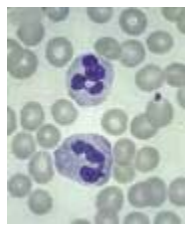
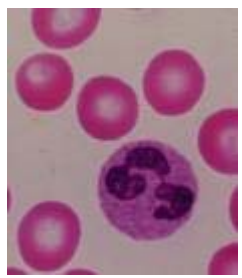
The Immune Response

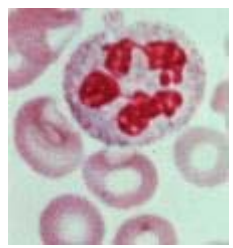
- The reaction to any foreign substance (living or non-living) regardless of pathologic consequences.
- Innate immunity (nonspecific)
- Acquired or adaptive immunity

Innate Immunity

- Physical barriers
- Anti-microbial proteins
- Coagulation factors
- Complement
- Phagocytes (macrophages and neutrophils)







Pathogen-Associated Molecular Patterns (PAMPs)

Pathogens, especially prokaryotes, have molecular structures that

- are not shared with their host;
- are shared by many related pathogens;
- are relatively invariant; that is, do not evolve rapidly

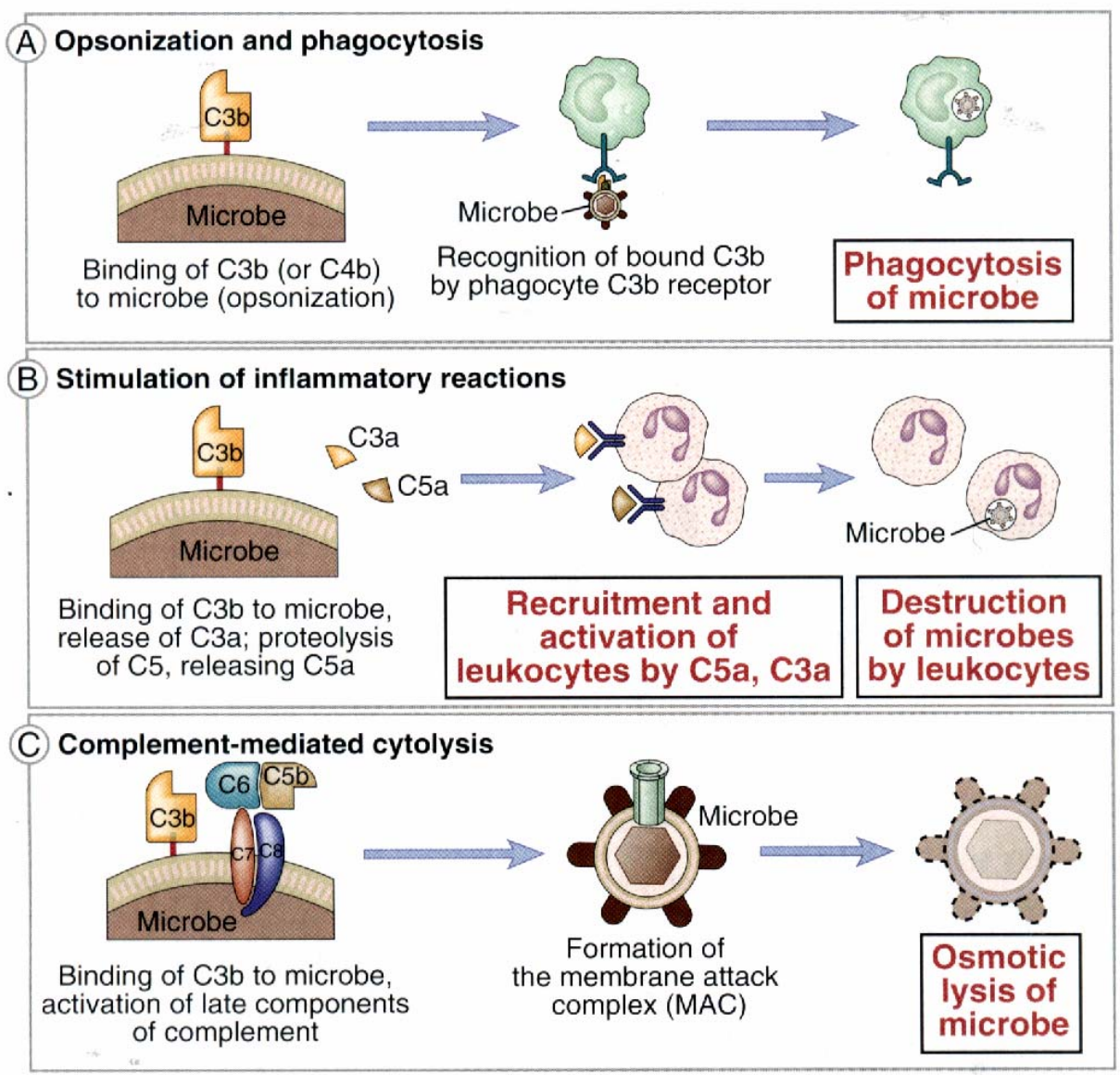
Examples:

- flagellin of bacterial flagella;
- peptidoglycan of gram-positive bacteria ;
- lipopolysaccharide (**LPS**, also called **endotoxin**) of gram-negative bacteria;
- double-stranded RNA; &
- unmethylated DNA.

Pattern Recognition Receptors (PRRs)

- secreted molecules that circulate in blood and lymph;
- surface receptors on phagocytic cells like macrophages that bind the pathogen for engulfment;
- cell-surface receptors that bind the pathogen initiating a signal leading to the release of effector molecules (cytokines).

Complement-Mediated Stimulation of Inflammation



Complement Activities

- Indentification/opsinization of foreign bodies (C3, C4);
- Recruitment/activations (C3a, C5a);
- Lysis of pathogens/cytotoxicity (C5b-9 (MAC);
- Clearing immune complexes and apoptotic cells (C1q, C3b,C4b);
- Augment T and B cell responses (C3,C4, C3a,C5a).

General Features:

AMPLIFICATION: (zymogen cascade)

SOLID-STATE: increases local protein concentration

SOLUBLE SIGNALS: cleaved fragments act as signaling molecules to enhance and regulate inflammation

MULTIPLE INHIBITORS: host cells contain numerous complement inhibitors, inhibitors also present in circulating serum

The Pathways

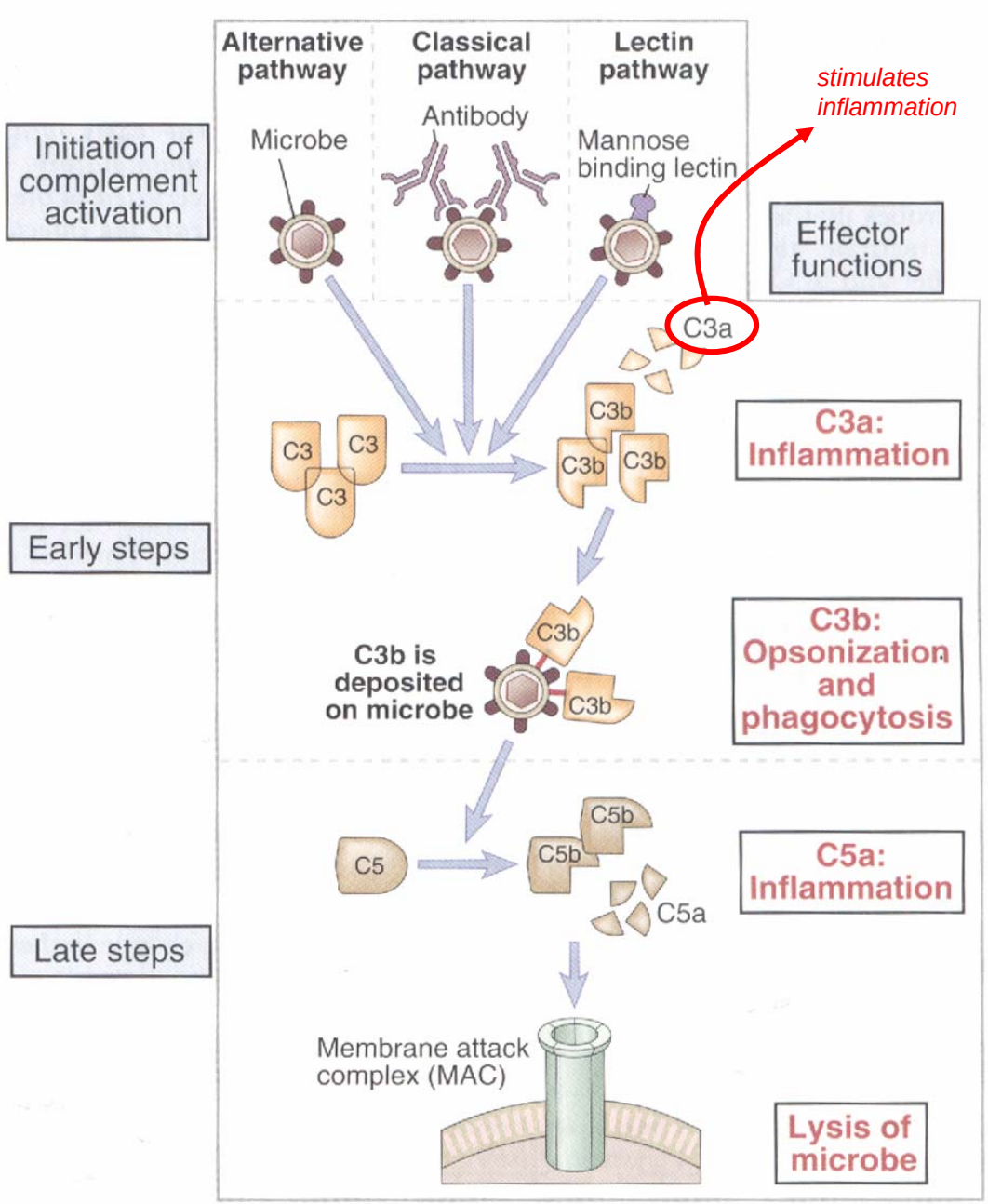


Figure from Abbas, Cellular and Molecular Immunology, 5th ed 2003

Complement Pathways

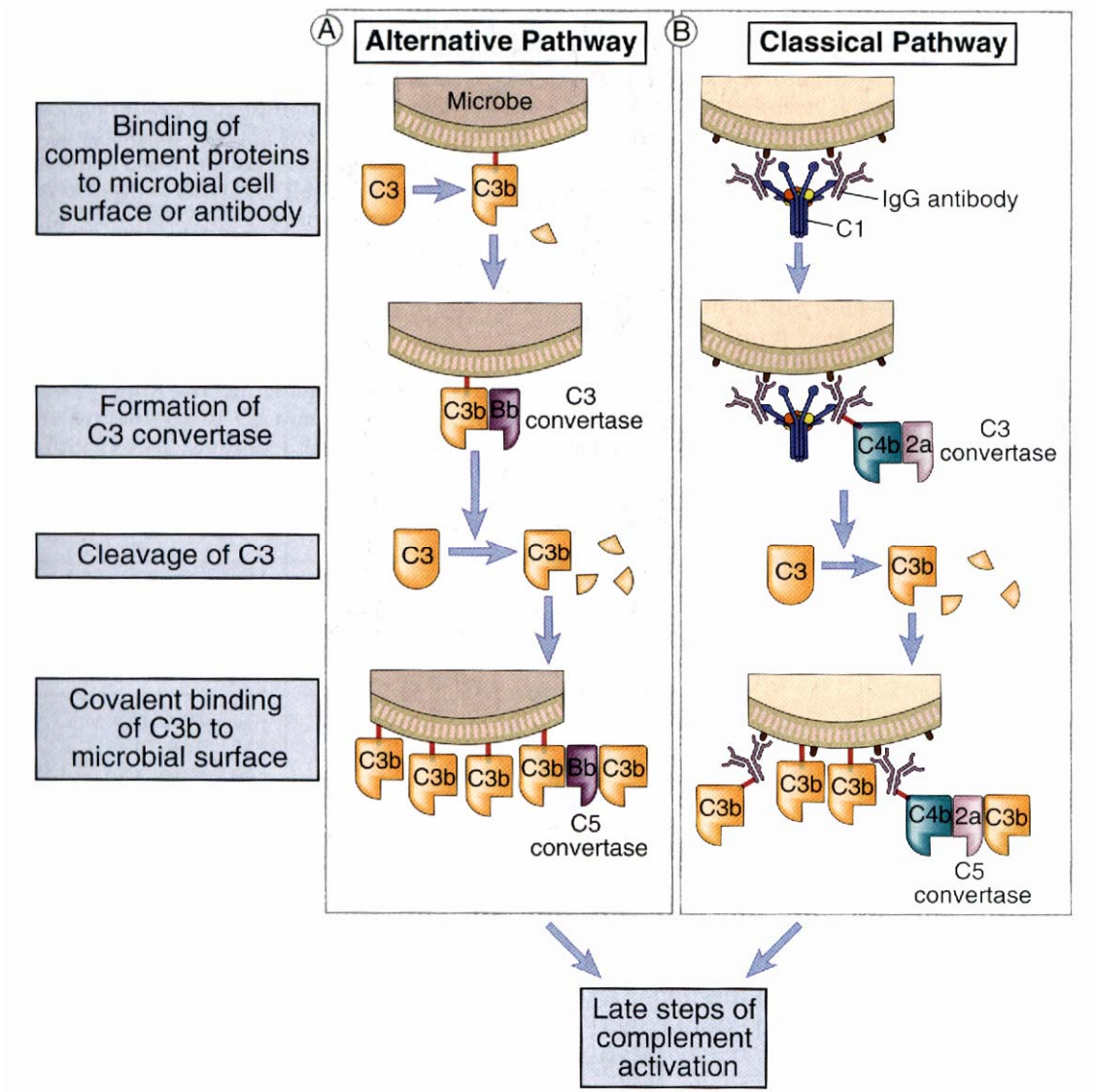


Figure from Abbas, Cellular and Molecular Immunology, 5th ed 2003

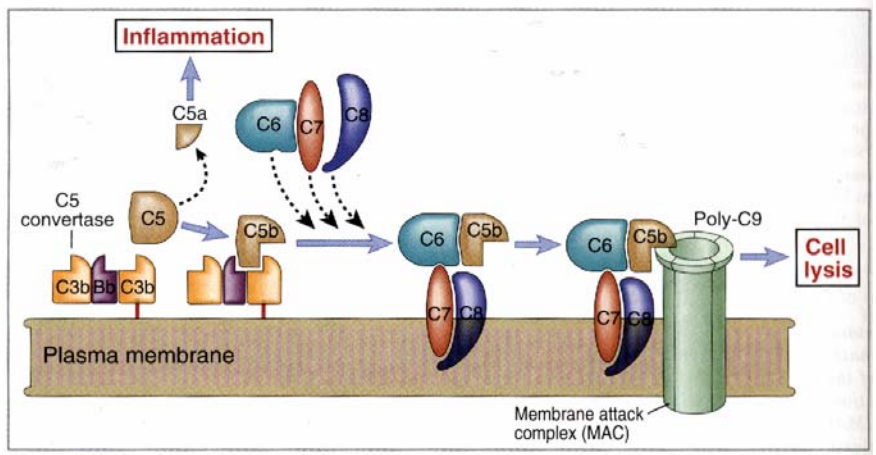
Late Stages of Complement Activation

potent inflammatory mediator

- C5 convertase cleaves C5, *releases C5a*, C5b remains bound
- C5b transiently maintains conformation that binds C6 and C7
- C5bC6C7 is highly hydrophobic, inserts into lipid bilayer
- binds C8, stabilizes insertion of complex into membrane
- C9 polymerizes at sites of C5b-C8
    - forms pores (100 angstrom), creates channels
    - osmotic lysis (rupture)
    - rapid calcium entry -> activates caspases -> apoptosis

Table 14–5. Proteins of the Late Steps of Complement Activation

Protein	Structure	Serum concentration (μg/mL)	Function
C5	190-kD dimer of 115- and 75- kD chains	80	C5b initiates assembly of the MAC C5a stimulates inflammation (anaphylatoxin)
C6	110-kD monomer	45	Component of the MAC: binds to C5b and accepts C7
C7	100-kD monomer	90	Component of the MAC: binds to C5b,6 and inserts into lipid membranes
C8	155-kD trimer of 64-, 64-, and 22-kD chains	60	Component of the MAC: binds to C5b,6,7 and initiates the binding and polymerization of C9
C9	79-kD monmer	60	Component of the MAC: binds to C5b,6,7,8 and polymerizes to form membrane pores



Complement Regulation

- Complement regulatory proteins – soluble and membrane bound
- Importance of rapid regulation of complement – soluble inhibitors abundant in serum
- Cleaved products are normally only reactive for brief periods – ensures limited diffusion and local concentration
    - ex. C3b thioester reactivity is very short-lived

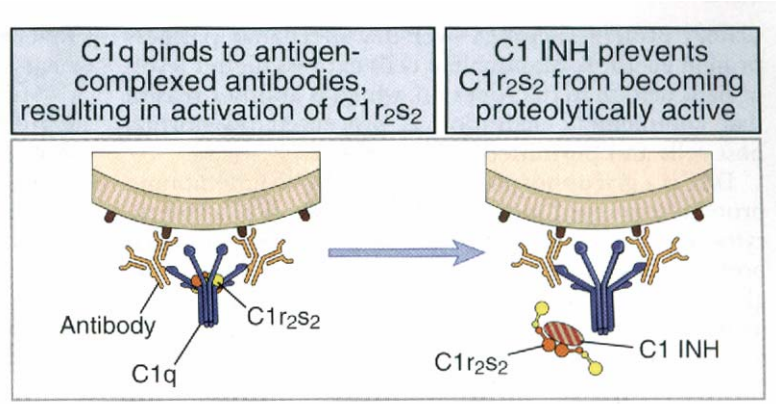
Table 14–7. Regulators of Complement Activation

Receptor	Structure	Distribution	Interacts with	Function
C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 µg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
Factor I	88-kD dimer of 50- and 38-kD subunits	Plasma protein; conc. 35 µg/mL	C4b, C3b	Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors
Factor H	150 kD; multiple CCPRs	Plasma protein; conc. 480 µg/mL	C3b	Binds C3b and displaces Bb Cofactor for factor I–mediated cleavage of C3b
C4-binding protein (C4BP)	570 kD; multiple CCPRs	Plasma protein; conc. 300 µg/mL	C4b	Binds C4b and displaces C2 Cofactor for factor I–mediated cleavage of C4b
Membrane cofactor for protein (MCP, CD46)	45-70 kD; four CCPRs	Leukocytes, epithelial cells, endothelial cells	C3b,C4b	Cofactor for factor I–mediated cleavage of C3b and C4b
Decay-accelerating factor (DAF)	70 kD; GPI linked, four CCPRs	Blood cells, endothelial cells, epithelial cells	C4b2b, C3bBb	Displaces C2b from C4b and Bb from C3b (dissociation of C3 convertases)
CD59	18 kD; GPI linked	Blood cells, endothelial cells, epithelial cells	C7, C8	Blocks C9 binding and prevents formation of the MAC

Abbreviations: CCPR, complement control protein repeat; conc., concentration; GPI, glycophosphatidylinositol; MAC, membrane attack complex.

C1 Inhibitor (C1 INH)

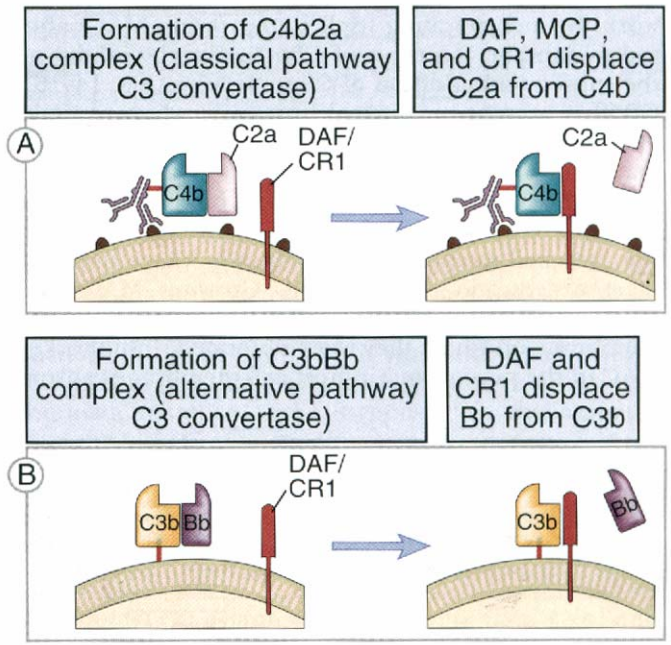
- C1 INH is a serine protease inhibitor (serpin class)
- mimics normal substrate of C1r and C1s
- C1q binds antibody, C1r and C1s become active
- C1 INH competes for normal substrate (C4)
- becomes cleaved and attaches to C1 complex
- C1r-C1s tetramer dissociates from C1q
- Limits classical pathway activation



- hereditary angioneurotic edema
  - deficiency of C1 INH
  - acute edema in skin and mucosa
  - abdomina pain, vomiting, diarrhea
  - airway obstruction
  - mechanism?
    - over-production of C2 fragment (C2 kinin)
    - remember C1 cleaves C2 when bound to C4b
    - causes excessive vascular permeability

Inhibitors of C3 Convertase

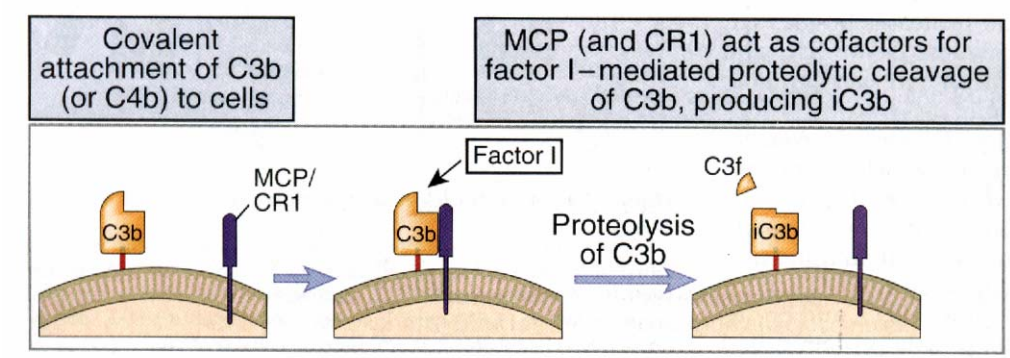
- C3b is commonly deposited on normal host cells
    - remember, C3b is spontaneously generated at low rates
- if not quickly inhibited, complement will destroy normal host tissue
- Membrane Cofactor Protein (MCP/CD46), Type I Complement Receptor (CR1), Decay Accelerating Factor (DAF), C4-Binding Protein (C4BP)
    - bind to C3b on cell surface
    - competitively inhibit and/or displace binding of other components of the C3 convertase – Bb (alternative path) or C2a (classical path)
    - *engineered CR1 used as pharmaceutical*
- Factor H is abundant soluble plasma protein (0.5mg/mL)
    - inhibits binding of Bb to C3b
    - Why then does factor H not inhibit C3 convertase formation on microbe surfaces?
        - Factor H has higher affinity for sialic acid rich surfaces
    - *Factor H has been applied to biomaterial surfaces*



- Paroxysmal nocturnal hemoglobinuria
    - deficiency in enzyme required for forming glycophosphatidylinositol-linked membrane proteins (GPI)
    - failure to express DAF, complement-mediated lysis of erythrocytes
    - recurrent intravascular hemolyisis, chronic hemolytic anemia, venous thrombosis

Factor I

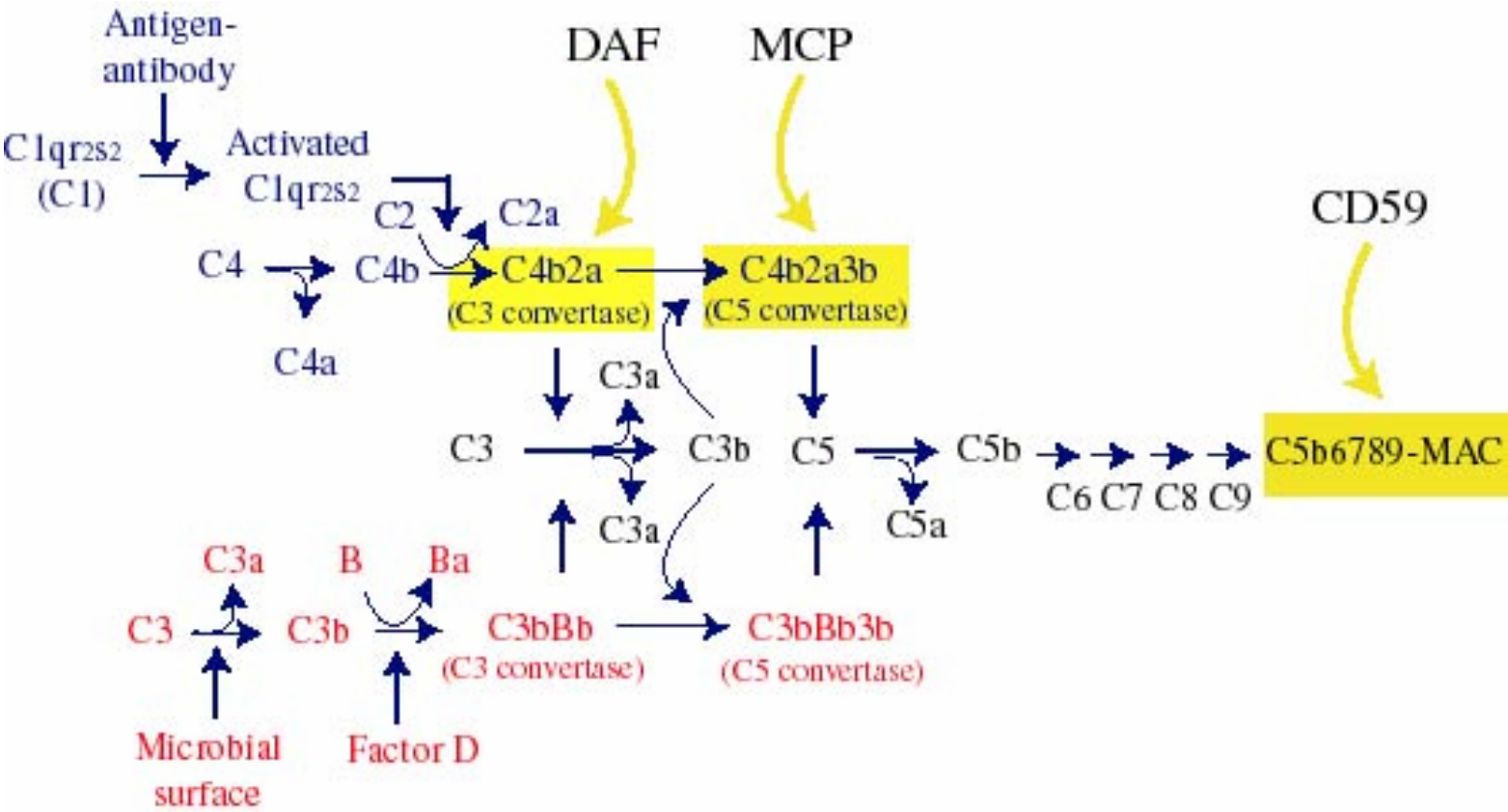
- serine protease
- MCP, Factor H, C4BP, and CR1 are cofactors for Factor I cleavage of C3b or C4b
- C3b cleaved fragments generated
    - iC3b, C3d, and C3dg
    - do not activate complement
    - but are recognized by phagocytes
- Thus, further complement activation is halted without affecting leukocyte clearance of foreign particles
- Complement inhibitors can be swamped
    - over-production of complement or antibodies can overcome the inhibitory system
    - results in various disease states



Pattern Recognition Receptors (PRRs)

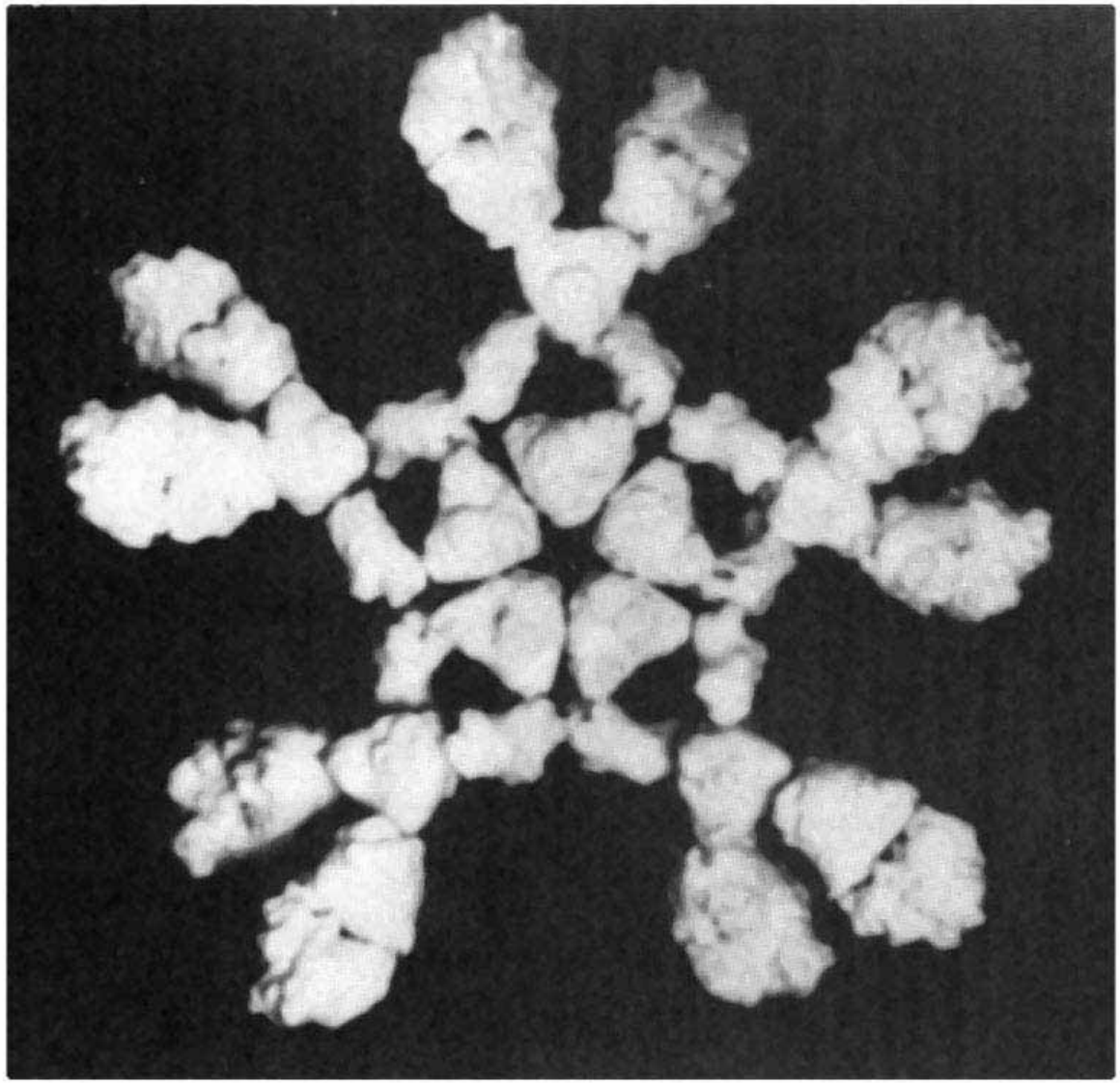
- secreted molecules that circulate in blood and lymph;
- surface receptors on phagocytic cells like macrophages that bind the pathogen for engulfment;
- cell-surface receptors that bind the pathogen initiating a signal leading to the release of effector molecules (cytokines).

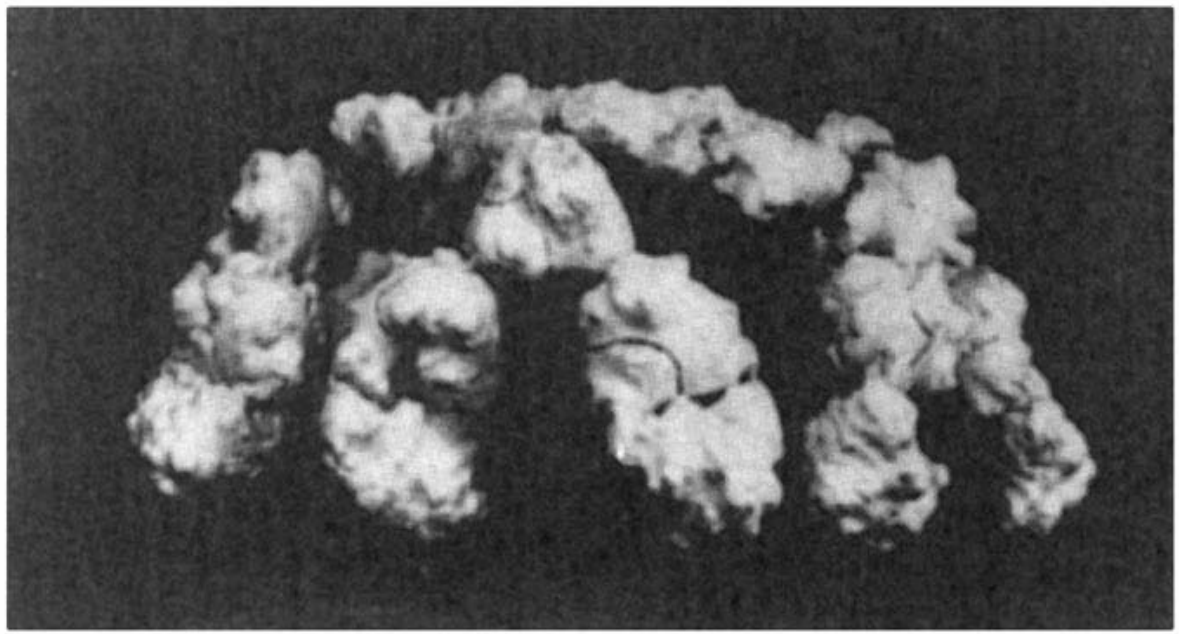
Complement

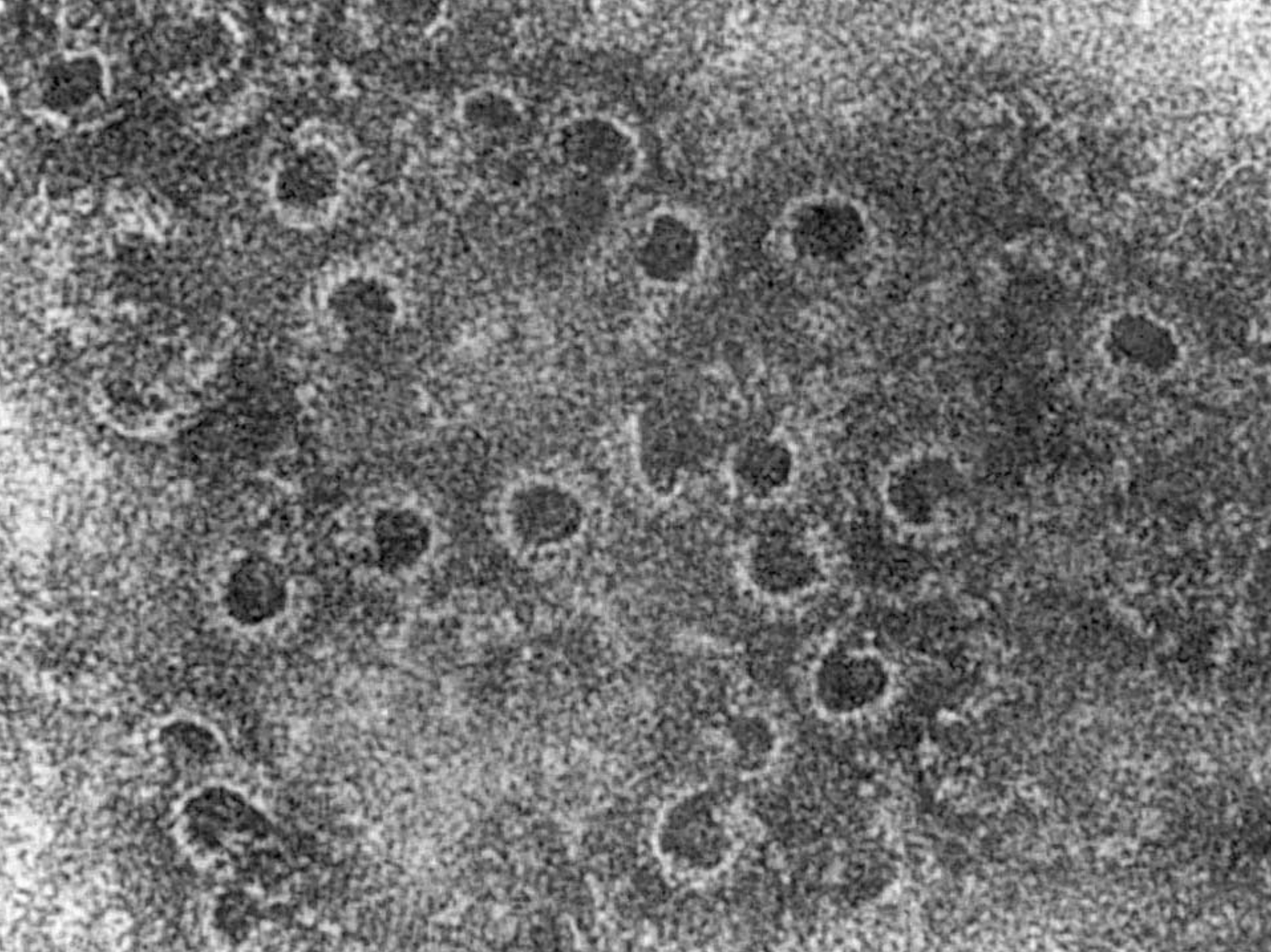

Antigen-
antibody
C1qr2s2
(C1)
Activated
C1qr2s2
C2
C2a
DAF
MCP
CD59
C4
C4b
C4b2a
(C3 convertase)
C4b2a3b
(C5 convertase)
C4a
C3a
C3
C3b
C5
C5b
C6 C7 C8 C9
C5b6789-MAC
C5a
C3a
C3a
B
Ba
C3
C3b
C3bBb
(C3 convertase)
C3bBb3b
(C5 convertase)
Microbial
surface
Factor D

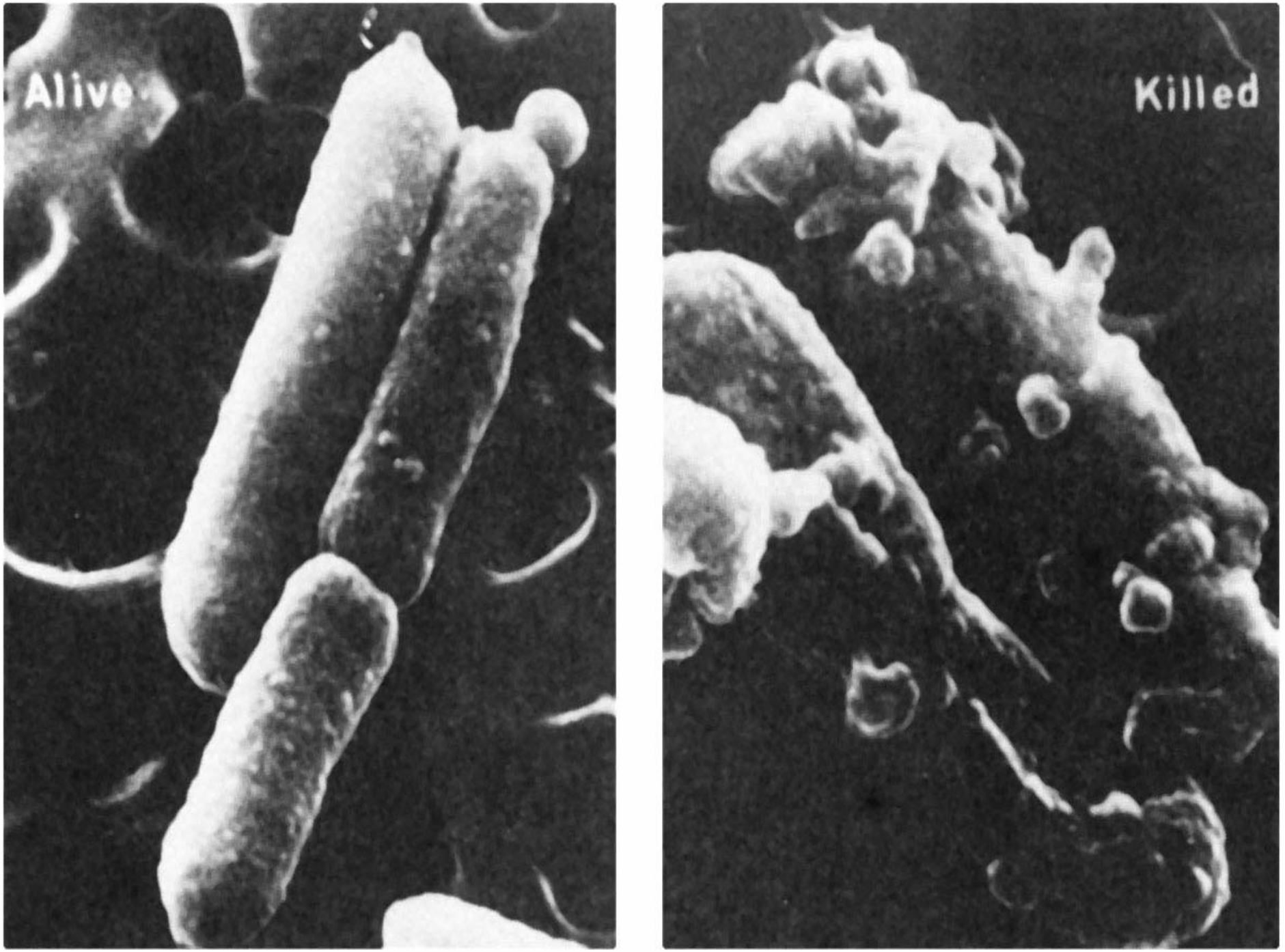
Complement Activities

- Indentification/opsinization of foreign bodies (C3, C4);
- Recruitment/activations (C3a, C5a);
- Lysis of pathogens/cytotoxicity (C5b-9 (MAC);
- Clearing immune complexes and apoptotic cells (C1q, C3b,C4b);
- Augment T and B cell responses (C3,C4, C3a,C5a).

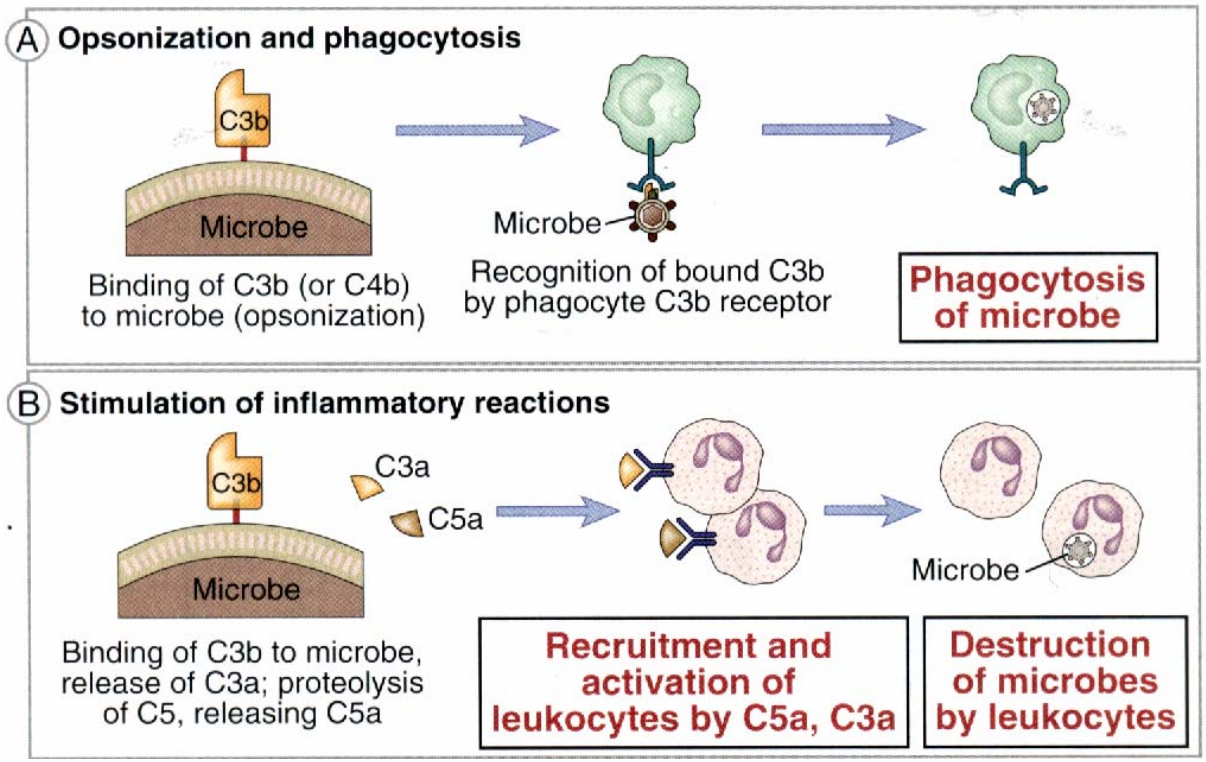







Alive
Killed

Complement-Mediated Stimulation of Inflammation



General Features:

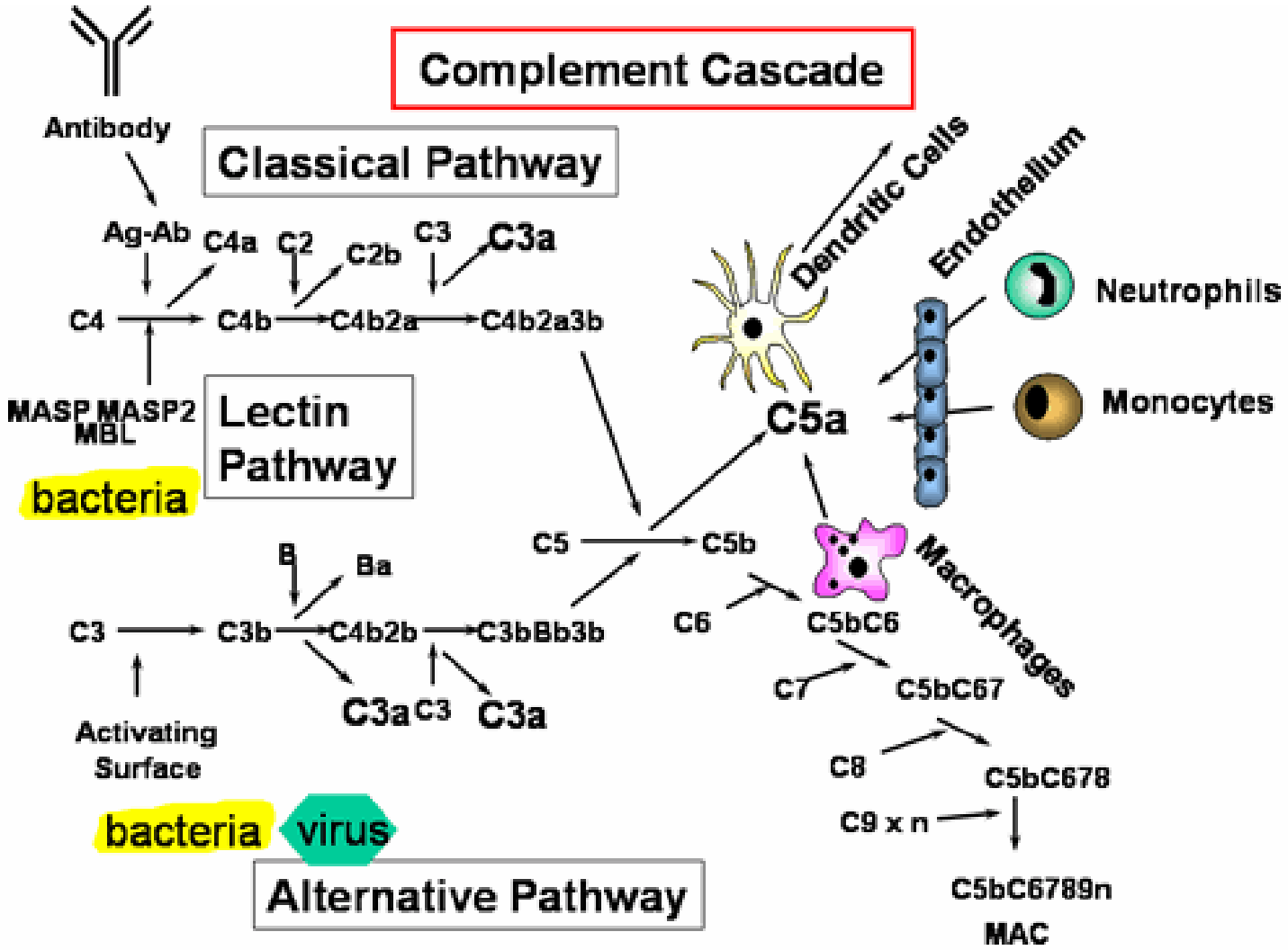
AMPLIFICATION: (zymogen cascade)

SOLID-STATE: increases local protein concentration as components bind to implant surfaces and promote phagocyte/ macrophage attachment and activation

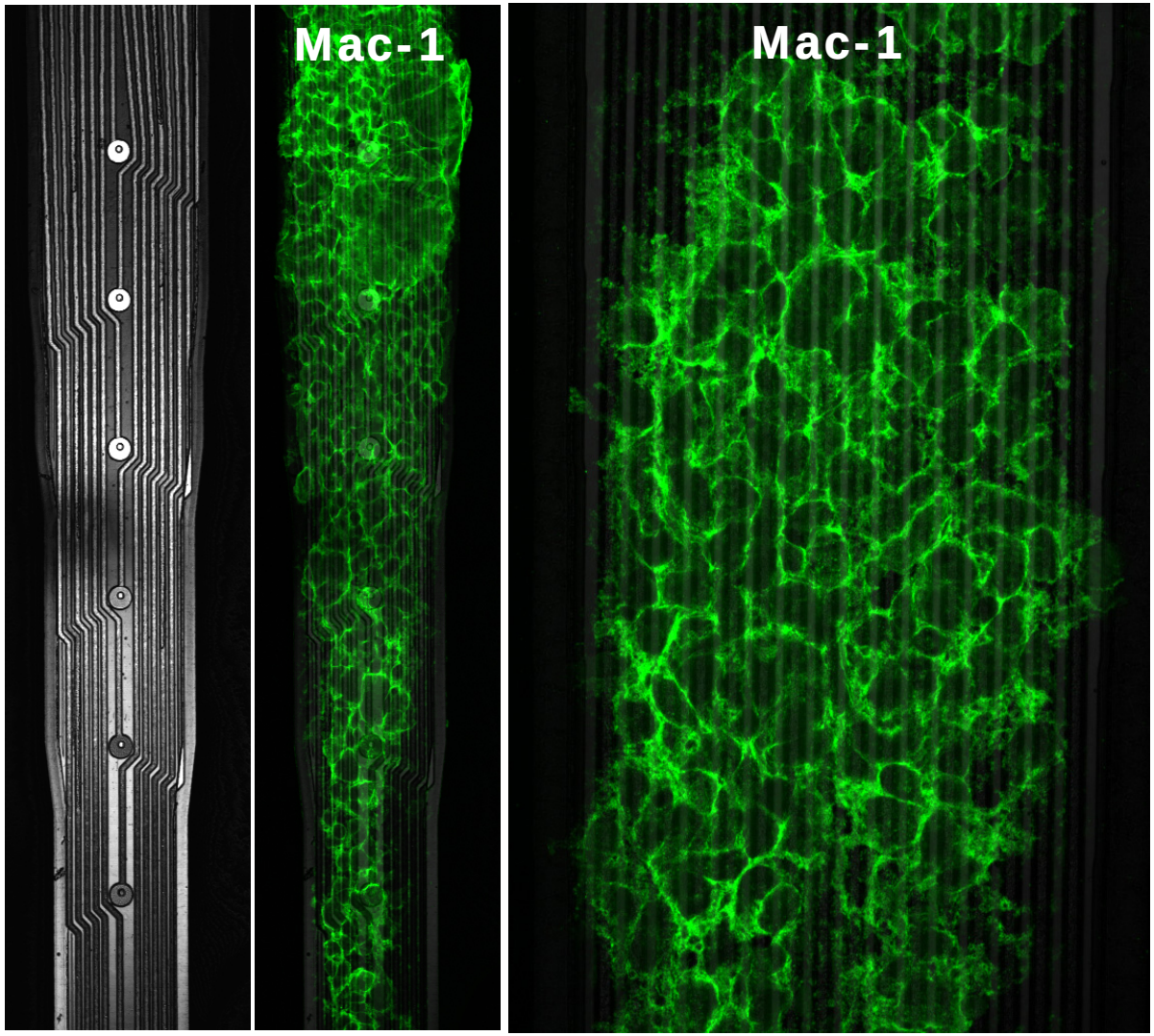
SURFACE DAMAGE: enzymatic and oxidative reactions.

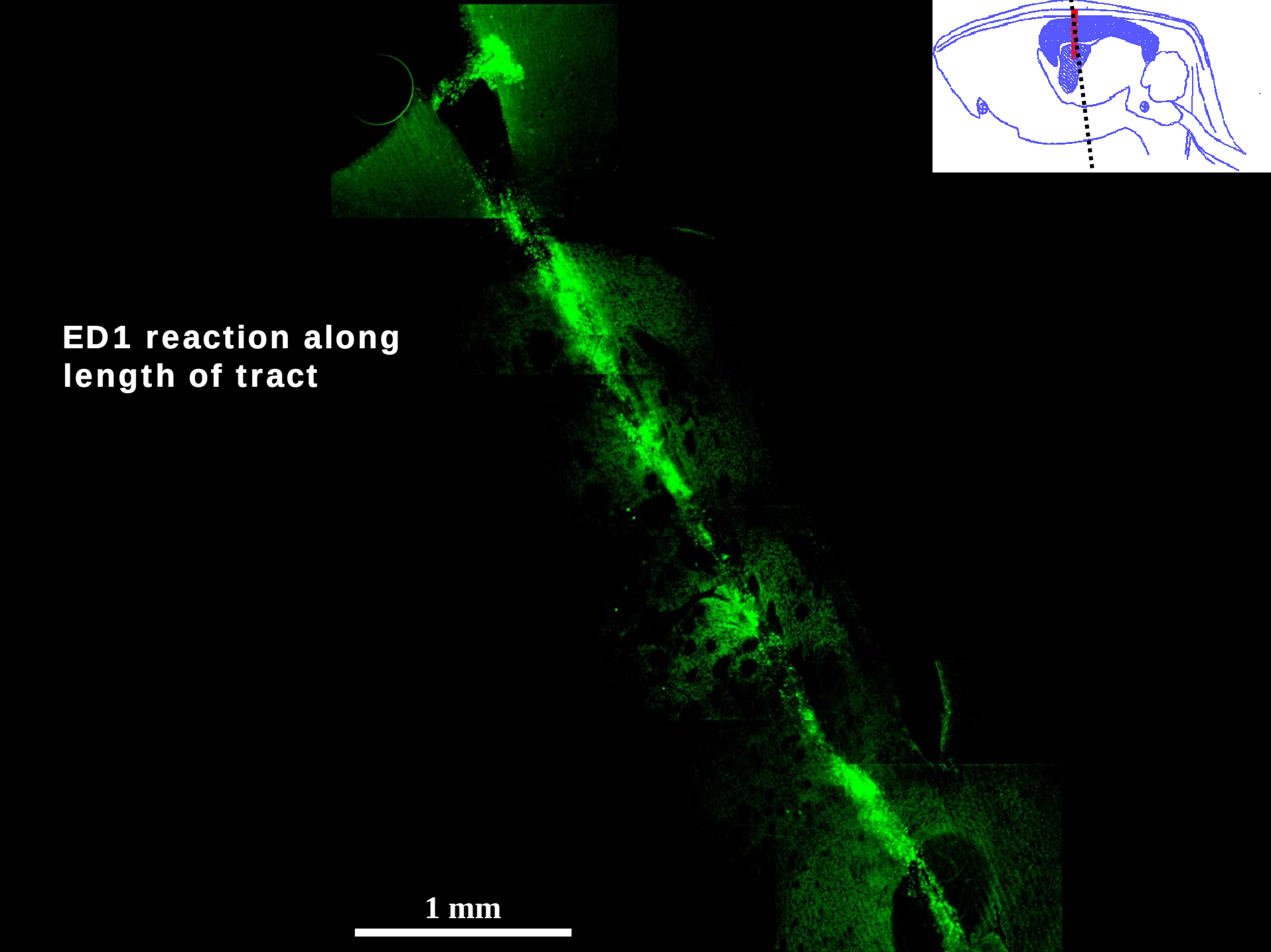
FRUSTRATED PHAGOCYTOSIS: Macrophages are unable to remove implant.

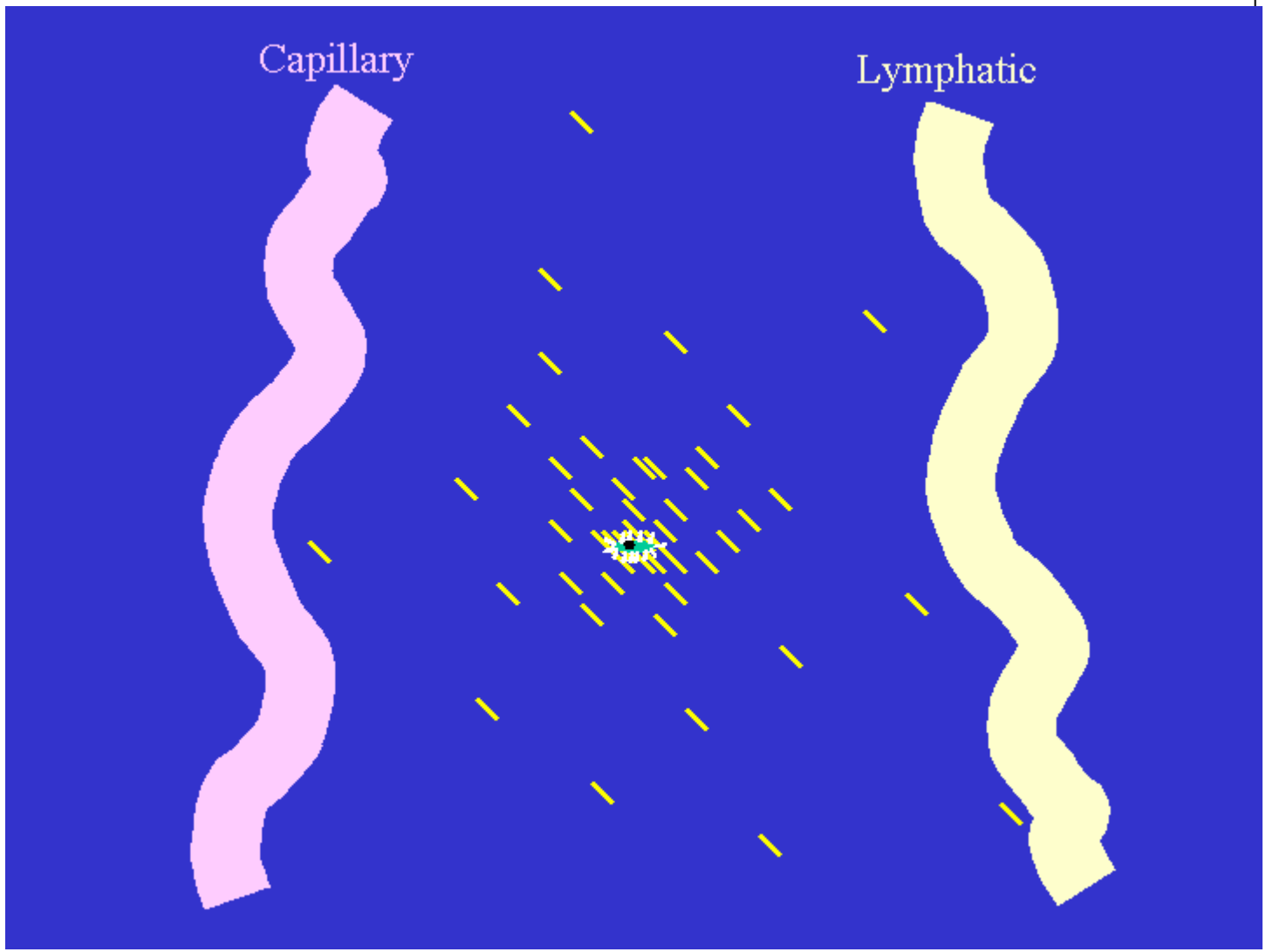
SOLUBLE SIGNALS: cleaved fragments act as signaling molecules to enhance and sustain inflammation

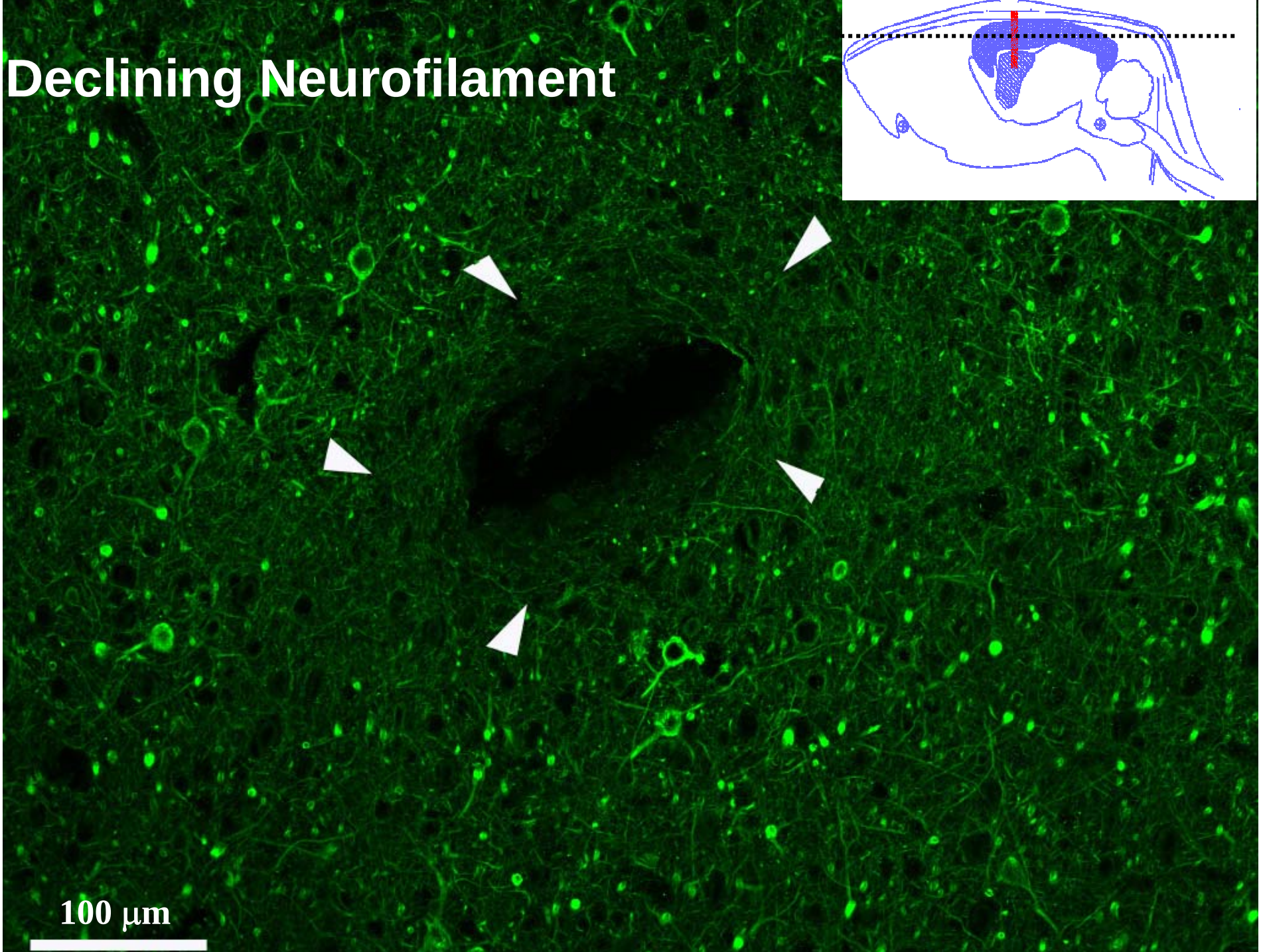

Complement Cascade
Antibody
Classical Pathway
Ag-Ab
C4a
C2
C2b
C3
C3a
C4
C4b
C4b2a
C4b2a3b
MASP
MASP2
MBL
Lectin Pathway
bacteria
Dendritic Cells
Endothelium
Neutrophils
Monocytes
C5a
C5
C5b
B
Ba
C3
C3b
C4b2b
C3bBb3b
C6
C5bC6
Macrophages
C7
C5bC67
C3a
C3
C3a
Activating Surface
C8
C5bC678
C9 x n
C5bC6789n
MAC
bacteria
virus
Alternative Pathway

Mac-1^{+} Microglia on Retrieved Microelectrodes

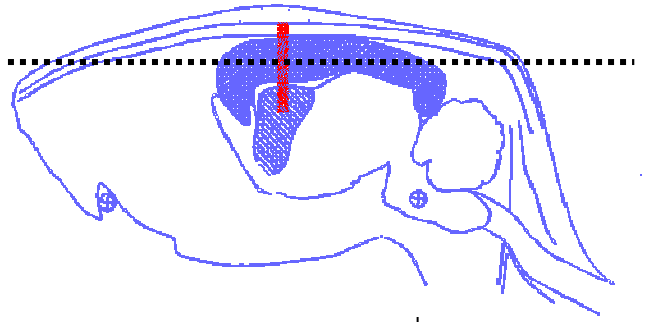


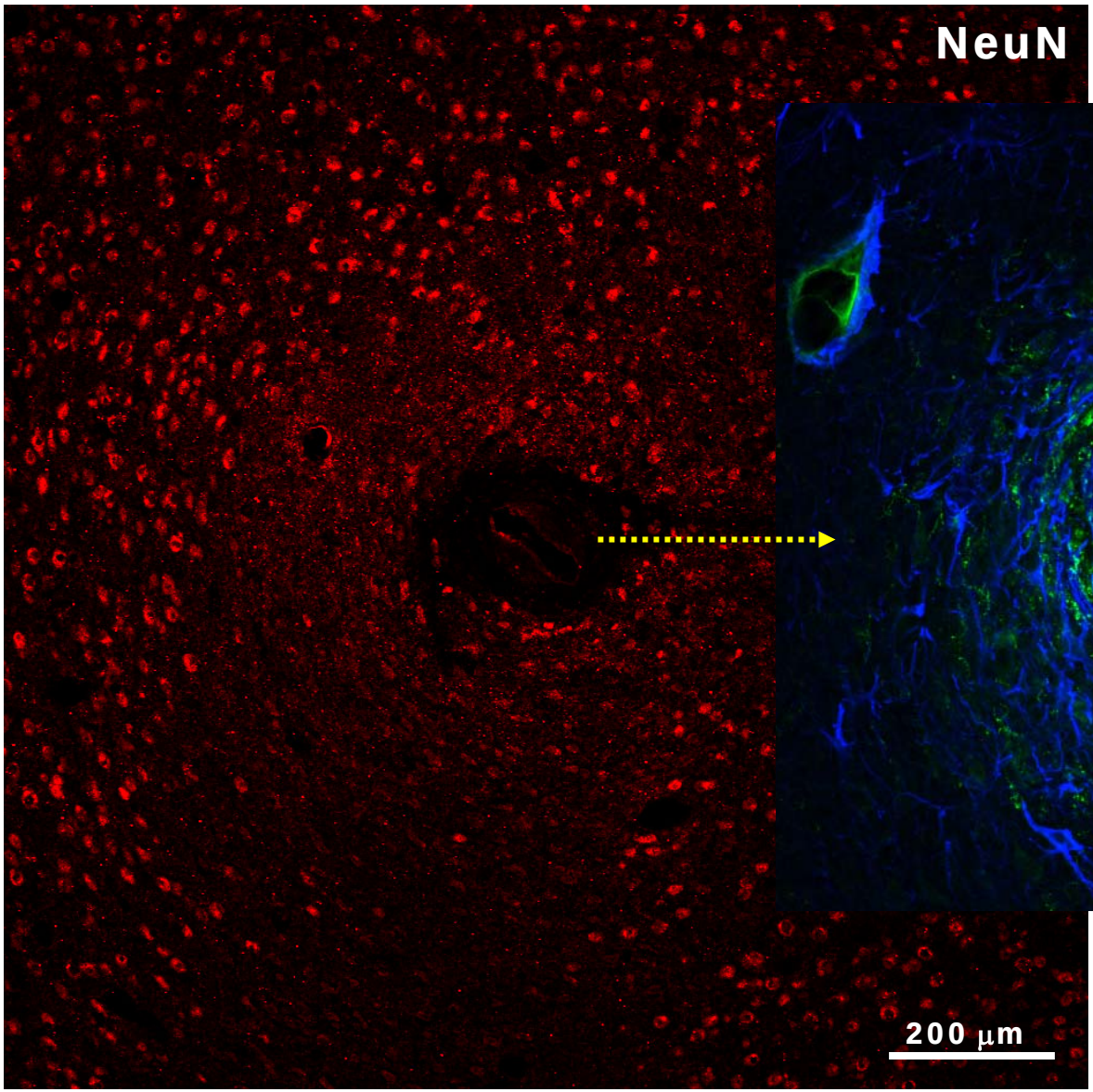

ED1 reaction along
length of tract
1 mm

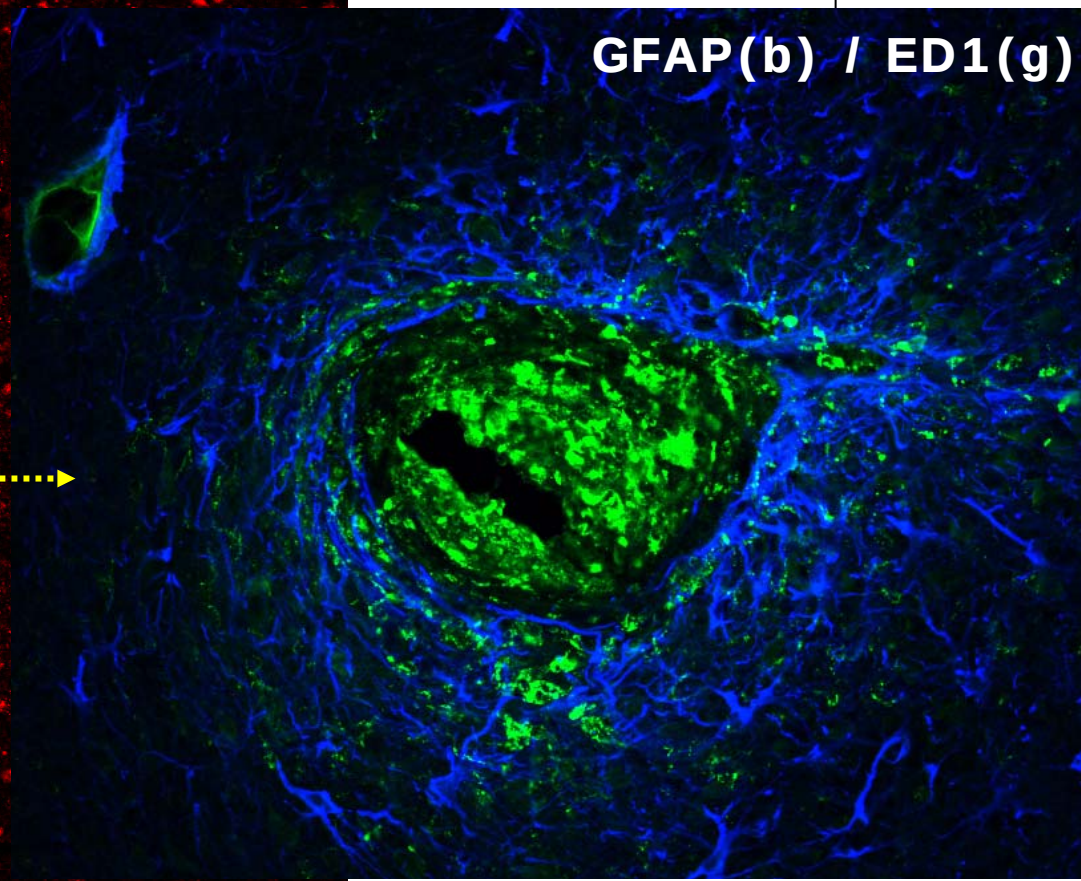

Capillary
Lymphatic


Declining Neurofilament
100 μm

Neuronal Density and Inflammation





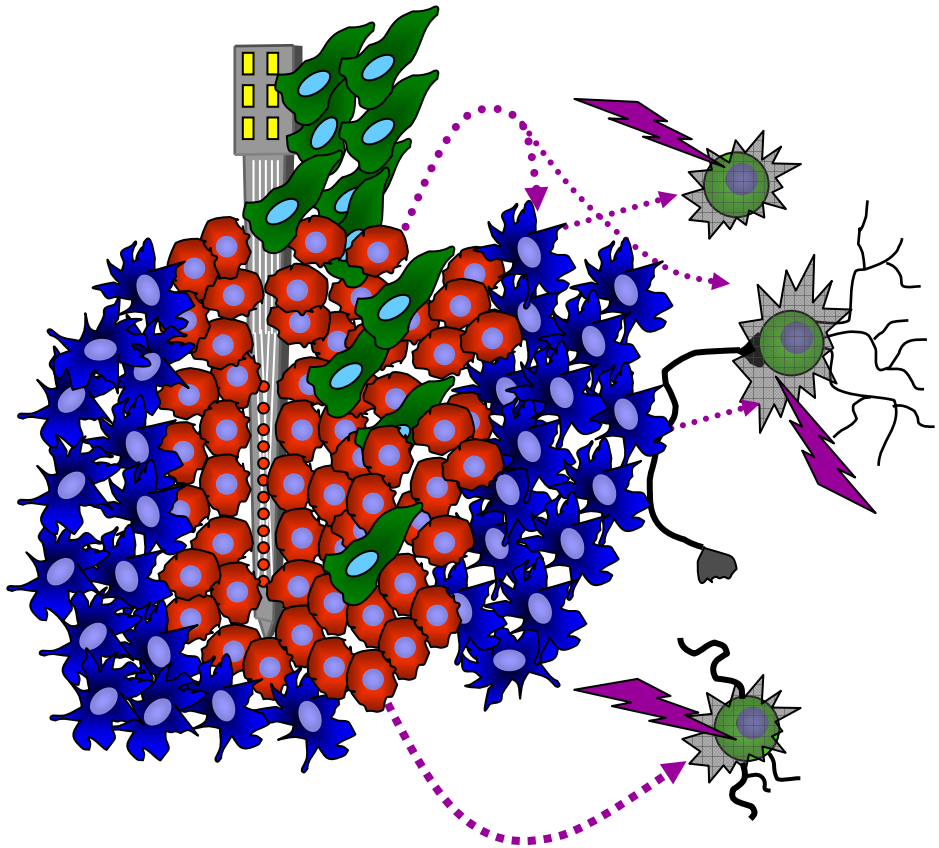


Neurotoxicity Around Implants?

Questions:

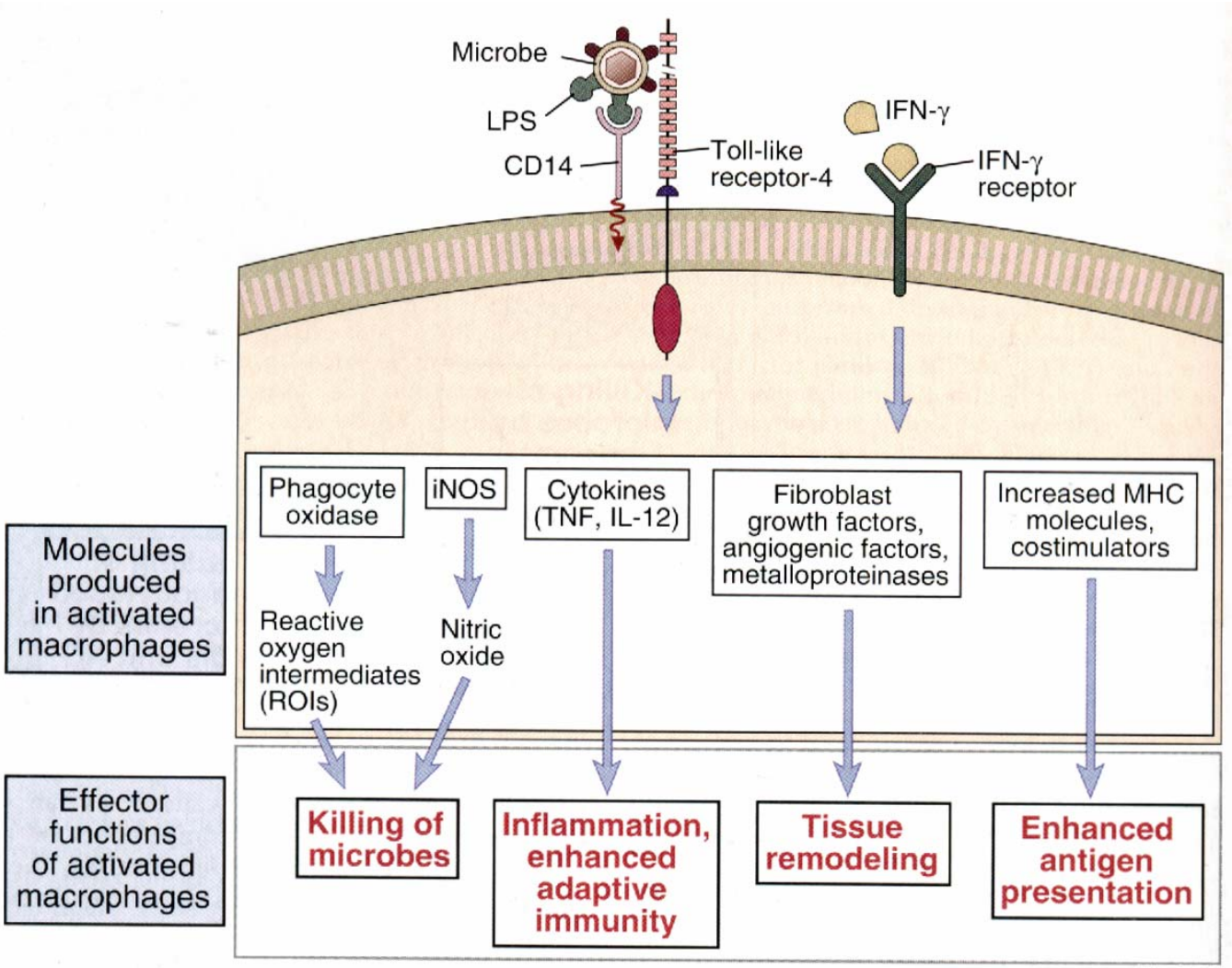
1. Are microglia at the interface of a neural implant chronically activated?

2. Are they neurotoxic?



directed and indirect neuronal cytotoxicity mediated by microglia

Major Activities of Leukocyte Secreted Factors



Inflammation at Biomaterial Interfaces

TISSUE	DEVICE	MATERIALS	PHENOMENA
BONE	articulating prostheses	polyethylene, titanium	osteolysis, loosening of implant
BLOOD	hemodialyis	cellulose acetate and others	complement deposition, neutropenia,
SUBCUTANEOUS	breast implants	silicone	fibrosis, calcification, contraction / extrusion
BRAIN	electrodes	silicon, various metals	encapsulation, loss of chronic recording

Important Contributing Factors: Plasma Protein Adsorption

TABLE 1 Properties of the "Big 12" Plasma Proteins W

Protein	Plasma concentration g/l–mg/ml	Plasma concentration μmol	Molecular weight (kDa)
Albumin	40	600	66
IgG	8–17	53–113	150
		100*	
LDL	4.0	2	2,000
HDL	3	18	170
α-Macroglobulin	2.7	3.3	725
Fibrinogen	2–3	6–9	340
		7.5	
Transferrin	2.3	30	77
α-Antitrypsin	2	40	(51)
			54
			(45)
Haptoglobins	2.0	20	100
	1.6–3.0	8–1.5	200
	1.2–2.6	3–6.6	400
C3	1.6	9	180
IgA	1–4	7–27	150
		15*	
IgM	0.05–2	0.06–2	900
		1*	

Note: Numbers used for calculation of $CD^{1/2}$ are indicated b

Adapted from Amrade, J. D. and Hlady, V., *Ann. N.Y. Ac*

Greco, Implantation Biology, 1994

The Journal of
THORACIC AND
CARDIOVASCULAR SURGERY

ARTICLE

Complement activation and neutropenia occurring during cardiopulmonary bypass

DE Hammerschmidt, DF Stroncek, TK Bowers, CJ Lammi-Keefe, DM Kurth, A Ozalins, DM Nicoloff, RC Lillehei, PR Craddock and HS Jacob

J Lab Clin Med. 1996
May;127(5):456-69.

Related Articles, Links

Infusion of ovine C5a into sheep mimics the inflammatory response of hemodialysis.

Johnson RJ, Burhop KE, Van Epps DE.

Baxter Healthcare Corporation, Round Lake, IL USA.

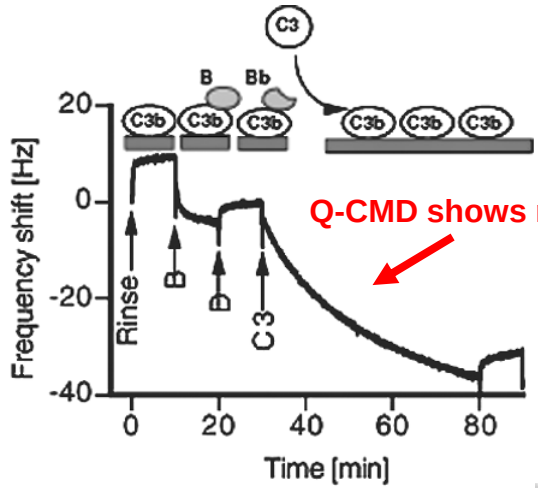
Important Contributing Factors: COMPLEMENT

C3 Adsorbed to a Polymer Surface Can Form an Initiating Alternative Pathway Convertase[1]

Jonas Andersson,* Kristina Nilsson Ekdahl,*† Rolf Larsson,* Ulf R. Nilsson,* and Bo Nilsson[2]*

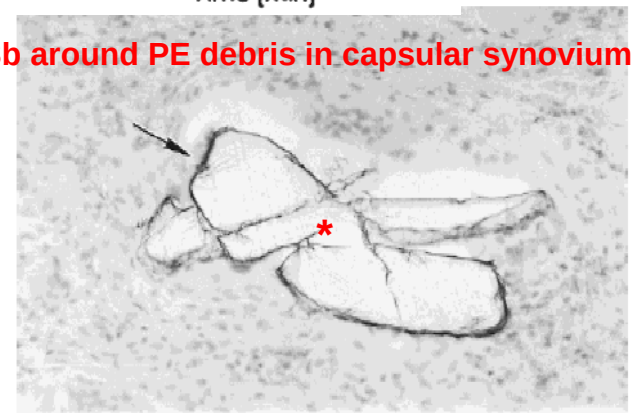
In situ complement activation by polyethylene wear debris

David H. DeHeer, [1,2] James A. Engels ,[1] Aaron S. DeVries, [2]Robert H. Knapp,[3] John D. Beebe [2]
[1]Grand Rapids Orthopaedic Surgery Residency Program, 1840 Wealthy Street SE, Grand Rapids, Michigan 49506
[2]Department of Biology, Calvin College, 3201 Burton Street SE, Grand Rapids, Michigan 49546
[3]Department of Pathology, Spectrum Health East Campus, 1840 Wealthy Street, SE, Grand Rapids, Michigan 49506



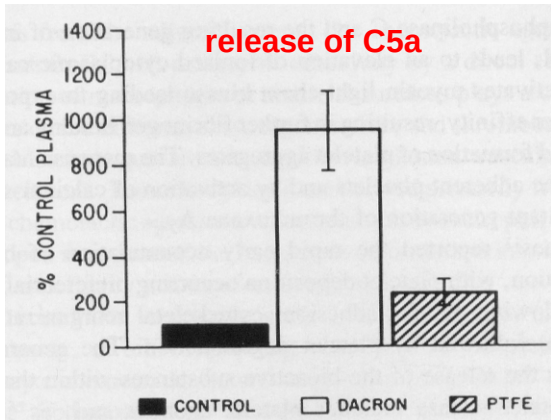
Q-CMD shows mass increase

iC3b around PE debris in capsular synovium



- Complement activation by alternative pathway
- Appears independent of “tick-over” pathway
- non-specficic C3 adsorption alone can trigger activation by factor B to generate a functional C3 convertase
- Adsorbed C3 is resistant to factor H and I
- Conformational change upon adsorption is likely cause
- C5a release also detected, potential to initiate leukocyte chemotaxis

release of C5a



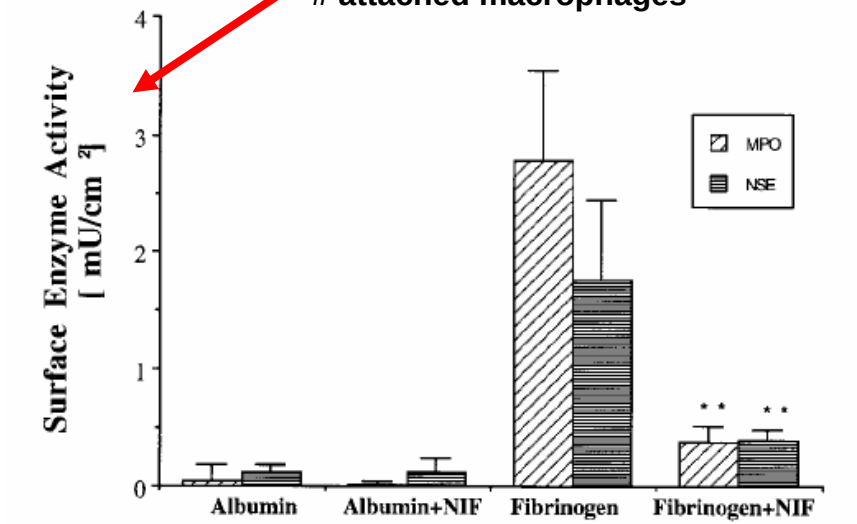
Greco, Implantation Biology, 1994

Important Contributing Factors: FIBRINOGEN

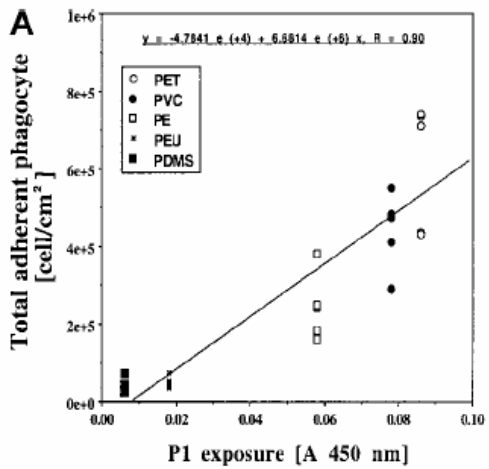
- fibrinogen deposition on biomaterial surfaces occurs rapidly
- conformational changes upon adsorption reveal adhesive domains (mimics thrombin mediated conversion to fibrin)
    - extent varies with material identity
- mediates macrophage attachment and increased cytokine production
- in this model (PET disc), macrophage attachment was normal in SCID mice (no IgG) and complement depleted mice (cobra venom factor)
- severe hypofibrinogenemic mice do not mount inflammatory response to PET unless fibrinogen is pre-adsorbed
- Hence, fibrinogen adsorption may be more influential in macrophage attachment than complement or antibodies

peritoneal PET disc model

 # attached macrophages



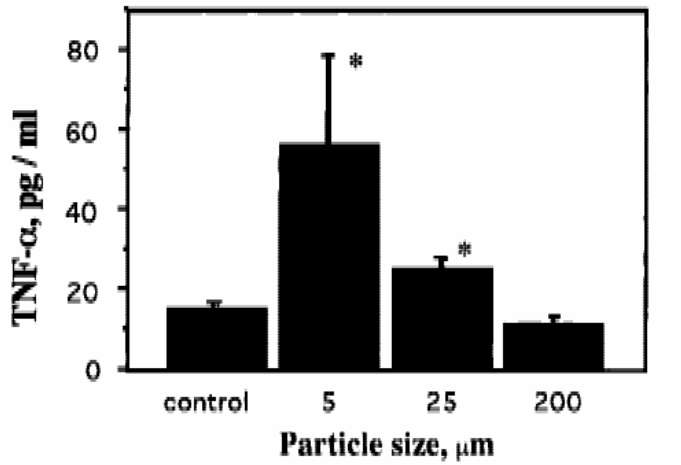
Mac-1 inhibitor

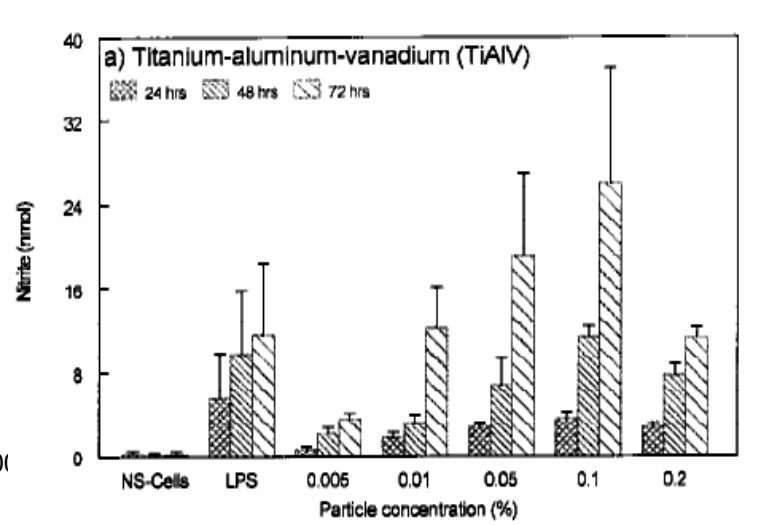


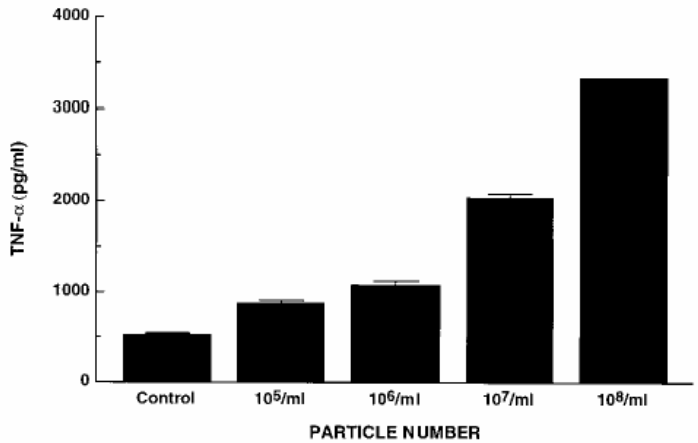
Macrophage attachment increases with amount of adhesive epitope exposure in fibrinogen

Important Contributing Factors: PARTICULATE SIZE AND CONCENTRATION

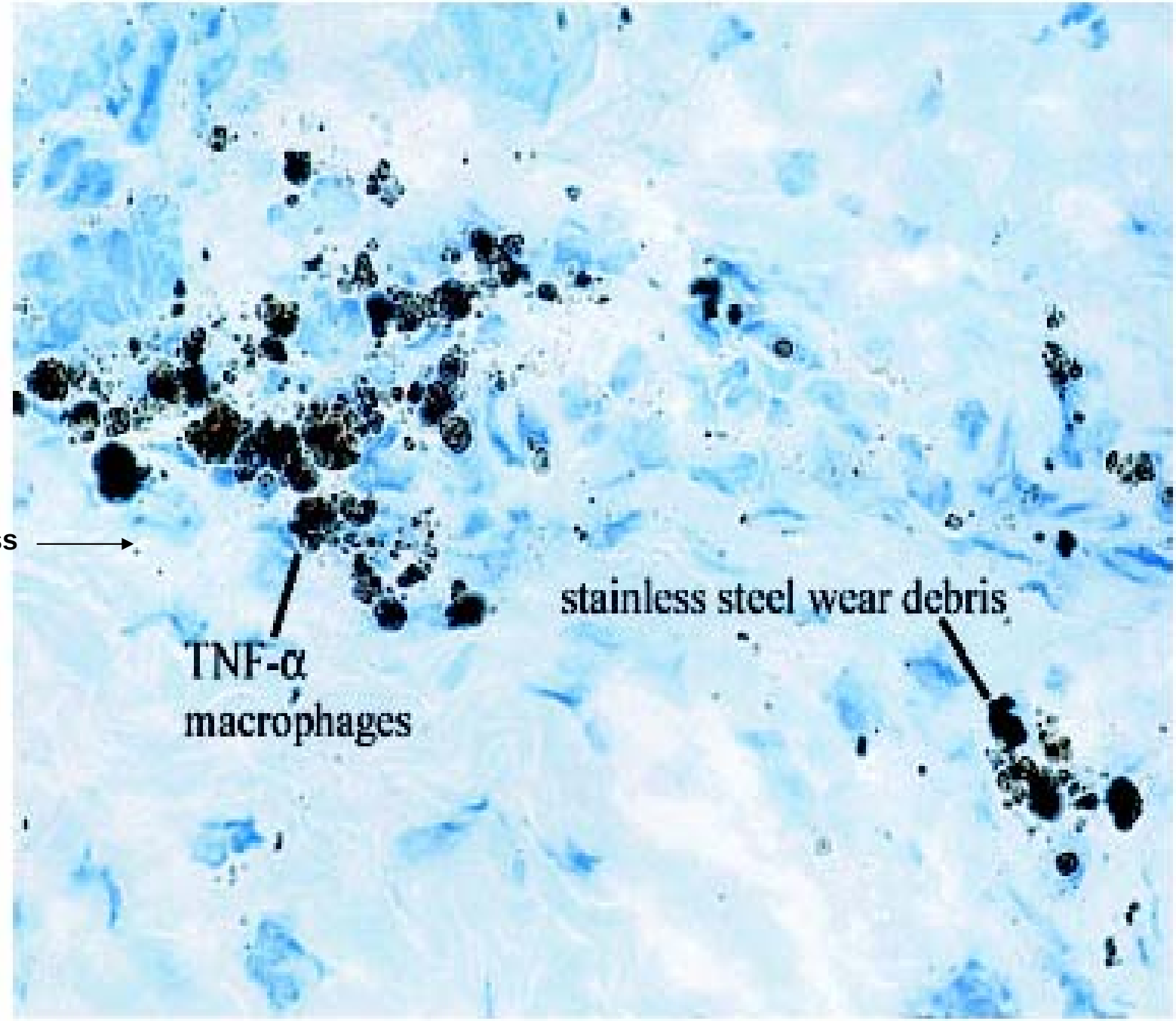
- Problems in joint prostheses
  - periprosthetic osteolysis
  - chronic inflammation
  - release of wear particles
  - loosening of implant
  - 30,000 revision surgeries/year in U.S.
- debris activates macrophages
  - TNF-alpha release recruits osteoclasts
  - Stimulates NO production -> PGE release
  - Osteoclasts degrade bone







Macrophages consume wear debris and express TNF-alpha surrounding a spinal implant



Important Contributing Factors: Motion

- **prostaglandins are products of cycloxegenase pathway**
- **induced by TNF-alpha**
- **potent pro-inflammatory mediator**
- **this is still a subject of debate – no definitive in vivo data**

Effect of mechanical perturbation on the release of PGE_2 by macrophages *in vitro*

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TABLE I
PGE_2 Release in Response to Mechanical Perturbation (4% Strain) of Nonactivated Macrophages

	Prostaglandin E_2 (pg/mL) Mean ± S.E.M.	
Sample	24-h Pre-incubation	24-h Post stretch
Control (n = 3)	119 ± 21	420 ± 122
Stretched (n = 3)	118 ± 26	748 ± 53

Elastic membrane, 4% stretch, 1 Hz strain, 1 hour

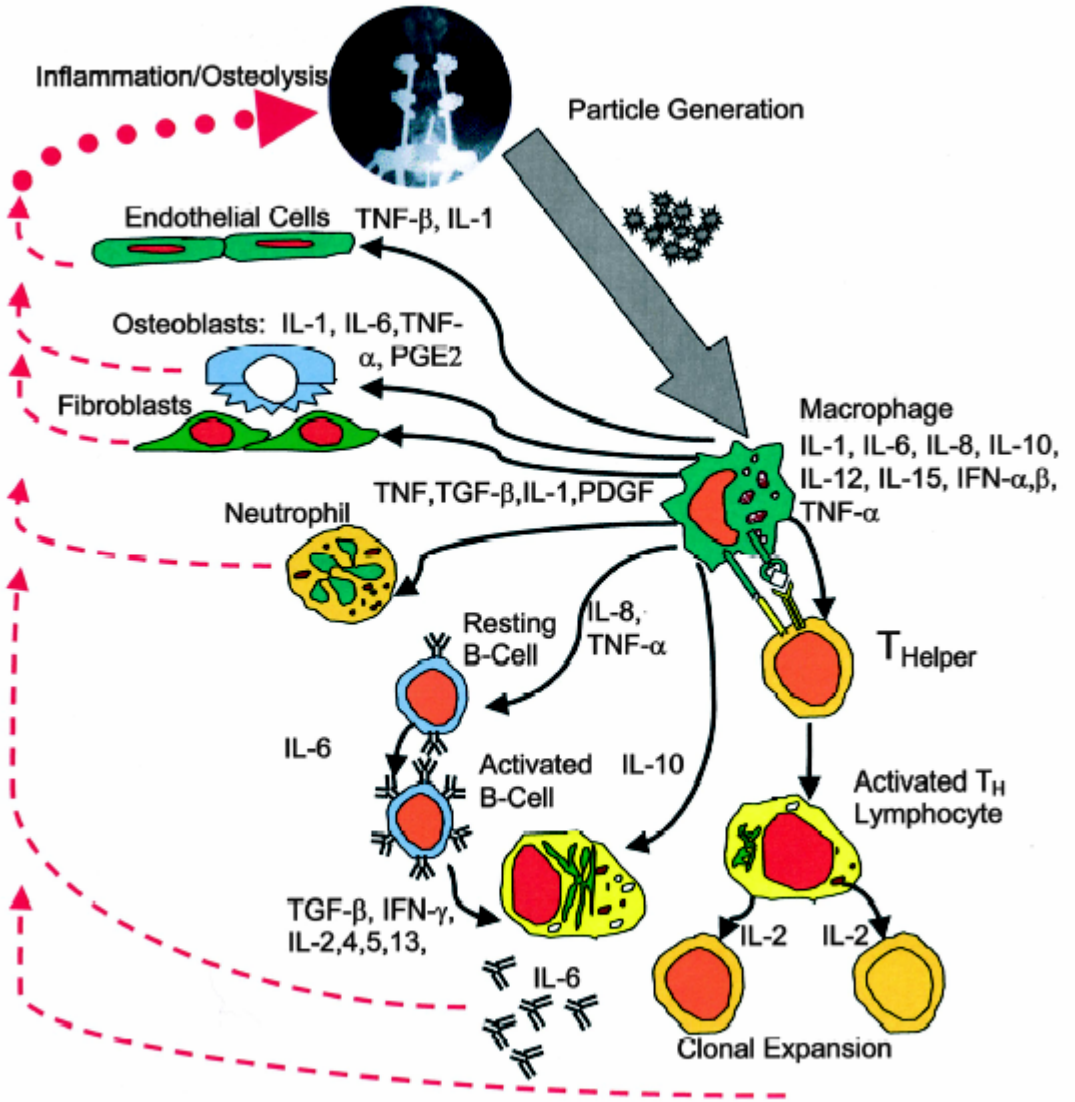

Figure 1. A schematic representation of the cycle of inflammation around implants from particle-induced osteolysis illustrates macrophage exhaustion, reactive oxygen intermediates, and proinflammatory cytokines recruit a host of local cell types and induce a widening zone of soft tissue damage and inflammation.[46]

Complement Regulation

- Complement regulatory proteins – soluble and membrane bound
- Importance of rapid regulation of complement – soluble inhibitors abundant in serum
- Cleaved products are normally only reactive for brief periods – ensures limited diffusion and local concentration
    - ex. C3b thioester reactivity is very short-lived

Table 14–7. Regulators of Complement Activation

Receptor	Structure	Distribution	Interacts with	Function
C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 µg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
Factor I	88-kD dimer of 50- and 38-kD subunits	Plasma protein; conc. 35 µg/mL	C4b, C3b	Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors
Factor H	150 kD; multiple CCPRs	Plasma protein; conc. 480 µg/mL	C3b	Binds C3b and displaces Bb Cofactor for factor I–mediated cleavage of C3b
C4-binding protein (C4BP)	570 kD; multiple CCPRs	Plasma protein; conc. 300 µg/mL	C4b	Binds C4b and displaces C2 Cofactor for factor I–mediated cleavage of C4b
Membrane cofactor for protein (MCP, CD46)	45-70 kD; four CCPRs	Leukocytes, epithelial cells, endothelial cells	C3b,C4b	Cofactor for factor I–mediated cleavage of C3b and C4b
Decay-accelerating factor (DAF)	70 kD; GPI linked, four CCPRs	Blood cells, endothelial cells, epithelial cells	C4b2b, C3bBb	Displaces C2b from C4b and Bb from C3b (dissociation of C3 convertases)
CD59	18 kD; GPI linked	Blood cells, endothelial cells, epithelial cells	C7, C8	Blocks C9 binding and prevents formation of the MAC

Abbreviations: CCPR, complement control protein repeat; conc., concentration; GPI, glycophosphatidylinositol; MAC, membrane attack complex.

The Wound Healing Continuum

- Initiation by mechanical injury/damage to vasculature
- Blood coagulation-clot formation
- Platelet activation and degranulation
- Inflammation-edema
- Removal of damaged matrix and necrotic cell components
- Cell proliferation and recruitment including endothelial, epithelial, stromal and inflammatory cells
- Continued removal of matrix
- Angiogenesis
- Matrix synthesis and deposition
- Epithelialization and wound contraction
- Decrease in cellularity-apoptotic pathway
- Tissue remodeling-elastin synthesis