated Virus (AAV) Vectors

AAV and Complement

Remember some of roles of complement we discussed:

- Complement is part of innate immune system
- Antibodies on surfaces are great activators of complement
- IgM is great at activating complement
- Complement is part of removal of circulating immune complexes (CIC)

AAVs and Thrombotic Microangiopathy's (TMA)

- Many reports of TMAs after AAV treatment
- Even fatalities
- Number of files put on hold
- Have been connected to complement
- Complement inhibitors have been used to treat the AE
- But how did this get connected to complement activation?

Current Consensus/Published Data on Complement Pathway Involved in AAV Reactions

- There isn't a consensus and data is still reaching critical mass
- Involvement of the Classica/Lectin Pathway(s)
  - Data has been presented that C4/C4a is not increased
  - Also data that presence of anti-AAV antibodies that correlate with complement adverse events
- Involvement of the Alternative Pathway
  - Data for Bb increase in circulation and C3 fragments in tissues
  - Remember the Alternative Pathway is also the amplification loop, regardless of initial point of activation
- Involvement of the Terminal Pathway
  - Growing data of increase in sC5b-9
  - Increase in sC5b-9 also seen in TMA/aHUS

AAV and Thrombotic Microangiopathy (TMA)

TMA – Just some basics

- Categorical description of shared clinical and histological features
- Different pathophysiology
- Common links:
  - Endothelial injury
  - Platelet activation and aggregation
  - Fibrin disposition
  - Mechanical trauma to RBCs in capillary lumen

TMA – Just some basics

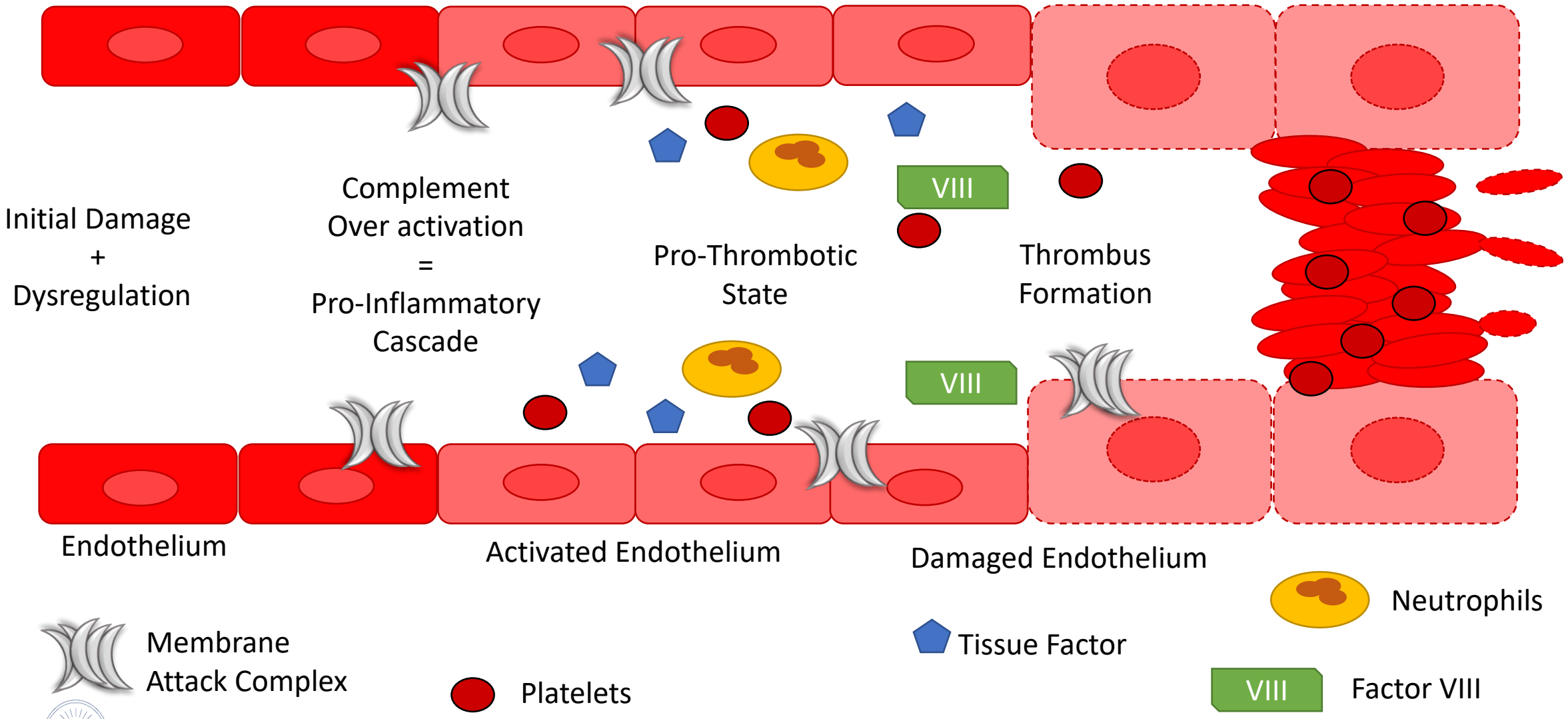
Types of TMA

- STEC-HUS
- Atypical HUS
- Acquired TTP (antibodies to ADAMTS13)
- Infections
- Stem Cell Transplant
- Others (catastrophic antiphospholipid antibody syndrome, HELLP [**H**emolysis, **E**levated **L**iver enzymes and **L**ow **P**latelets])
- Vasculitis
- Drug Induced (calcineurin inhibitors, ticlopidine, clopidogrel)

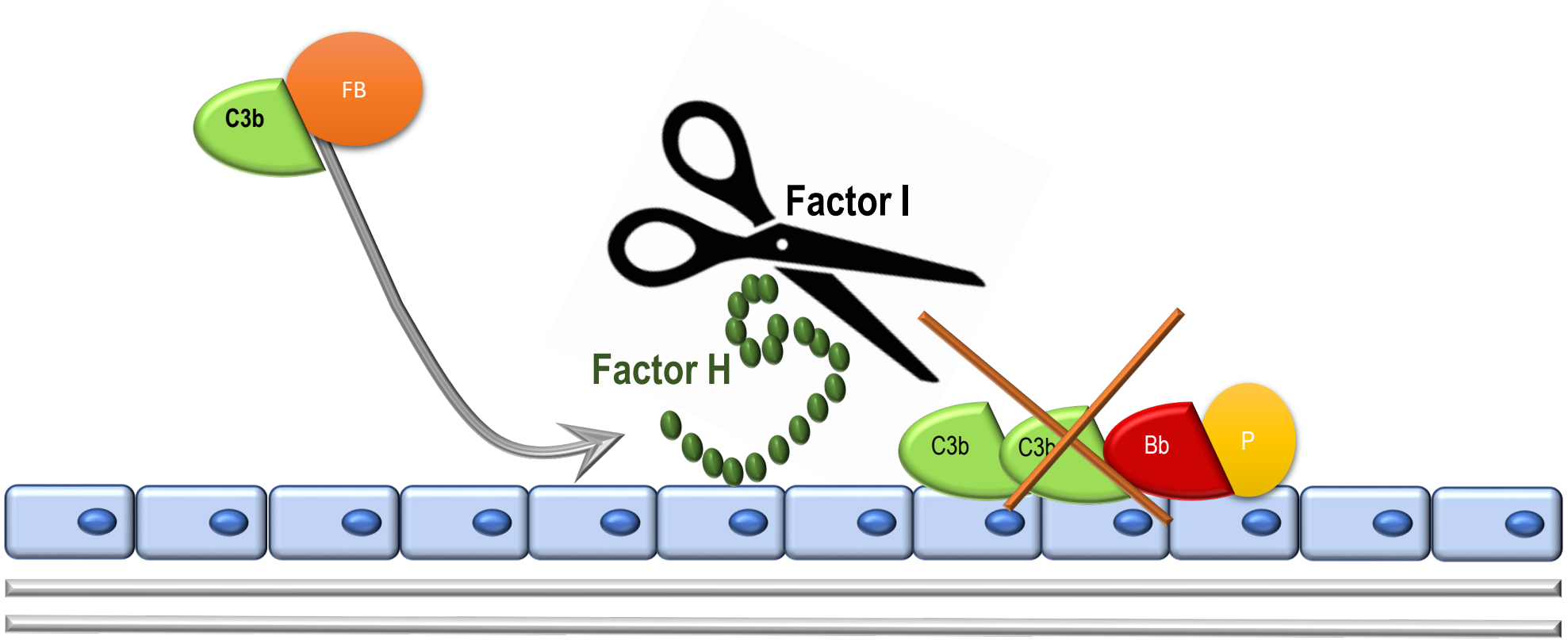
Complement and TMA

- Connected first the atypical Hemolytic Uremic Syndrome
- Form of TMA that looks like Shiga toxin related TMA
- Connected to genetic mutations in components of the alternative pathway of complement
- Loss of function for Factor H, Factor I, MCP
- Gain of function for C3 and Factor B
- *(other genes outside of our discussion)*

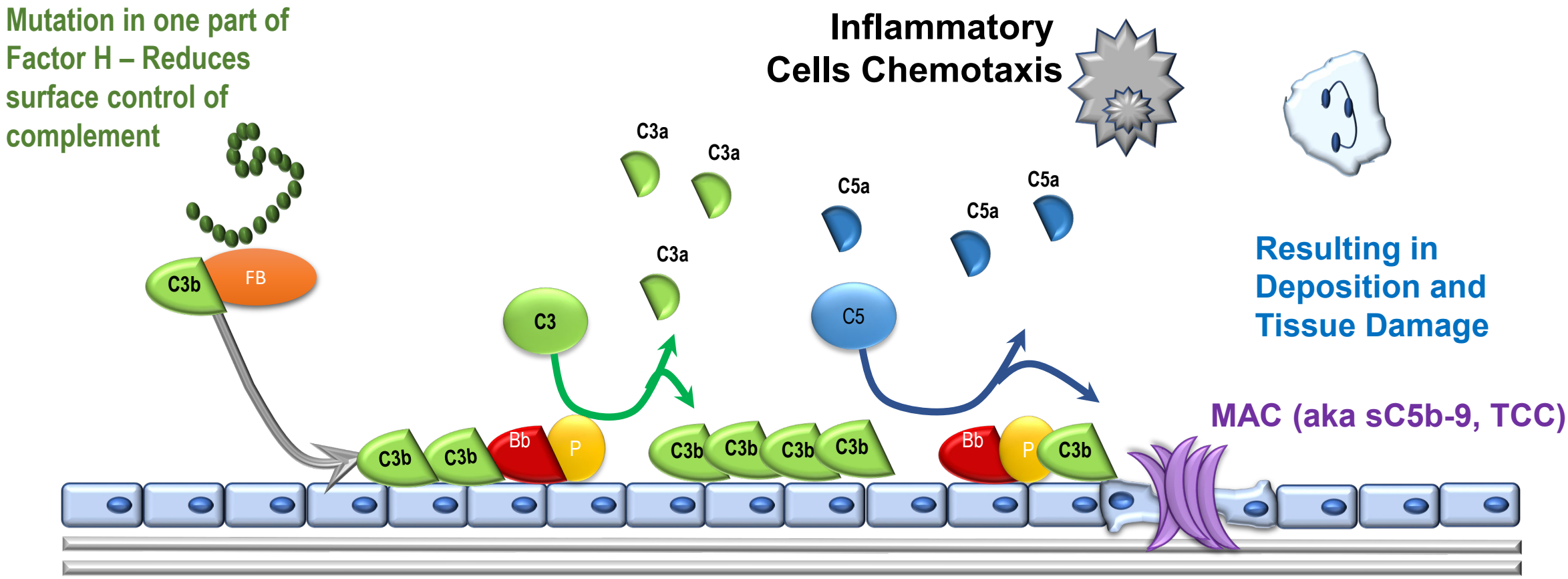
What is TMA?


Initial Damage
+
Dysregulation
Complement
Over activation
=
Pro-Inflammatory
Cascade
Pro-Thrombotic
State
VIII
Thrombus
Formation
VIII
Endothelium
Activated Endothelium
Damaged Endothelium
Neutrophils
Tissue Factor
Membrane
Attack Complex
Platelets
VIII
Factor VIII
Red blood cells

Complement Control in the Kidney - Normal



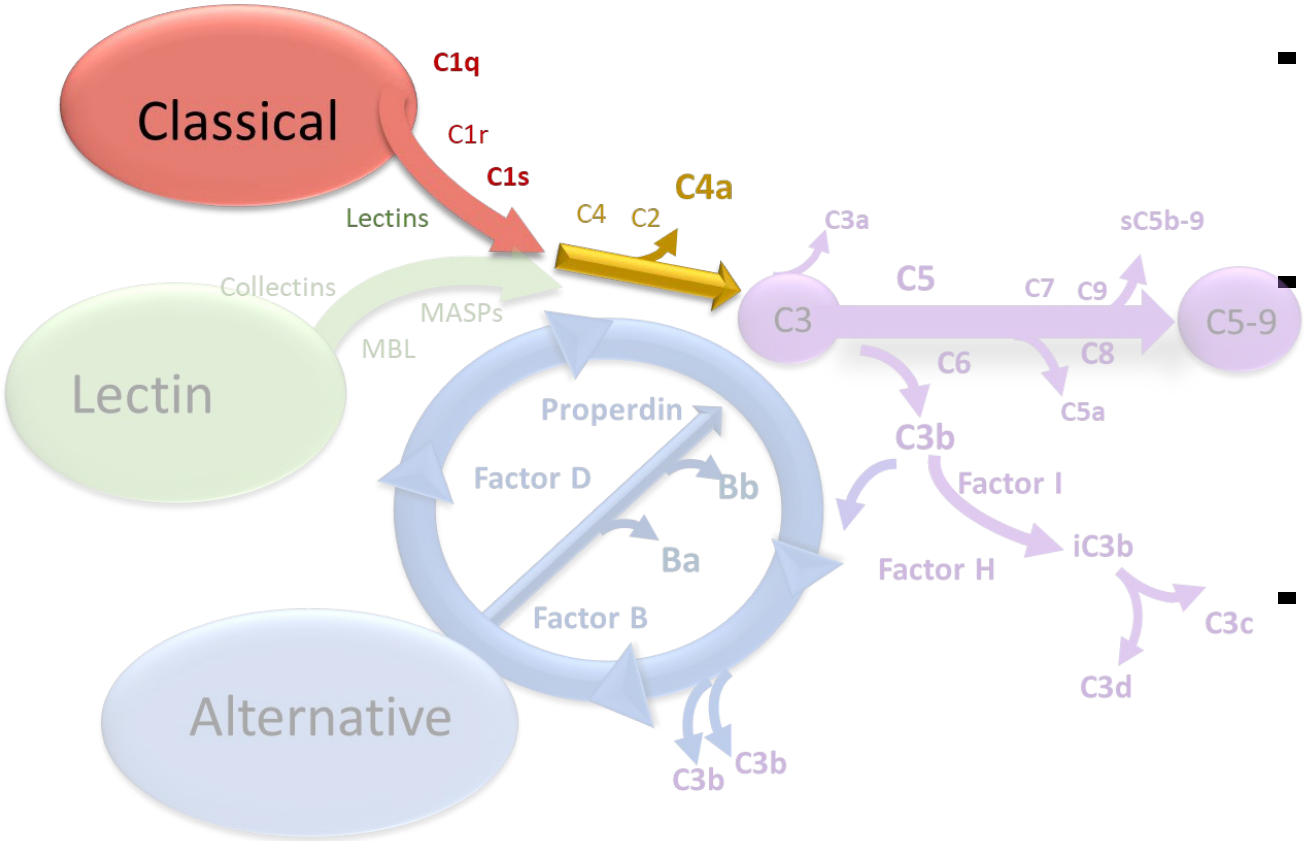
Loss of Complement Control in the Kidney – Mutant Factor H



So You Want to Check if Complement has a Role in an iAE?

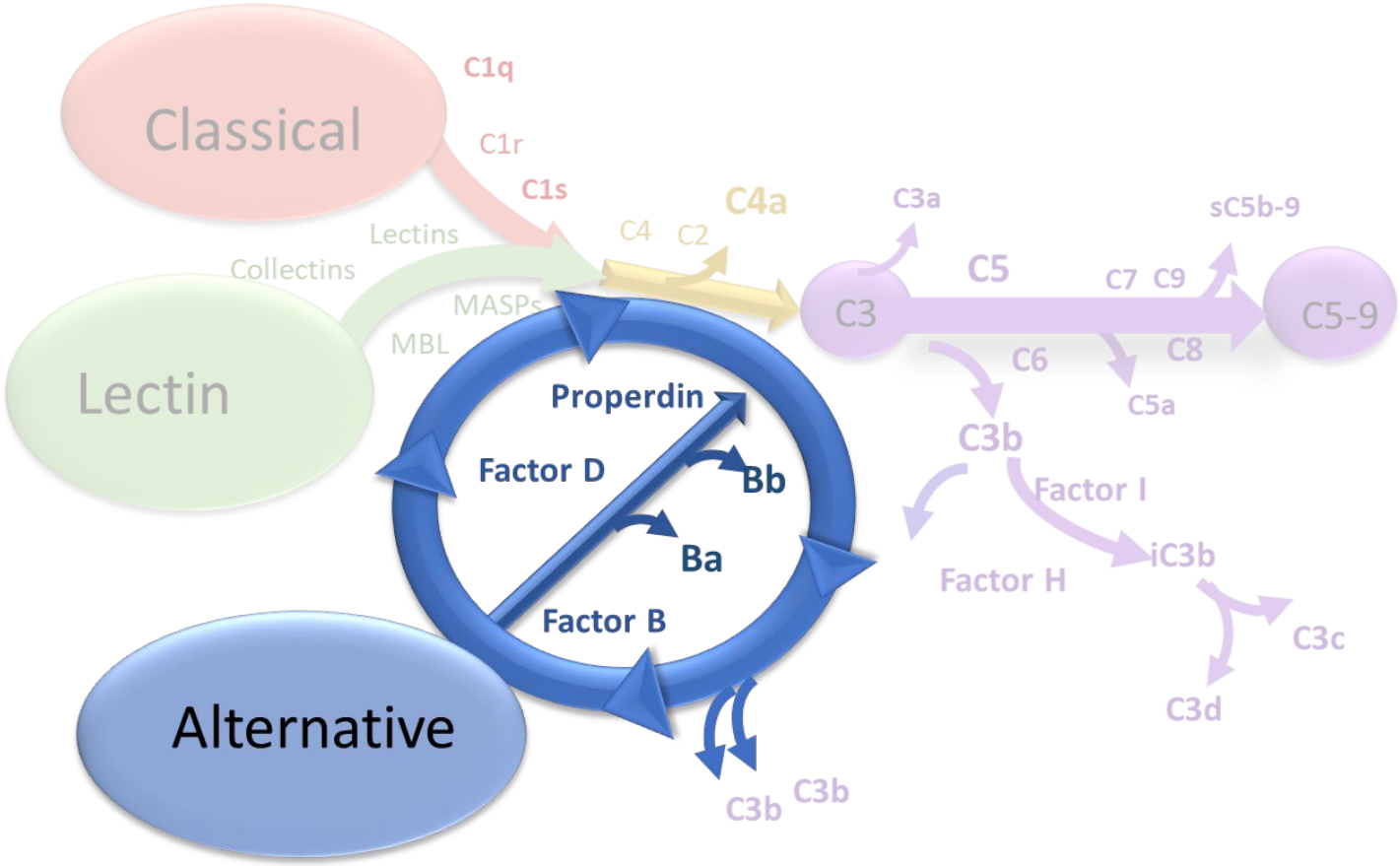
What to consider? Looking back at the potential biomarkers

Complement Classical Pathway Biomarkers



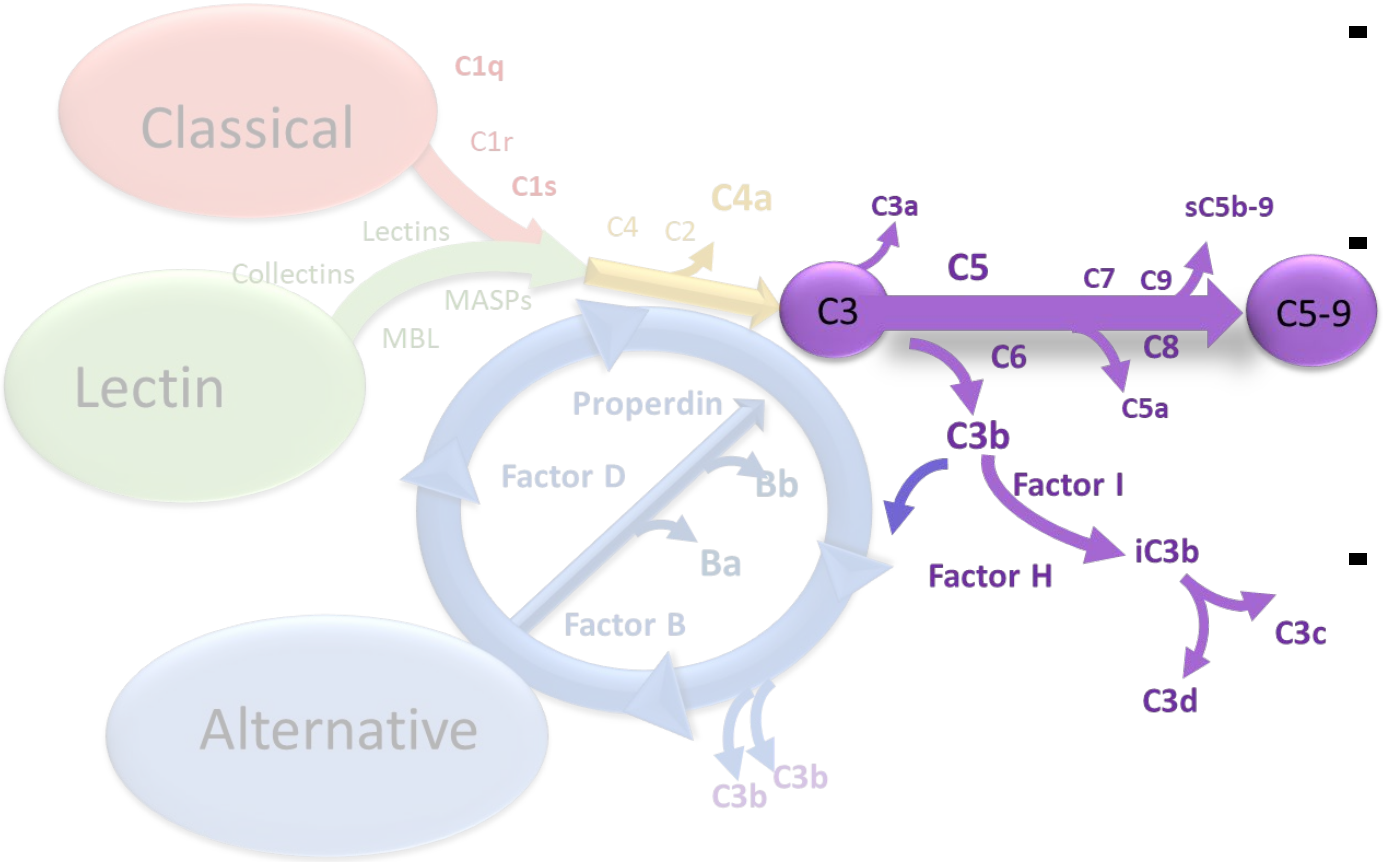
- CH50
  - Great screen but not specific
- C4a
  - Best marker we have for the classical pathway
- C1q and or CIC's
  - Can measure complement containing CIC and C1q to look at antibody involvement

Complement Alternative Pathway Biomarkers



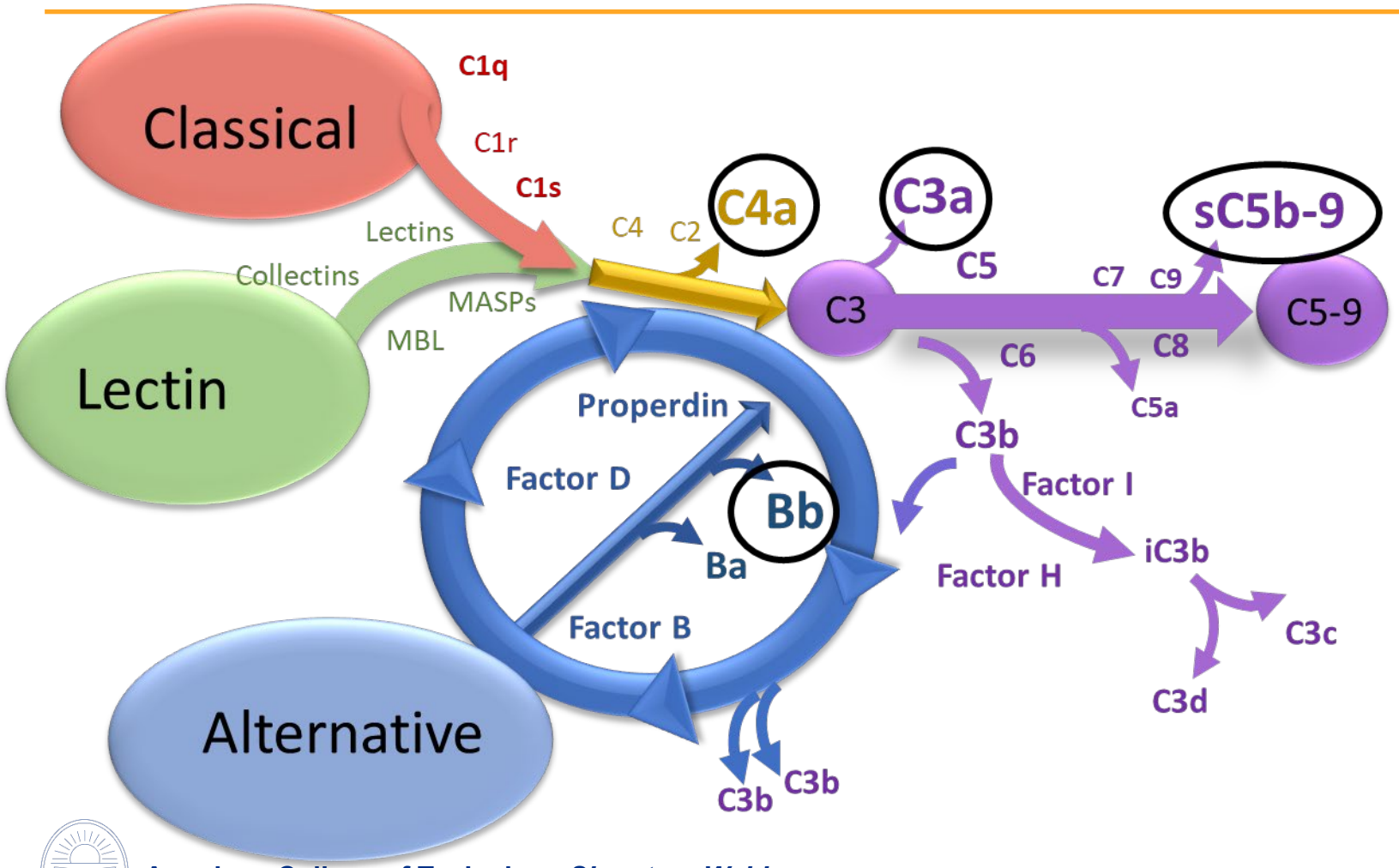
- No matter where it starts – if it is strong enough to be an AE – expect the alternative pathway is involved
- AH50
  - Decrease with activation
- Bb
  - Strong marker, and not cleared

Complement Terminal Pathway Biomarkers



- If the activation is strong – expect it to reach the terminal pathway
- C3a
  - It is a good marker of reaching the central point and is an anaphylatoxin
- sC5b-9
  - Good marker of terminal pathway activation
  - Do see inconsistent results if not performed right

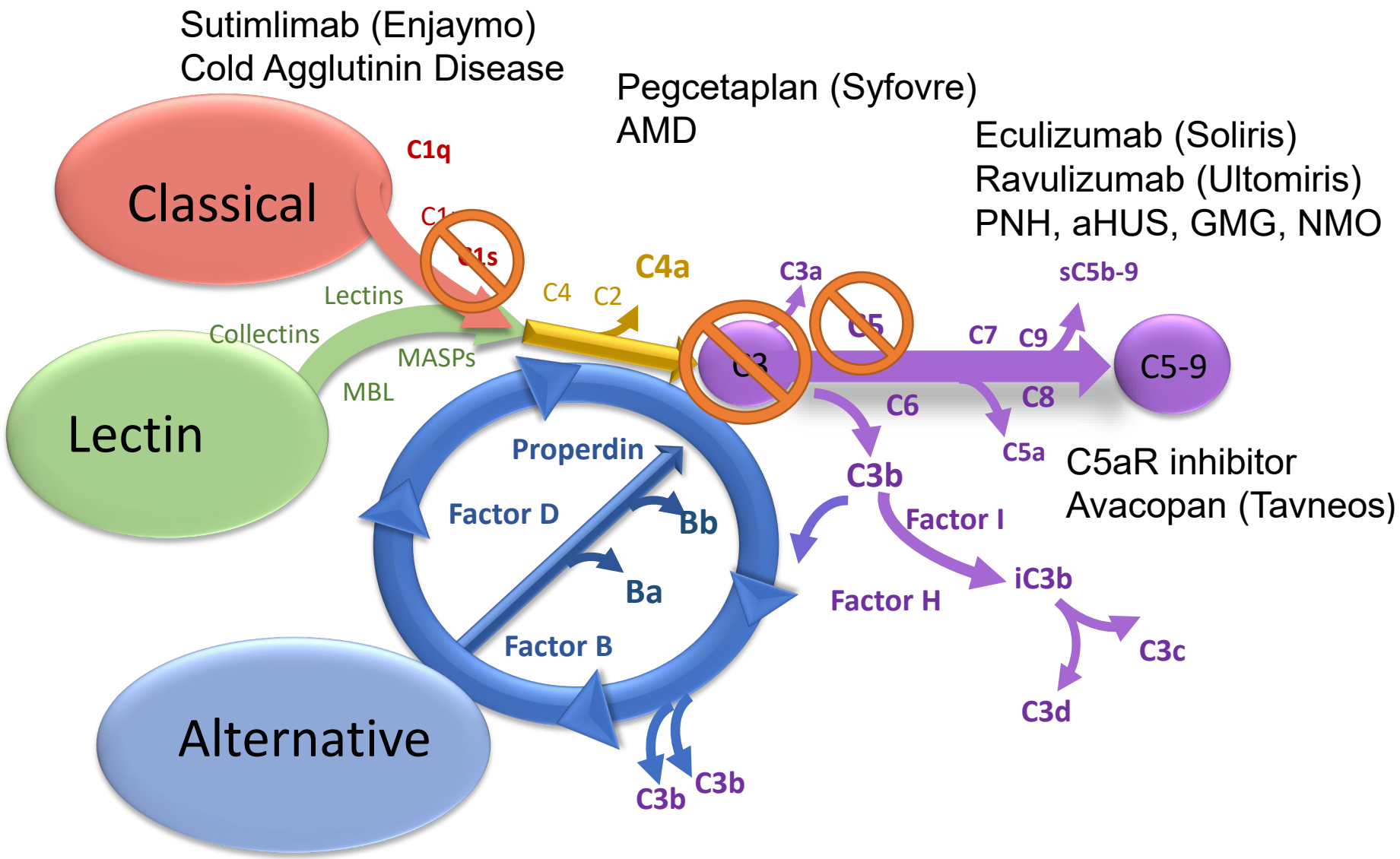
Complement Biomarkers Hit List



- C4a
- C3a
- Bb
- sC5b-9
- CH50
- C4
- C3
- Tissue staining

What can we do about complement AE?

Our growing arsenal of therapeutic complement inhibitors


Sutimlimab (Enjaymo)
Cold Agglutinin Disease
Pegcetaplan (Syfovre)
AMD
Eculizumab (Soliris)
Ravulizumab (Ultomiris)
PNH, aHUS, GMG, NMO
Classical
C1q
C1s
Lectins
Collectins
MASPs
MBL
Lectin
C4
C2
C4a
C3a
C3
C5
C7
C9
sC5b-9
C5-9
C6
C8
C5a
C5aR inhibitor
Avacopan (Tavneos)
Properdin
Factor D
Bb
Ba
Factor B
C3b
Factor I
Factor H
iC3b
C3c
C3d
C3b
C3b
Alternative

Complement Inhibitor of Choice for AE TMA

- Eculizumab (Soliris®)
- aHUS approval
- History success with bone marrow transplant TMA
- Some data of success in E. coli related TMA
- Ravulizumab is long acting so harder to titer
- The APL-9 being studied at this time