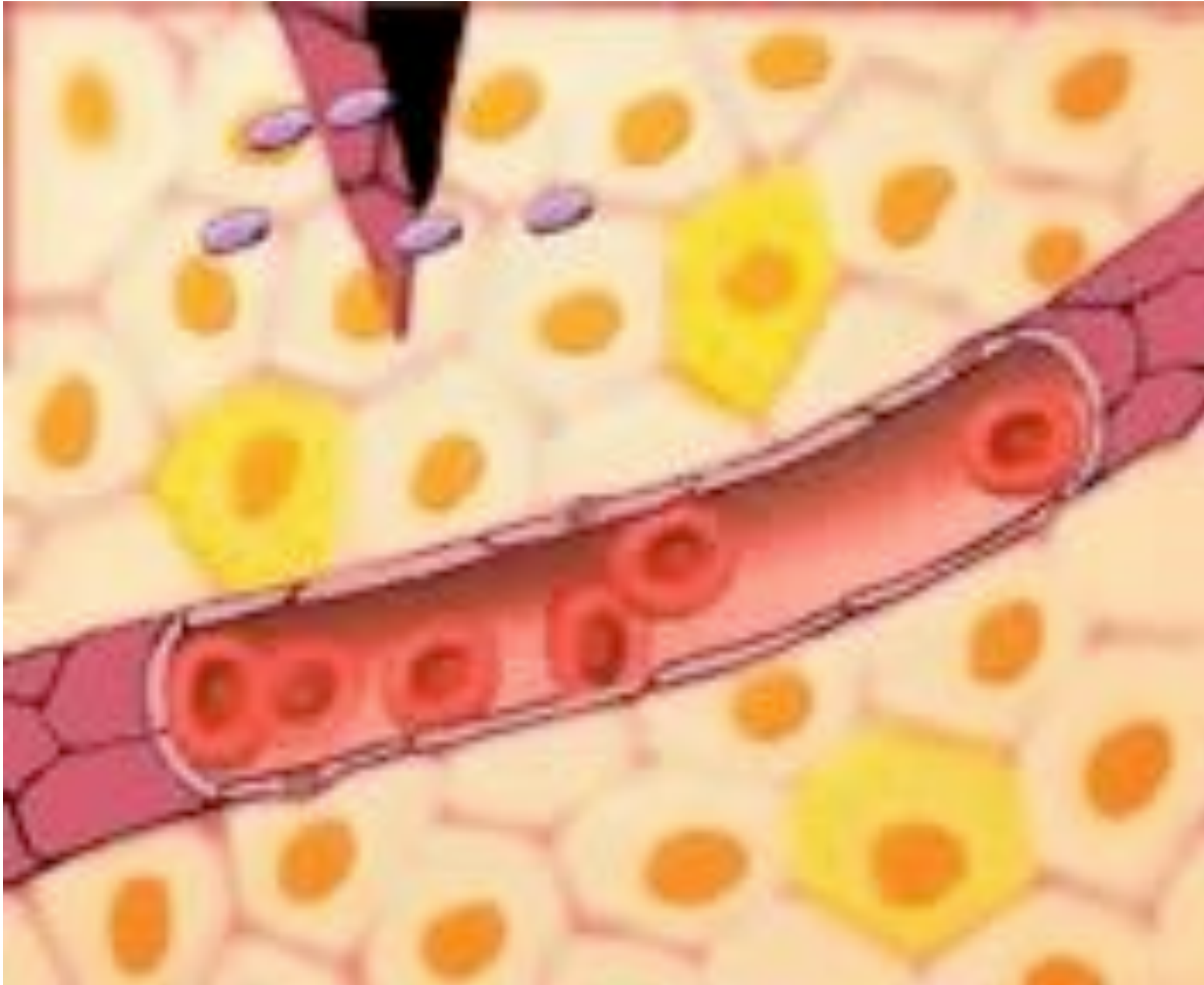
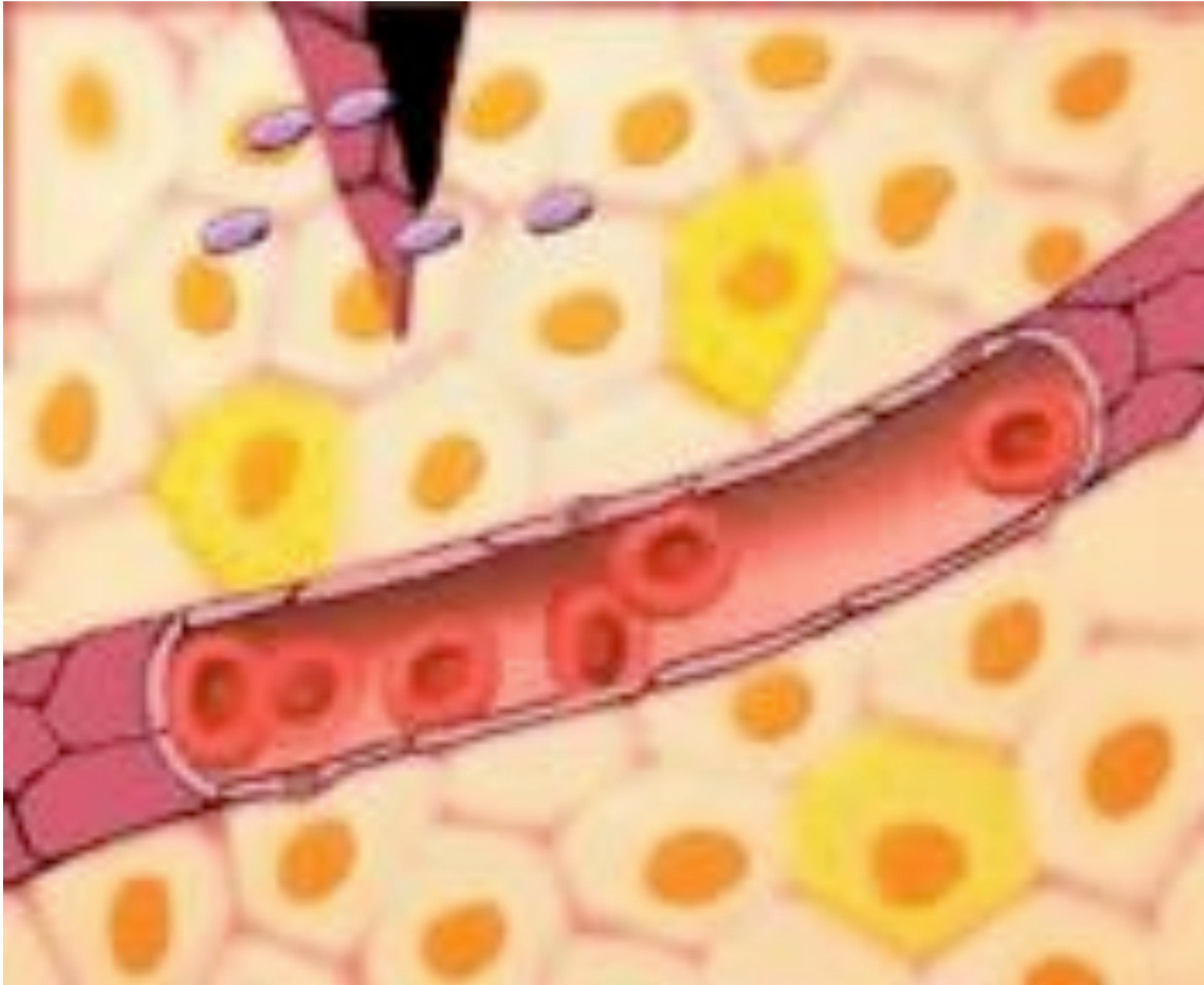


THE INNATE IMMUNITY AND INFLAMMATION RESPONSE!



THE INNATE IMMUNITY AND INFLAMMATION RESPONSE!



THE INNATE IMMUNITY AND INFLAMMATION RESPONSE BIOLOGY: THE ACUTE PHASE PROTEINS (APP).

Prof. Fabrizio Mainiero

**Full Professor of General Pathology and Physiopathology and Immunology
and Immunopathology**

**Department of Experimental Medicine
Università degli Studi "La Sapienza"
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00161 Roma**

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All the data here presented are for student use only

The more potent inducers of the inflammatory and immune response are microbial, such as viruses and bacteria, which are the major extracellular DAMPs or Danger-Associated Molecular Patterns and contain PAMPS or Pathogen Associated Molecular Patterns.....

Viruses infecting a cell,
multiplication and release

Streptococcus pneumoniae

Growth of pathogenic
bacteria shown in time-lapse

Speed = x 540

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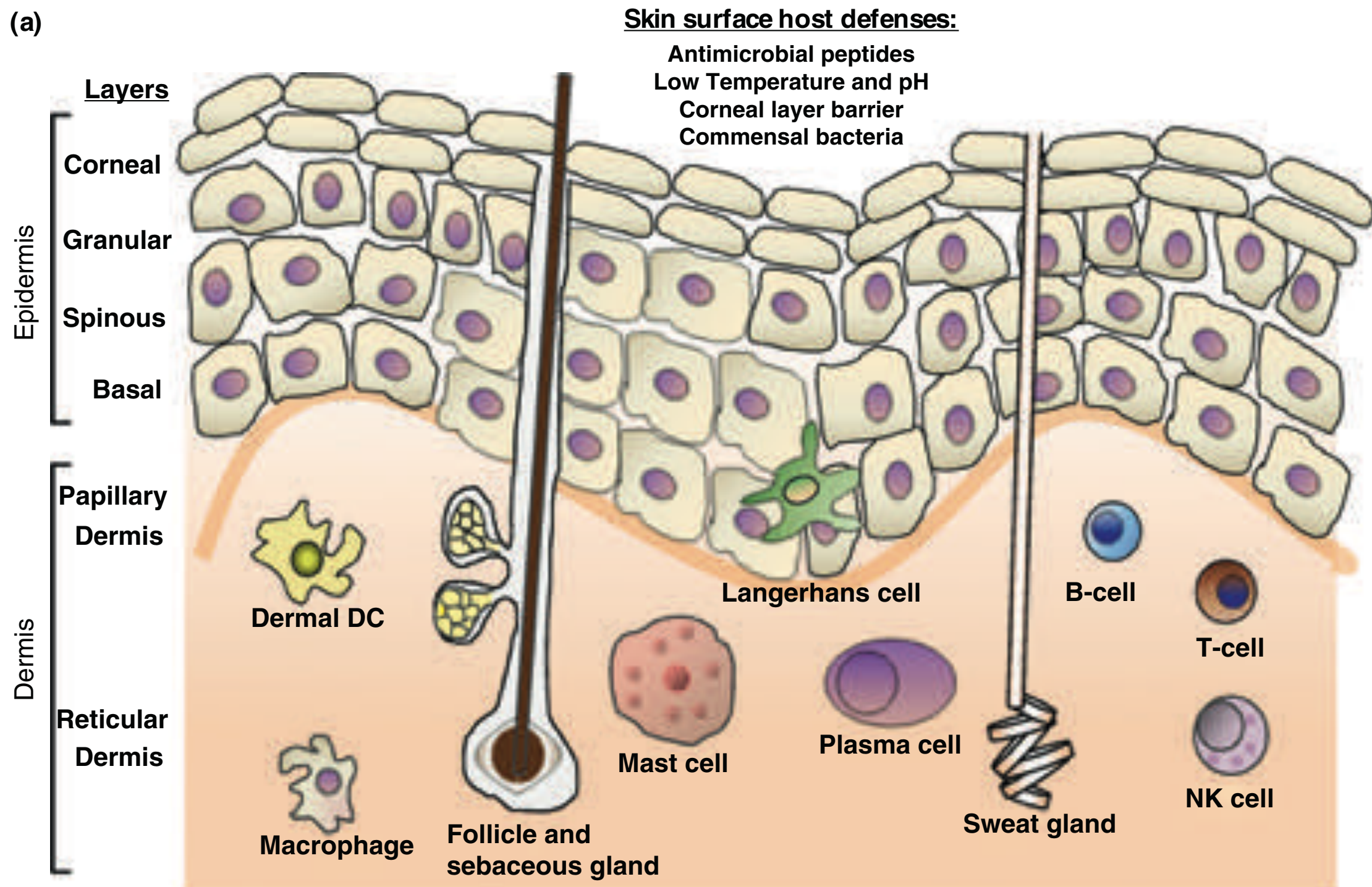
Streptococcus pneumoniae

Growth of pathogenic
bacteria shown in time-lapse




Speed = x 540

...upon the escape from the potent immunologic tissue barriers!

(a)



VIRUSES AND BACTERIA CAN GIVE TISSUE DAMAGE WITH THREE MAIN DIRECT MECHANISMS.....

| | Direct mechanisms of tissue damage by pathogens | | |
|----------------------|--|---|---|
| | Exotoxin production | Endotoxin | Direct cytopathic effect |
| Pathogenic mechanism |  |  |  |
| Infectious agent | <i>Streptococcus pyogenes</i> <i>Staphylococcus aureus</i> <i>Corynebacterium diphtheriae</i> <i>Clostridium tetani</i> <i>Vibrio cholerae</i> | <i>Escherichia coli</i> <i>Haemophilus influenzae</i> <i>Salmonella typhi</i> <i>Shigella</i> <i>Pseudomonas aeruginosa</i> <i>Yersinia pestis</i> | Varicella Varicella-zoster Hepatitis B virus Polio virus Measles virus Influenza virus Herpes simplex virus Human herpes virus 8 (HHV8) |
| Disease | Tonsillitis, scarlet fever Boils, toxic shock syndrome, food poisoning Diphtheria Tetanus Cholera | Gram-negative sepsis Meningitis, pneumonia Typhoid Bacillary dysentery Wound infection Plague | Smallpox Chickenpox, shingles Hepatitis Poliomyelitis Measles, subacute sclerosing panencephalitis Influenza Cold sores Kaposi's sarcoma |

... THEY CAN ENTER OUR CELLS, USING NOT ONLY SPECIFIC RECEPTORS

Table 1. Pathogenic microbes and their membrane receptor targets

| | Species | Virulence factor | Cell receptor ^a | Ref. |
|---------------------|--------------------------------------|------------------------------------|---|------|
| Bacteria | <i>E. coli</i> | Heat-labile enterotoxin, endotoxin | Ganglioside | [66] |
| | <i>V. cholera</i> | Cholera toxin | Ganglioside | [66] |
| | <i>Streptococcus, Staphylococcus</i> | Lipoteichoic acid, hemolysin | Phospholipid | [67] |
| Enveloped virus | Influenza | Hemagglutinin, neuraminidase | Ganglioside | [68] |
| | HIV | GP120 protein | Galactosyl ceramide | [69] |
| | Paramyxovirus | Attachment protein G | EphrinB2 protein | [70] |
| Non-enveloped virus | Polyomavirus, rhinovirus | Capsid coat protein | Ganglioside, ceramide, ICAM-1 and LDLR proteins | [71] |
| | Adenovirus | Capsid protein knob domain | CAR and LDLR proteins | [72] |

^aAbbreviations: ICAM-1, intercellular adhesion molecule 1; CAR, coxsackie virus and adenovirus receptor; LDLR, low-density lipoprotein receptor.

Inhibiting host–pathogen interactions using membrane-based nanostructures

Daniel A. Bricarello^{1,4}, Mira A. Patel² and Atul N. Parikh^{2,3,4,5}

¹Food Science and Technology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
²Chemical Engineering and Materials Science, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
³Biomedical Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
⁴Foods for Health Institute, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
⁵Molecular Physics, Department of Applied Physics, Linköping University, Linköping, S 581 83, Sweden

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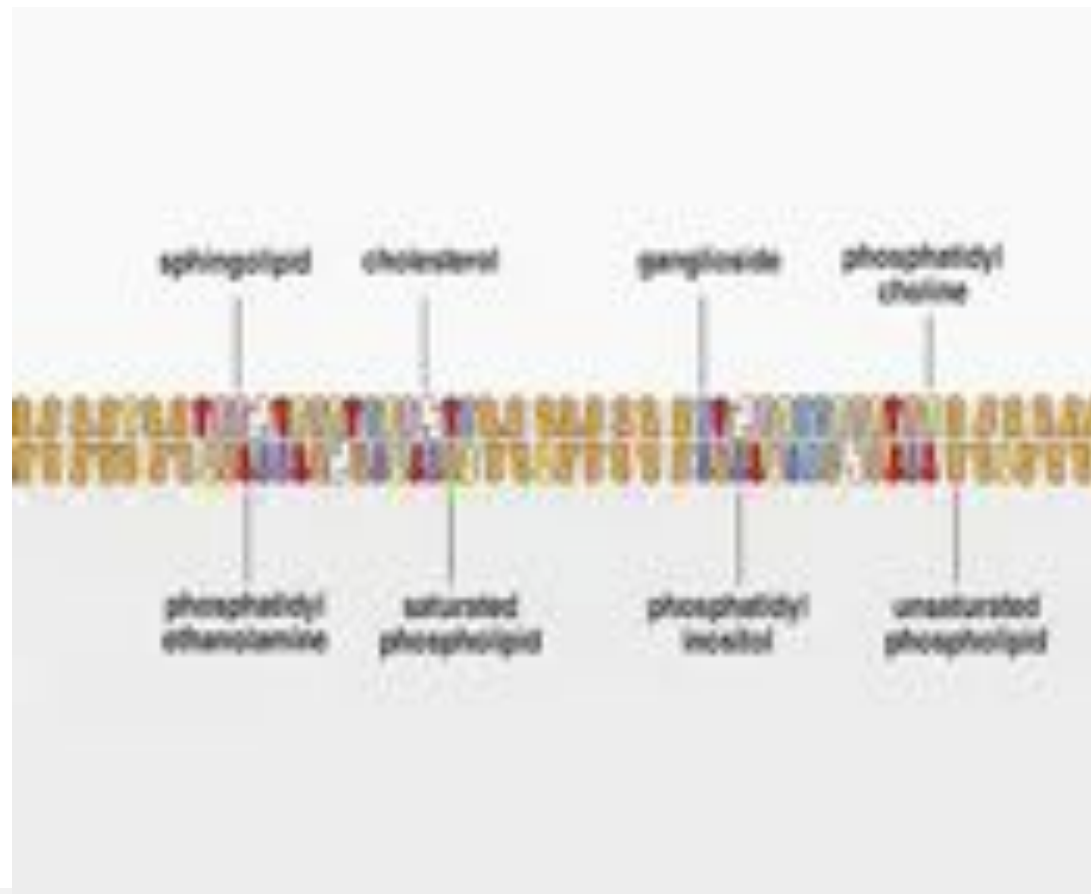
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....BUT ALSO RAFTS...

PATHOGENS: RAFT HIJACKERS!

Santos Mañes, Gustavo del Real & Carlos Martínez-A

Nature Reviews Immunology 3, 557-568 (2003)



I rafts lipidici sono delle strutture di membrana eterogenee, insolubili in detergenti non ionici come il Triton X-100 ed arricchite in colesterolo, glicosfingolipidi come GM1 o GM3 e proteine come le caveoline e le flotilline

Viruses

| | |
|-------------------------------------|----------------------------|
| Simian virus 40 | Entry/trafficking |
| Echovirus 11 | Entry/trafficking |
| Echovirus 1 | Entry |
| Avian sarcoma and leukemia virus | Entry |
| Semliki-forest virus | Entry/budding |
| Ectropic mouse leukaemia virus | Entry/budding |
| Human T-cell leukaemia virus type 1 | Entry/budding |
| HIV-1 | Entry/budding/transcytosis |
| Ebola and Marburg viruses | Entry/budding |
| Measles virus | Budding |
| Herpes simplex virus | Budding |
| Influenza virus | Budding |
| Epstein-Barr virus | Signalling |

Bacteria

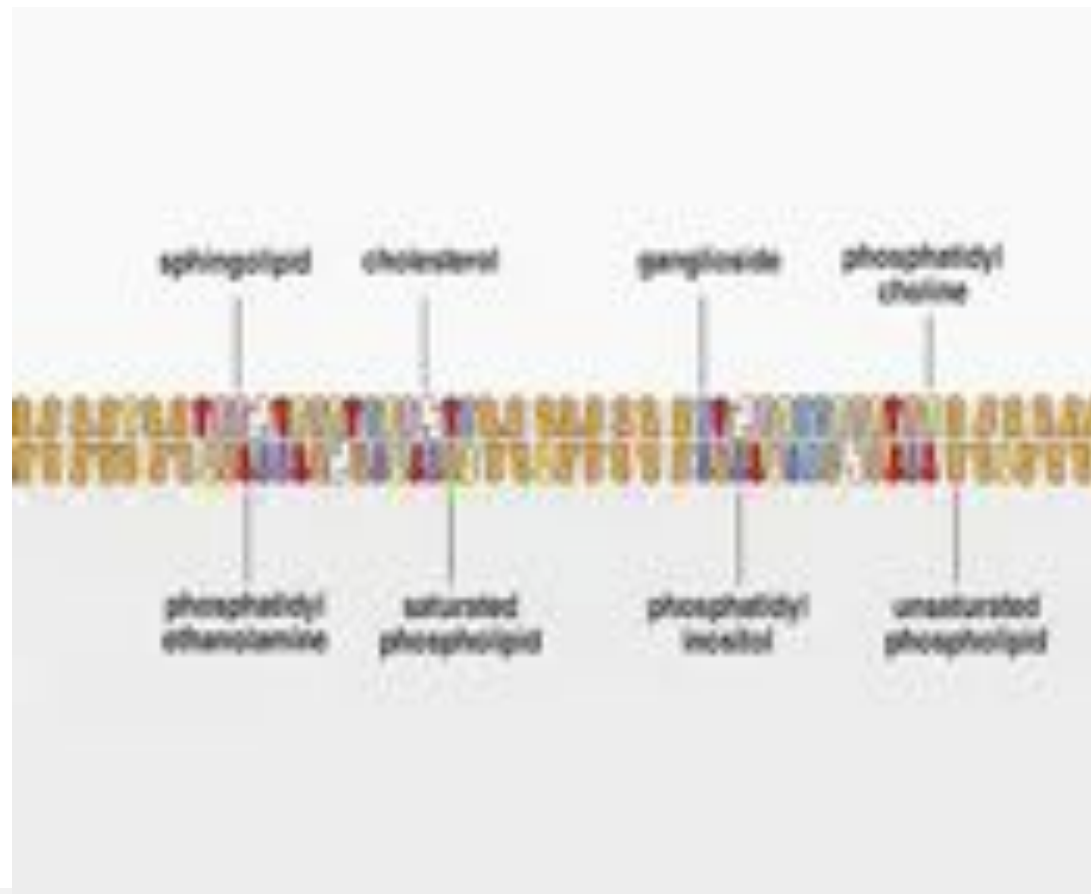
| | |
|--|---------------------------------|
| <i>Campylobacter jejuni</i> | Intracellular survival |
| <i>Legionella pneumophila</i> | Intracellular survival |
| <i>Pseudomonas aeruginosa</i> | Host response, signaling |
| <i>Brucella</i> spp. | Entry/intracellular survival |
| FimH and Dr* <i>Escherichia coli</i> | Entry/intracellular survival |
| <i>Salmonella typhimurium</i> | Entry/intracellular survival |
| <i>Shigella flexneri</i> | Entry/intracellular survival |
| <i>Chlamydia</i> spp. | Entry/intracellular survival |
| <i>Mycobacterium</i> spp. | Entry/intracellular survival |
| <i>Vibrio cholerae</i> (cytotoxin) | Toxin binding/oligomerization |
| <i>Aeromonas hydrophila</i> (aerolysin) | Toxin binding/oligomerization |
| <i>Clostridium</i> spp. | Toxin binding/oligomerization |
| <i>Streptococcus pyogenes</i> (streptolysin O) | Toxin oligomerization |
| <i>Bacillus anthracis</i> (anthrax toxin) | Toxin oligomerization |
| <i>Bacillus thuringiensis</i> (Cry1A toxin) | Toxin binding/oligomerization |
| <i>Helicobacter pylori</i> (vacuolating cytotoxin) | Toxin oligomerization/signaling |
| <i>Listeria monocytogenes</i> (listeriolysin O) | Toxin oligomerization/signaling |

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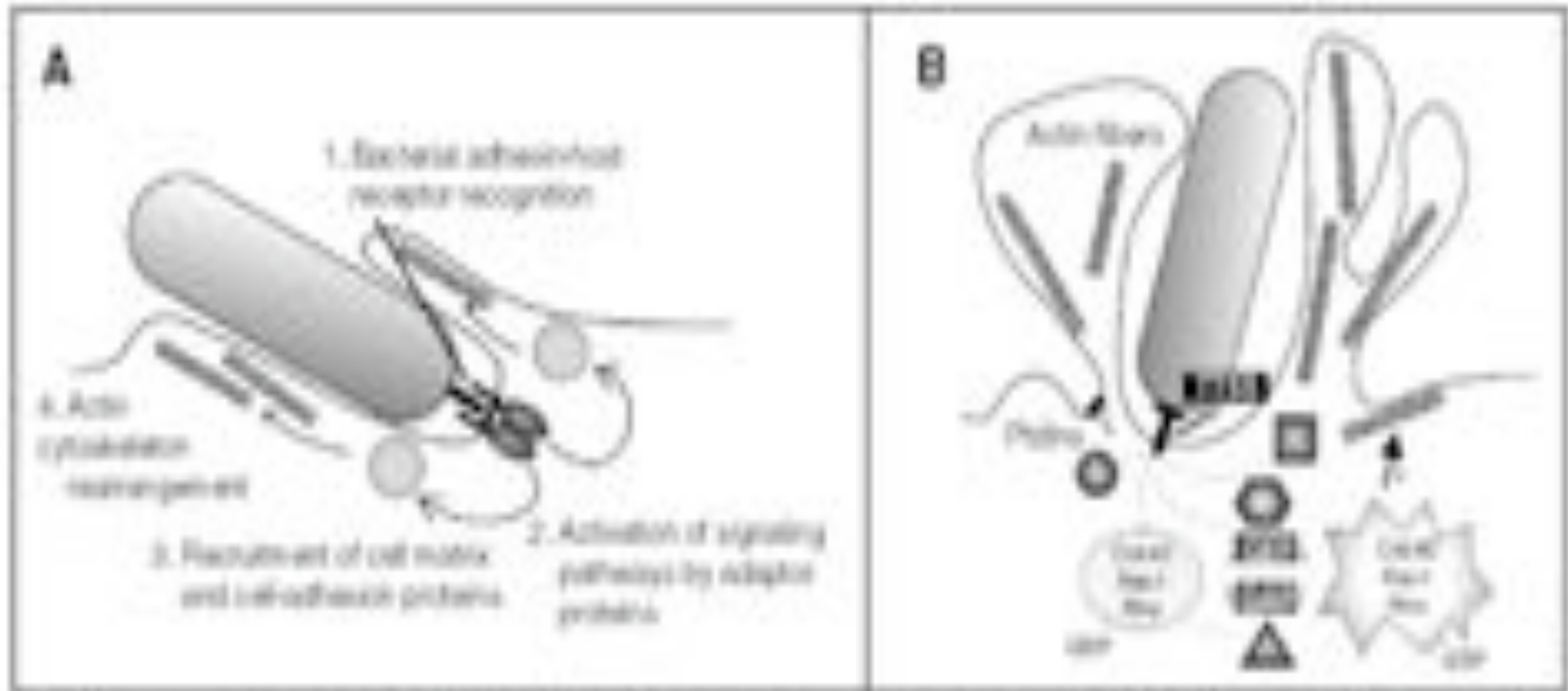
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| <i>Shigella flexneri</i> | Entry/intracellular survival |
| <i>Chlamydia</i> spp. | Entry/intracellular survival |
| <i>Mycobacterium</i> spp. | Entry/intracellular survival |
| <i>Vibrio cholerae</i> (cytolysin) | Toxin binding/oligomerization |
| <i>Aeromonas hydrophila</i> (aerolysin) | Toxin binding/oligomerization |
| <i>Clostridium</i> spp. | Toxin binding/oligomerization |
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..AND, IN THE CASE OF BACTERIA, EVEN TWO MECHANISMS!

ZIPPER MECHANISM

TRIGGER MECHANISM



The bacteria to enter the cells, move and reshape the cytoplasm and modulate the functions using proteins that mimic the functions of the structural and signaling proteins (such as small G proteins Rho, Rac and Cdc42) and their effectors (such as Wasp, Arf-6 and Arp2) that control the reorganization of the cytoskeleton.

Microbial pathogenesis and cytoskeletal function

Samantha Gruenheid and B. Brett Finlay

Nature 422, 775-781 (17 April 2003)

Bacterial Invasion: The Paradigms of Enteroinvasive Pathogens

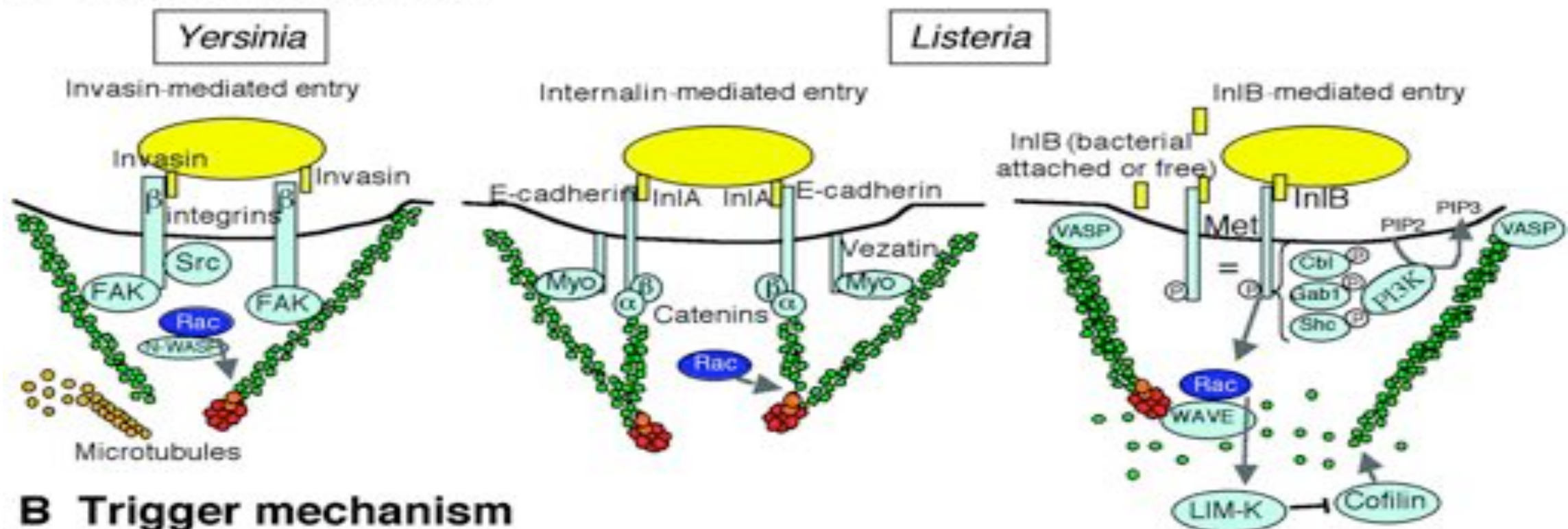
Pascale Cossart and Philippe J. Sansonetti

Mechanisms used by bacteria to enter cells.

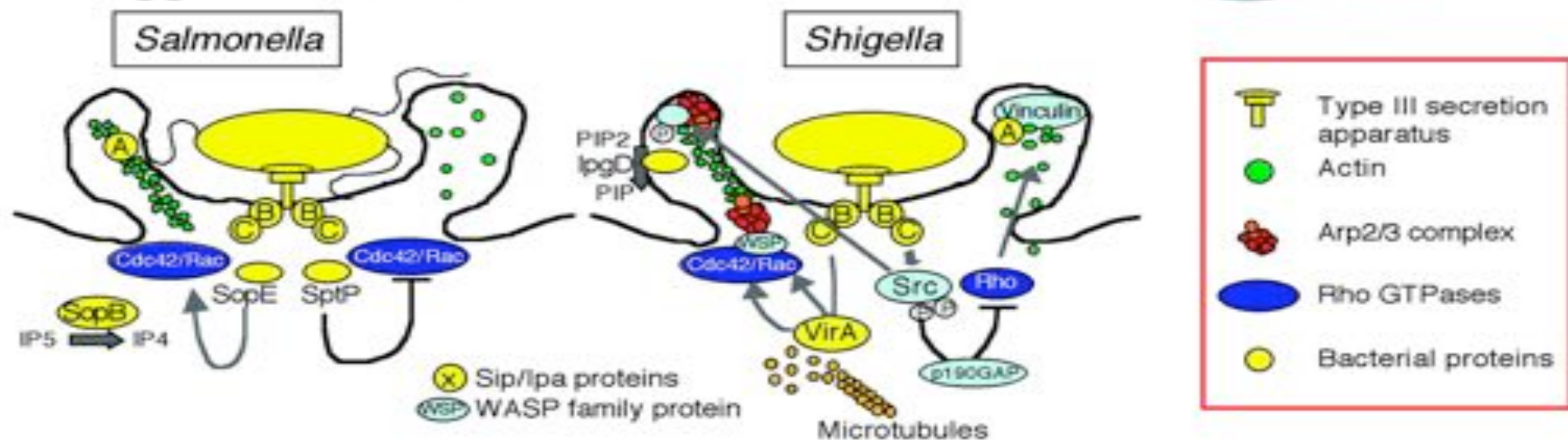
(A) The zipper mechanism used by *Yersinia* and *Listeria*.

(B) The trigger mechanism used by *Salmonella* and *Shigella*.

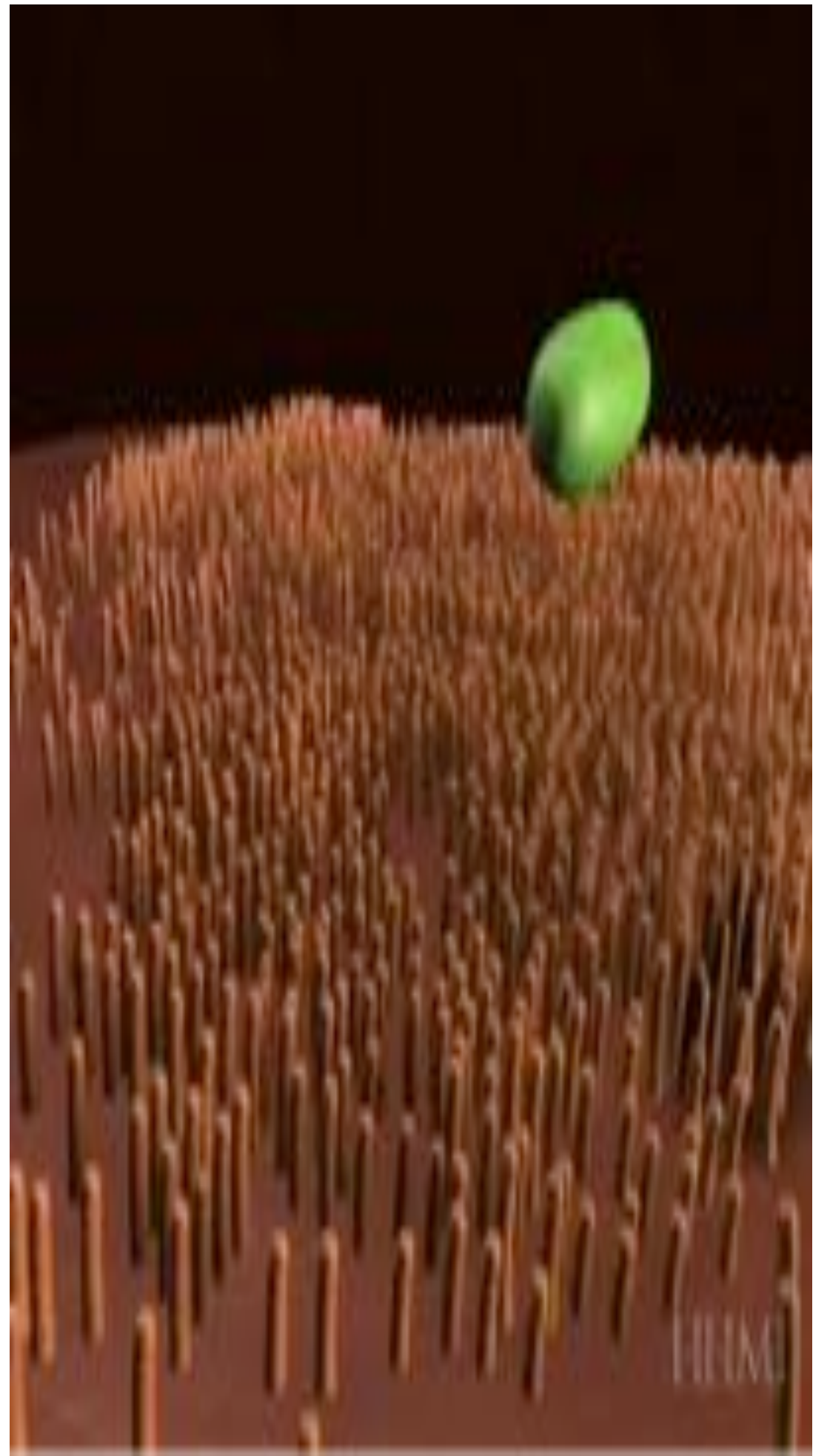
A Zipper mechanism



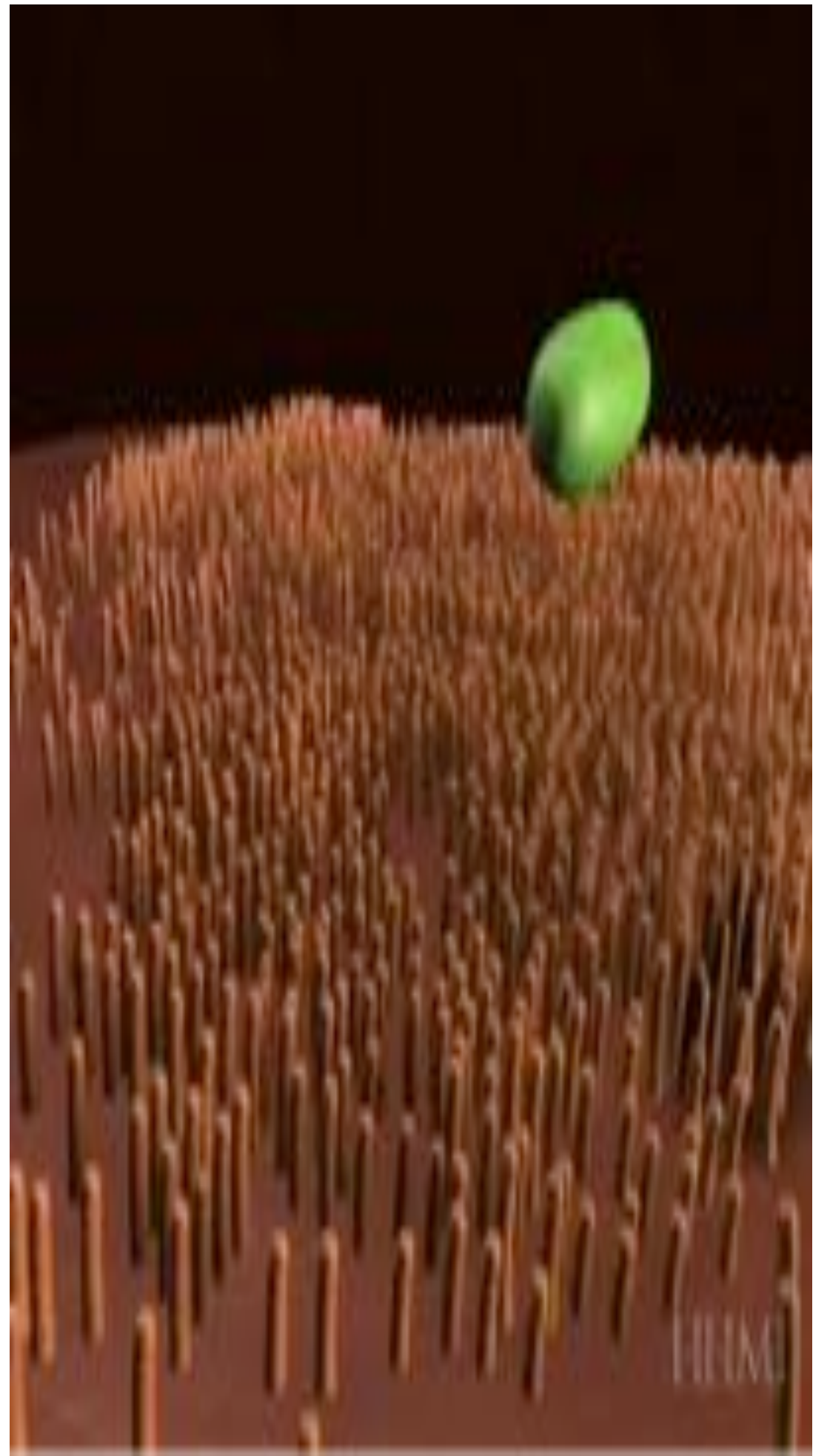
B Trigger mechanism



The invasion and cell migration of *Salmonella*!



The invasion and cell migration of *Salmonella*!

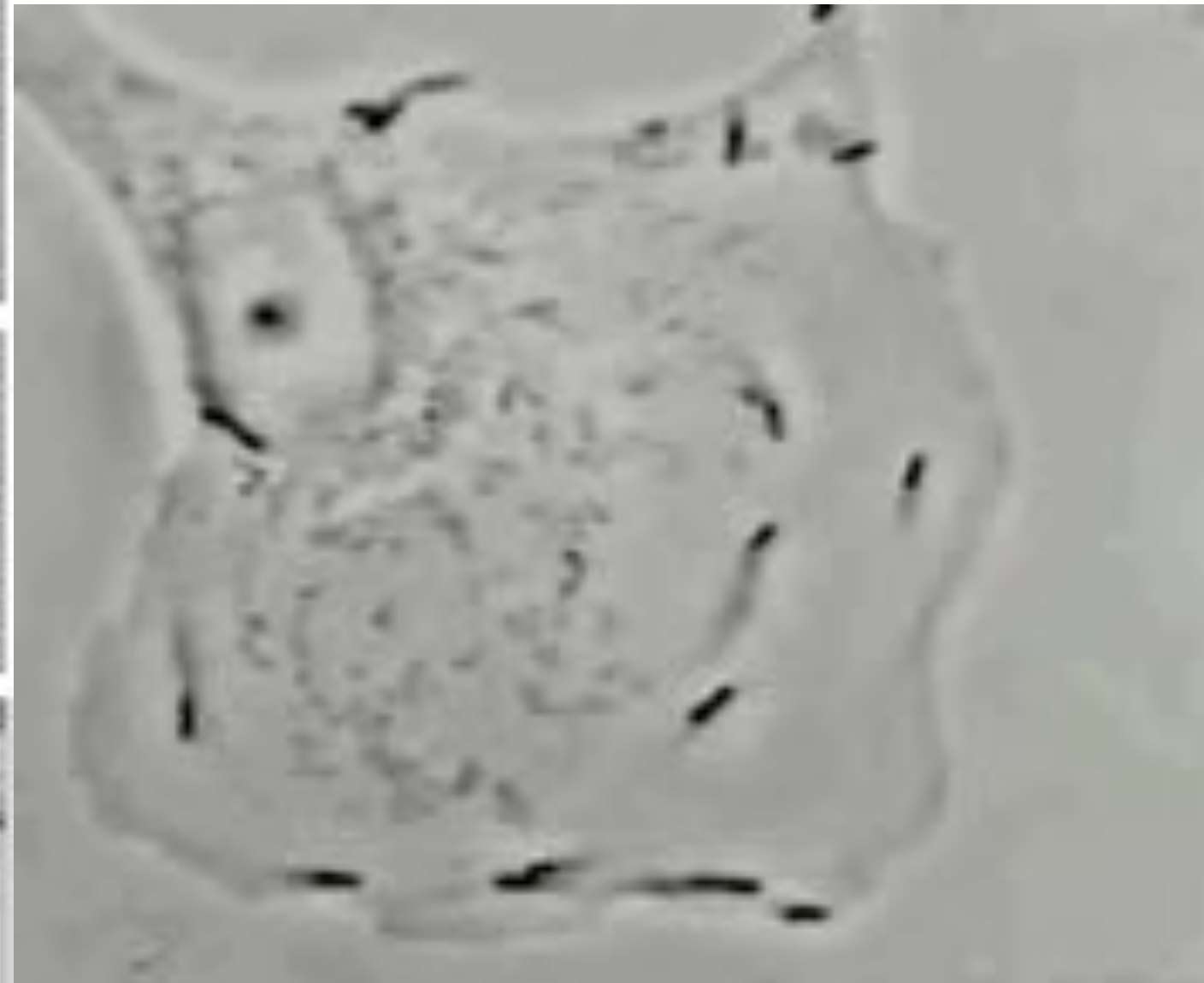
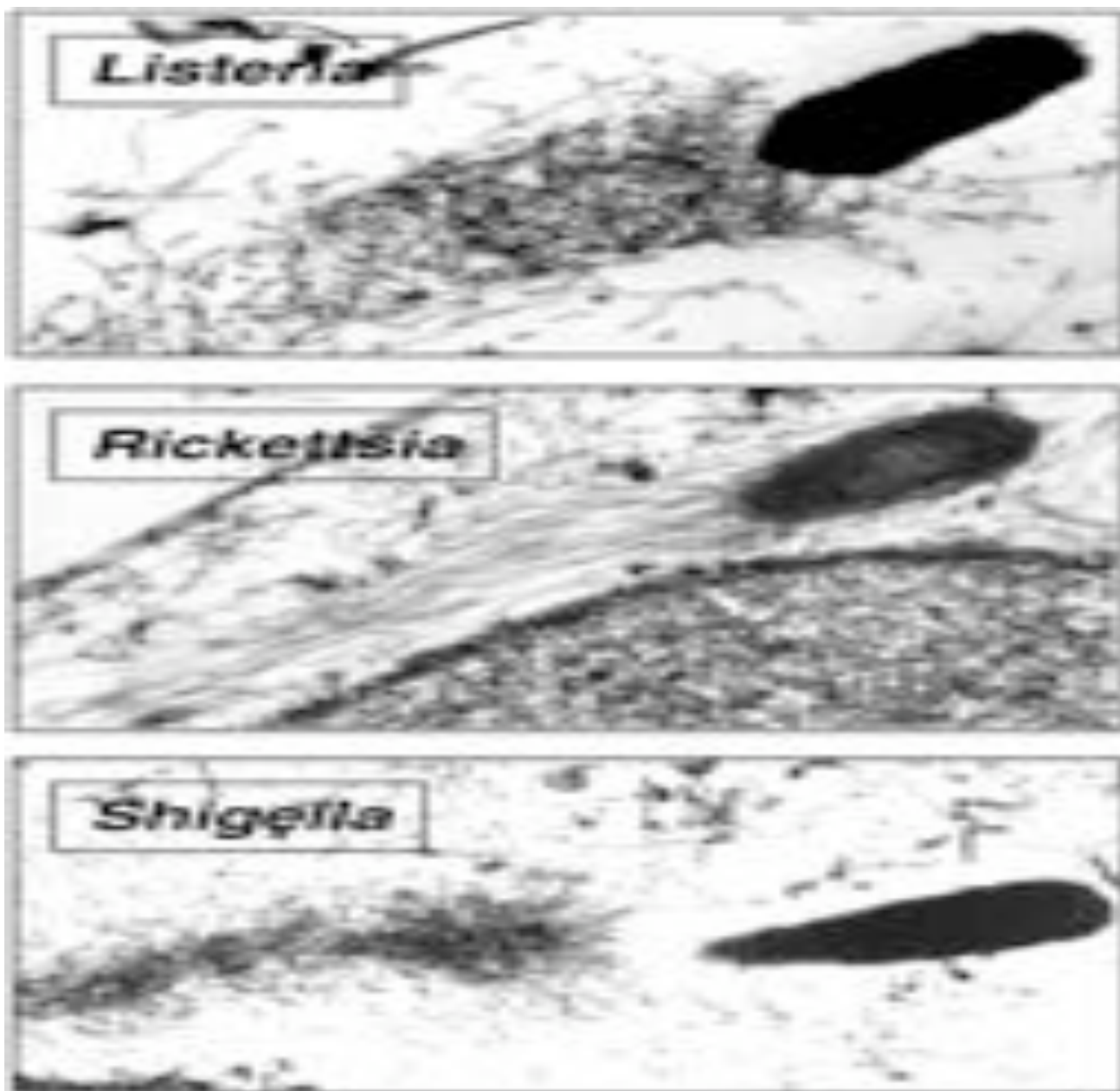


Bacteria reshape the cytoplasm of invaded cells, they reorganize their actin and migrate in the cells themselves!

Actin-based motility of *Listeria*, *Rickettsia*, and *Shigella*.

Electron micrographs of actin tails labeled with fragment S1 of myosin!

The invasion and cell migration of *LISTERIA*!

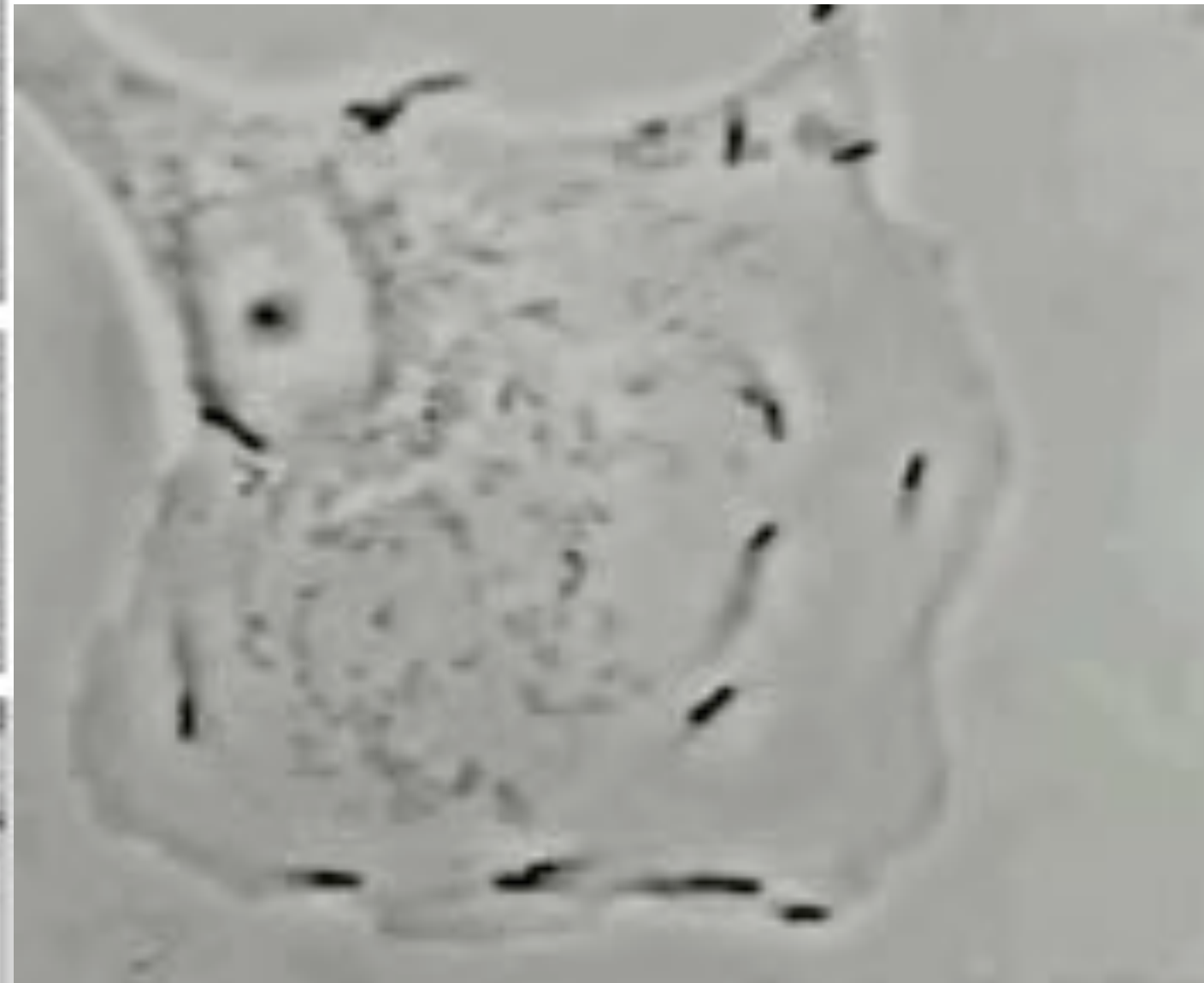
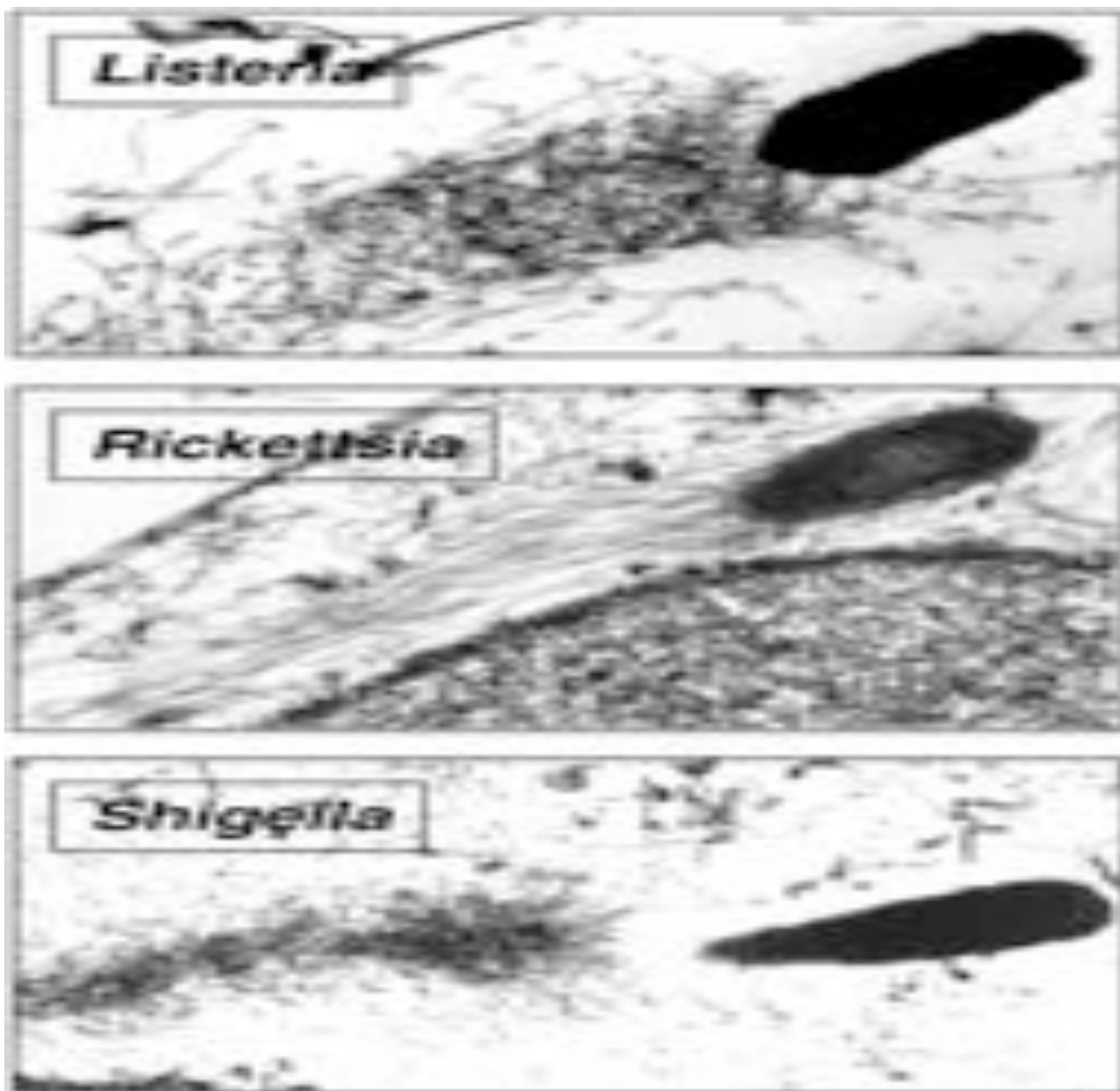


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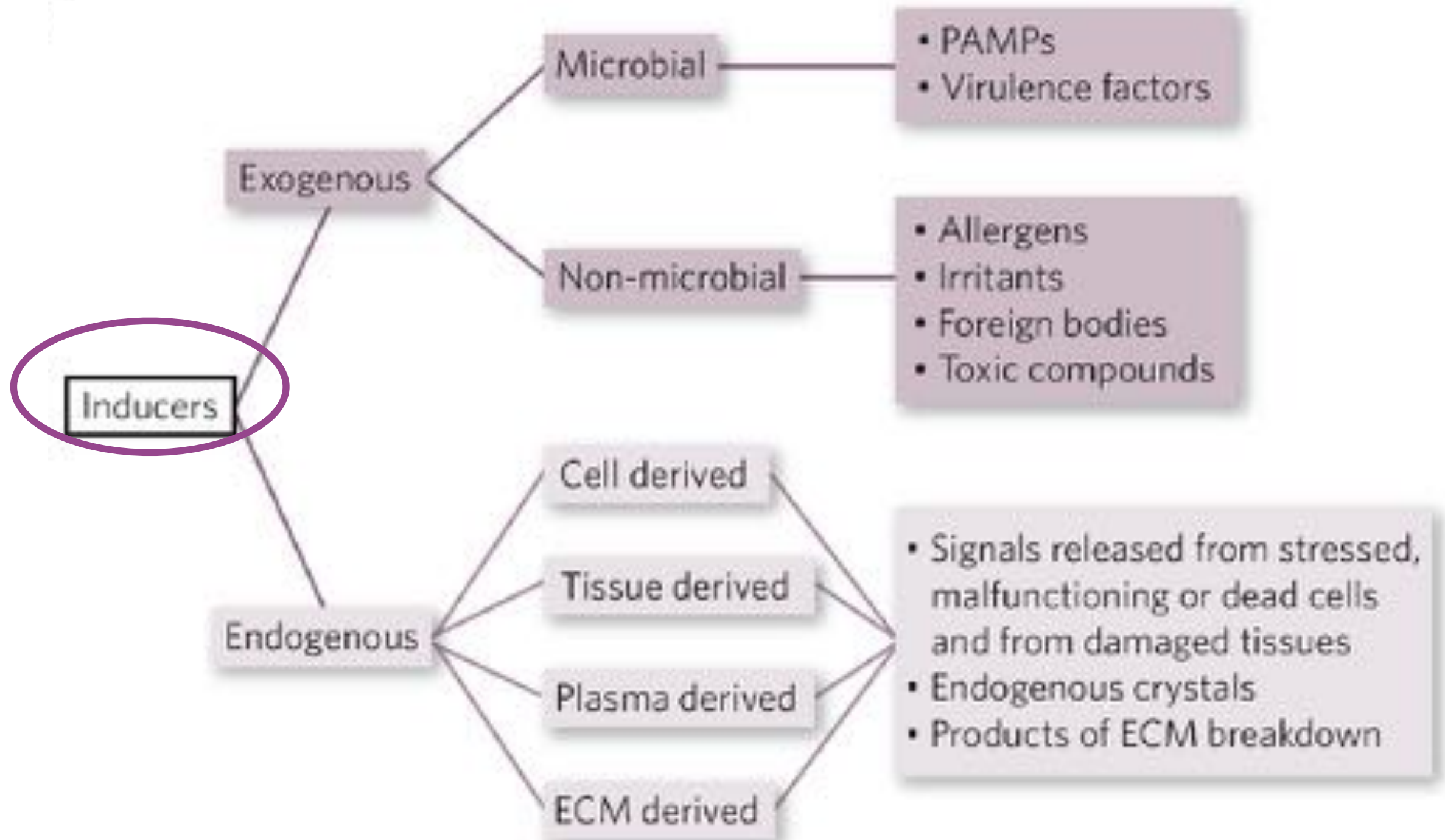
Electron micrographs of actin tails labeled with fragment S1 of myosin!

The invasion and cell migration of *LISTERIA*!



In addition to microbial pathogens, more inducers can activate the inflammatory and the immune response!

Inducers → Sensors → Mediators → Effectors



In addition to microbial pathogens, more inducers can activate the inflammatory and the immune response!

Inducers → Sensors → Mediators → Effectors

**Many endogenous inducers activate or are
"alarmins"**

or

DAMP, danger-associated molecular patterns!

Currently known alarmins include **defensins**, **cathelicidins**, **eosinophil-derived neurotoxin**, **lactoferrin**, some **high-mobility group (HMG) proteins**, **granulysin**, and probably also **ATP** and **histamine**, while endogenous mediators that may eventually prove to be alarmins include some members of the **S100 family proteins**, **heat-shock proteins**, and certain degraded products of extracellular matrix (e.g. **hyaluronan** and **heparan sulfate**).

Extracellular HMGB1 functions as an alarmin!

Biochimica et Biophysica Acta 1799 (2010) 157–163



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Review

The alarmin functions of high-mobility group proteins

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ABSTRACT

High-mobility group (HMG) proteins are non-histone nuclear proteins that bind nucleosomes and regulate chromosome architecture and gene transcription. Over the past decade, numerous studies have established that some HMG proteins can be released extracellularly and demonstrate distinct extracellular biological activities. Here, we will give a brief overview of HMG proteins and highlight their participation in innate/inflammatory and adaptive immune responses. They have the activities of alarmins, which are endogenous mediators that are rapidly released in response to danger signals initiated by infection and/or tissue damage and are capable of activating innate and adaptive immunity by promoting the recruitment and activation of antigen-presenting cells (APCs).

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1. HMG superfamily proteins

HMG proteins consist of a superfamily of nucleosome-binding proteins that were discovered more than 30 years ago [1]. HMG proteins are classified into HMGA, HMGB, and HMGN families [2,3]. HMGA family consists of four members (HMGA1a, 1b, 1c, and 2), each containing two to three “AT” hooks in the N-terminal portion of the molecule which enable HMGA to preferentially bind AT-rich regions of DNA. HMGB family has three members (HMGB1–3), each containing two “box” domains (A and B boxes) in the N-terminal portion of the molecule. The HMGN family contains five members (HMGN1, 2, 3, 4, and NBD-45) and is characterized by a cationic nucleosome-binding domain in the N-terminal portion of the molecule. Common to and characteristic of all HMG proteins is a C-terminal tail rich in acidic amino acid residues.

HMG proteins are ubiquitously present in almost all embryonic tissues. The expression of most members, such as HMGA1, 2, HMGB2, 3, and HMGN1, 2, is downregulated during ontogenic development [3–10]. In adults, HMGB1 is expressed at a high level in all cell types, whereas other HMG proteins are more selectively expressed in highly proliferative tissues that undergo constant turnover and differentiation, such as lymphoid tissues, testis, stem cells, and epithelial cells [3,4,8,10,11]. HMGN proteins appear late in evolution and are only found in vertebrates [3]. Inside the nucleus, HMG proteins exert diverse functions such as controlling chromatin architecture and dynamics, modifying the transcription of certain genes, and regulating DNA repair, cell differentiation, and ontogenic development, which will be the subjects of other reviews in this series.

Some HMG proteins, such as HMGB1 and members of HMGN family can be released from either injured necrotic cells or activated monocytes/macrophages, dendritic cells, and NK cells. HMGB1 release can be initiated by PAMPs, bacteria, ischemia/reperfusion-induced hypoxia, or proinflammatory cytokines [12–20]. While the extracellular release of HMGN family members has not been studied in detail, HMGB1 release by activated monocytes/macrophages involves a crucial initial acetylation on many of the 43 lysine residues of HMGB1 in the nucleus, followed by redistribution of HMGB1 from the nucleus to endolysosomes and finally exocytosis [14,17,21]. Release of HMGB1 by hepatocytes under hypoxic conditions relies on the generation of reactive oxygen species and calcium/calmodulin-dependent kinases [19]. LPS-induced HMGB1 release by monocytes and macrophages requires phosphorylation of HMGB1 by PKC and the presence of Ca²⁺ [22]. Recently, in a model of mouse lung inflammation caused by *Klebsiella pneumoniae*, the systemic release of systemic HMGB1 was found to be strictly dependent on NLRP3 and ASC, two critical components of the NLRP inflammasome inflammatory pathway [20].

HMG proteins in the extracellular milieu, in particular HMGB1, have also been shown during the last decade to have diverse activities in mediating cell migration, tumor invasiveness, neuronal innervation, inflammation, immunity, wound healing and repair [17,23–25]. We will focus on the alarmin functions of extracellular HMG proteins and their participation in inflammation and immunity.

2. Alarmin concept

During the 1990s, two popular models were developed to explain how the immune system is activated to mount innate and adaptive immune responses. The “infectious non-self” model proposed by Charles Janeway suggested that immune responses are initiated by

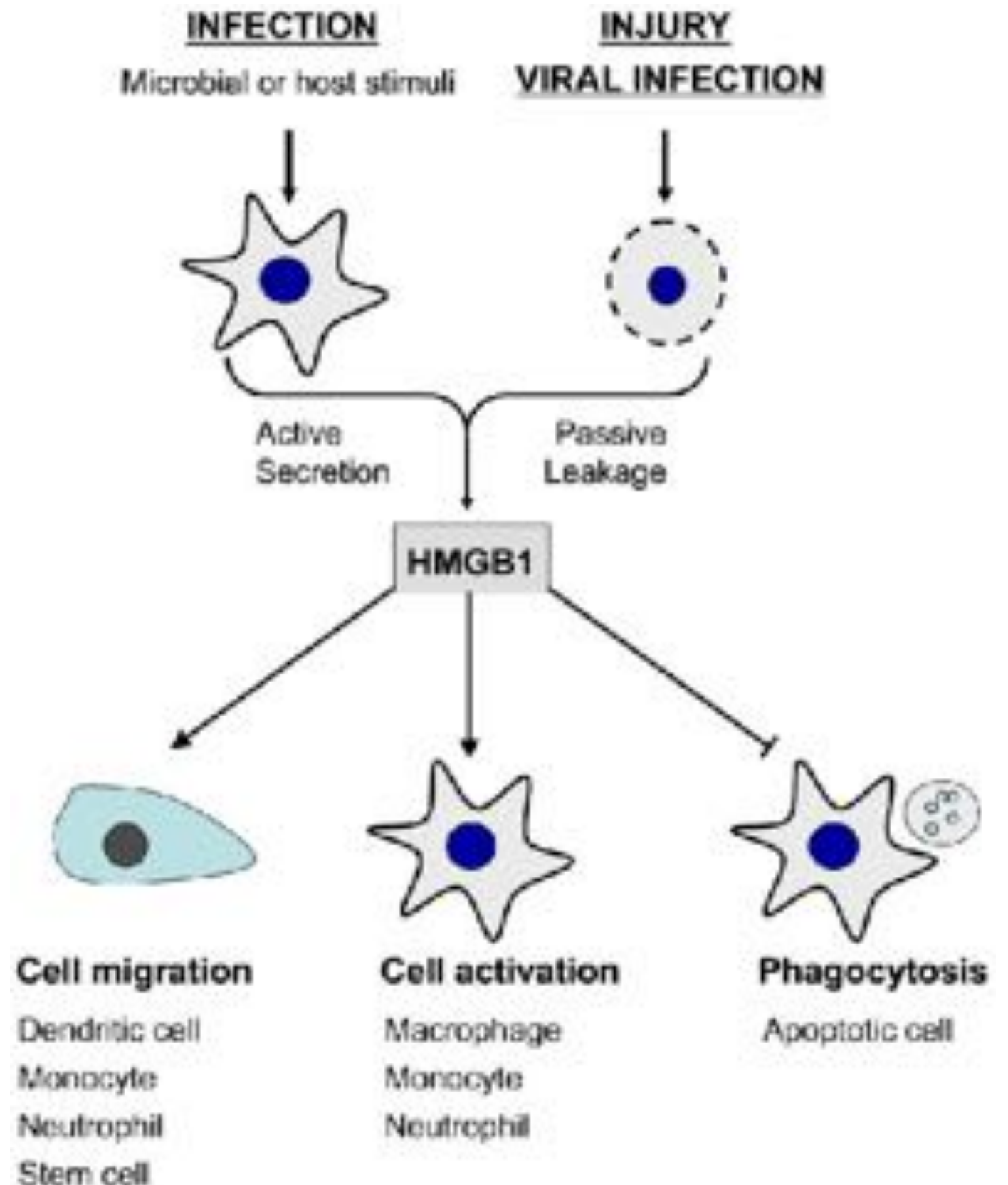
* Corresponding author. Tel.: +1 301 846 1551; fax: +1 301 846 7042.
E-mail address: oppenhei@ncicfcrf.gov (J.J. Oppenheim).

Extracellular HMGB1 functions as an alarmin!

HMGB1 is actively secreted by innate immune cells in response to exogenous microbial products (e.g., LPS or CpG-DNA) or endogenous host stimuli (TNF, IFN- γ , or hydrogen peroxide), and passively released by damaged or virus-infected cells. Extracellular HMGB1 sustains an inflammatory response by stimulating migration of innate immune cells, facilitating innate recognition of bacterial products, activating various innate immune cells, and suppressing phagocytosis of apoptotic cells. Thus, HMGB1 can function as an alarmin signal to recruit, alert and activate various innate immune cells.

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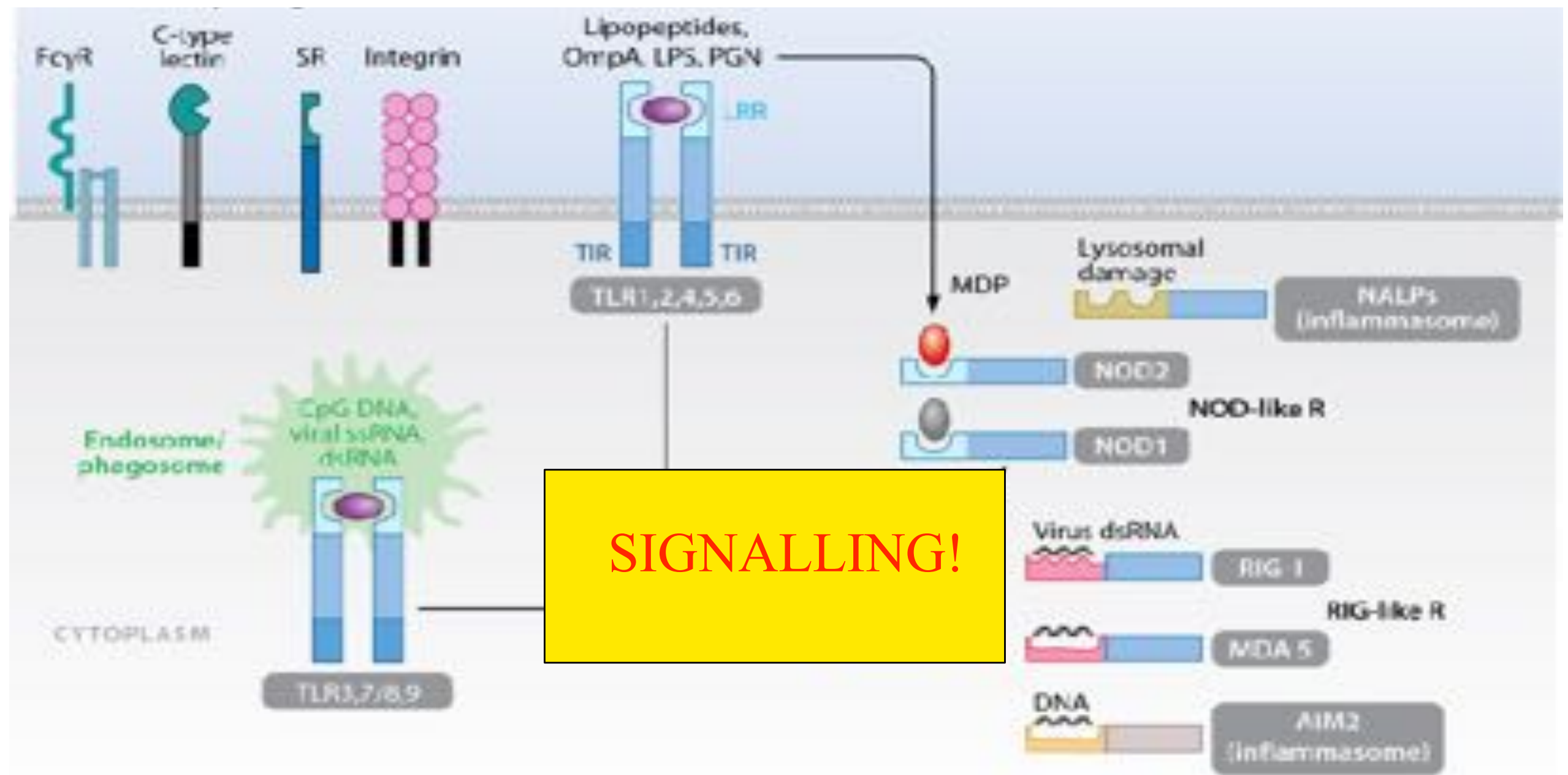
Pathogenetic inducers **can activate** **the inflammatory and the immune response** **through RECEPTORS!**

Our body feels the damage (mainly from biological, chemical and physical stimuli) through RECEPTORS!

The MAIN RECEPTORS of the damage (by stimuli biologicals, chemicals, physicals etc) are:

- **MEMBRANE RECEPTORS**
- **CYTOPLASMIC RECEPTORS**

THE MAIN CYTOPLASMIC AND MEMBRANE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION!

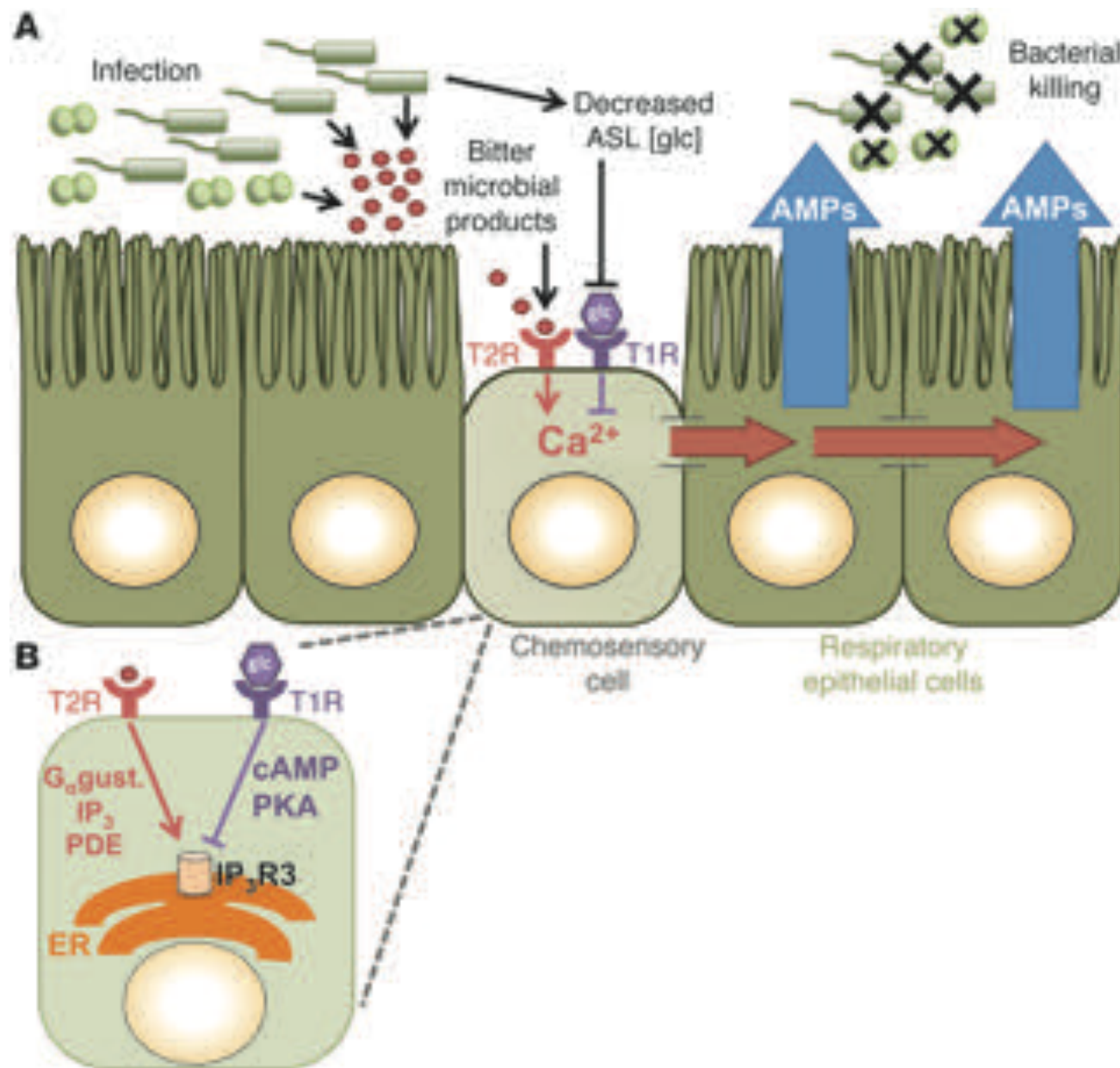


NEW! BITTER AND SWEET TASTE RECEPTORS AS RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION!



Bitter and sweet taste receptors regulate human upper respiratory innate immunity!

Bitter taste receptors (T2Rs) are emerging as novel regulators of innate immunity in the respiratory tract. T2Rs are expressed in respiratory ciliated cells and in solitary chemosensory cells (SCCs), which also express the T1R2 and T1R3 subunits comprising the human sweet taste receptor. Activation of the T2Rs in mouse nasal SCCs stimulates a trigeminal nerve-mediated reduction in respiratory rate.



Proposed model of T2R bitter receptor- and T1R sweet receptor-based regulation of AMP secretion in the human nose.

(A) From left to right, bitter chemicals released by microbes during infection activate T2Rs in the sinonasal epithelium, likely including those expressed in nonciliated chemosensory epithelial cells, likely the SCCs. This results in a calcium response that propagates to the surrounding epithelial cells, causing secretion of multiple AMPs, including β -defensins 1 and 2, that are capable of direct bacterial killing. Glucose in the the airway surface liquid (ASL) normally governs the T2R-mediated response through T1R2/3 activation. However, during acute infections, bacteria may consume glucose and decrease the ASL glucose concentration, which relieves the T1R2/3-mediated inhibition of T2Rs and allows the activation of the antimicrobial response.

(B) Proposed mechanism for T2R and T1R signaling in sinonasal chemosensory cells. T2R signaling is dependent upon $G_{\alpha}gust$ and IP_3R calcium release channels that likely include the IP_3R3 isoform. T1R signaling likely uses an alternative G protein that acts through cAMP/PKA and may inhibit IP_3R3 -mediated calcium signaling.

J Clin Invest. 2014 Mar 3;124(3):1393-405.

Bitter and sweet taste receptors regulate human upper respiratory innate immunity.

Lee RJ, Kofonow JM, Rosen PL, Siebert AP, Chen B, Doghramji L, Xiong G, Adappa ND, Palmer JN, Kennedy DW, Kreindler JL, Margolskee RF, Cohen NA.

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T2Rs are expressed in ciliated cells and chemosensory cells of the respiratory tract: bitter chemicals released by microbes during upper respiratory tract infections activate T2Rs and induce epithelial secretion of antimicrobial peptides, such as β -defensins 1 and 2

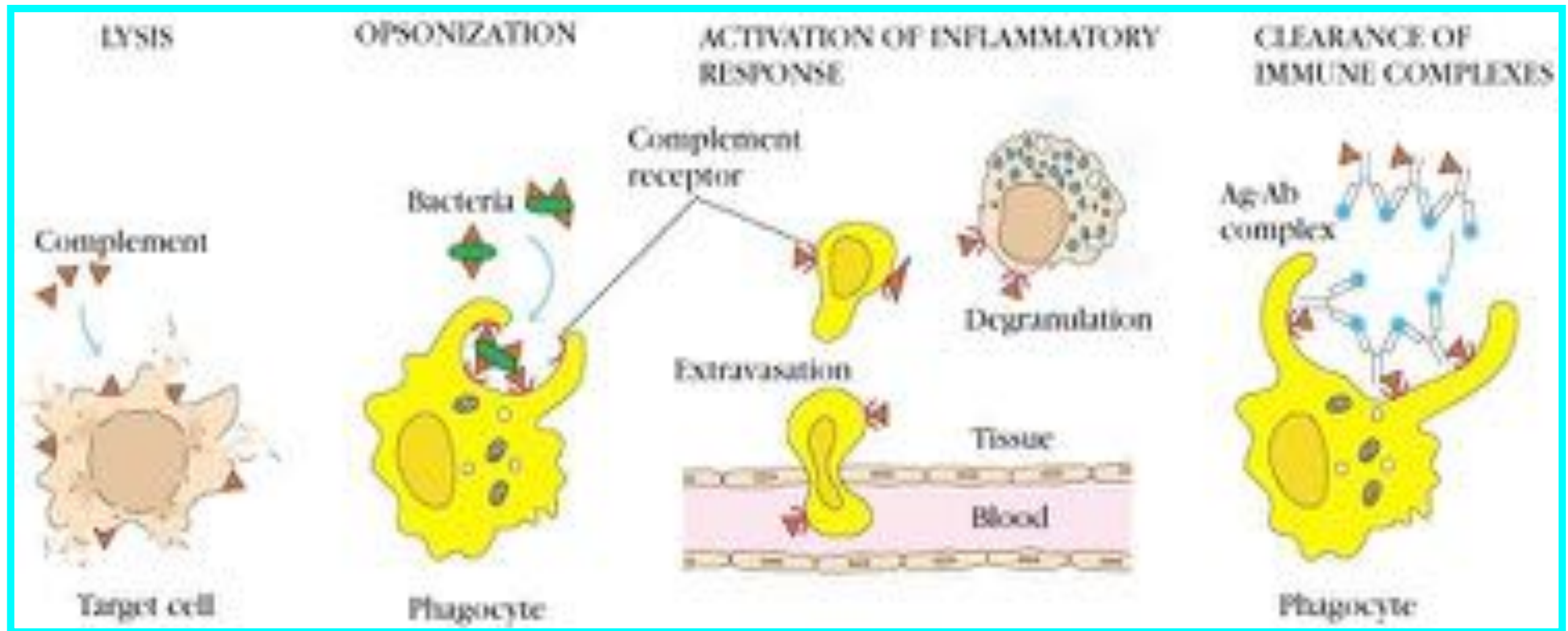
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protein that acts through cAMP/PKA and may inhibit IP3R3-mediated calcium signaling.

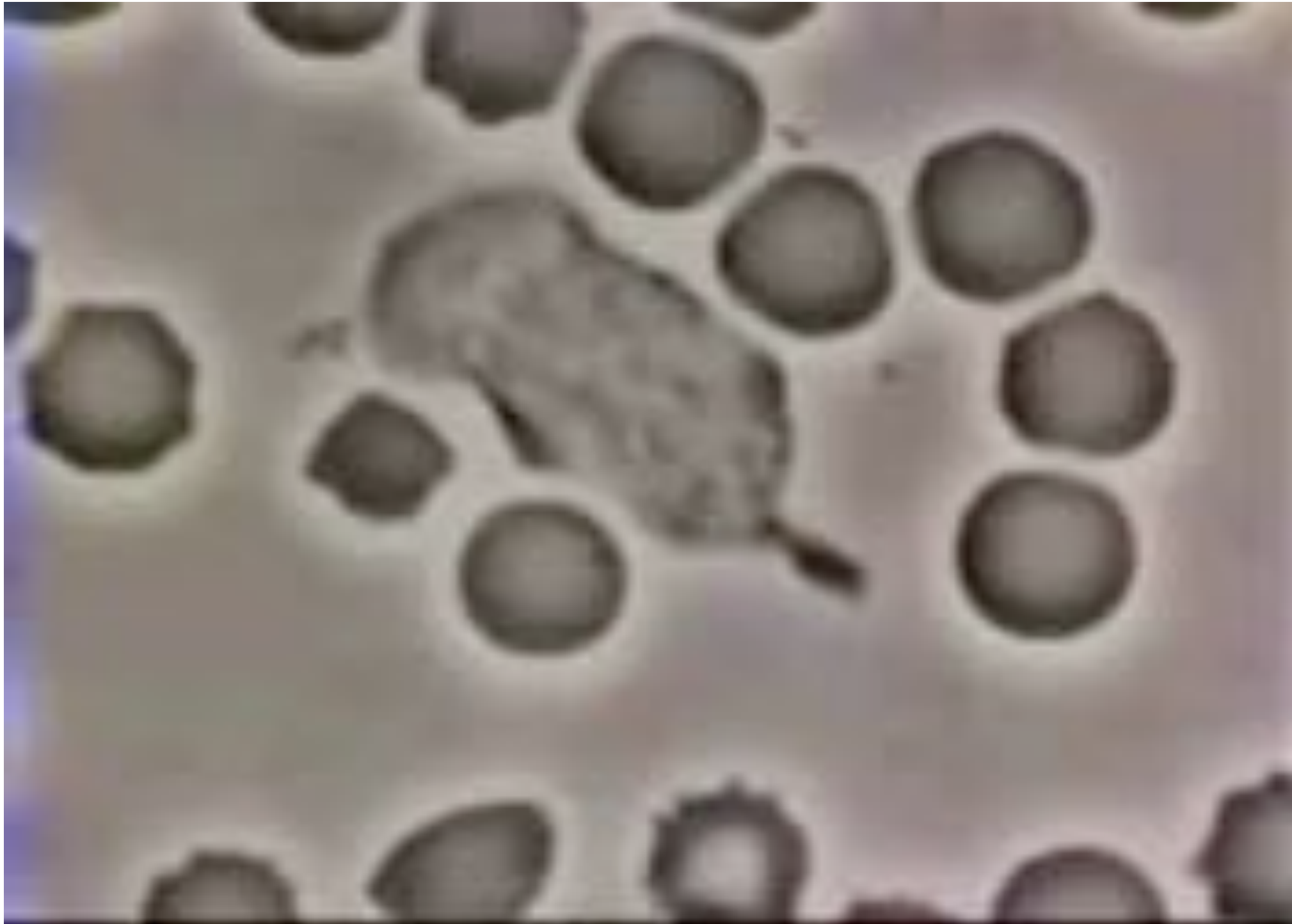
THE MAIN CYTOPLASMIC AND MEMBRANE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION ACTIVATE AND REGULATE:

- **THE COMPLEMENT SYSTEM!**



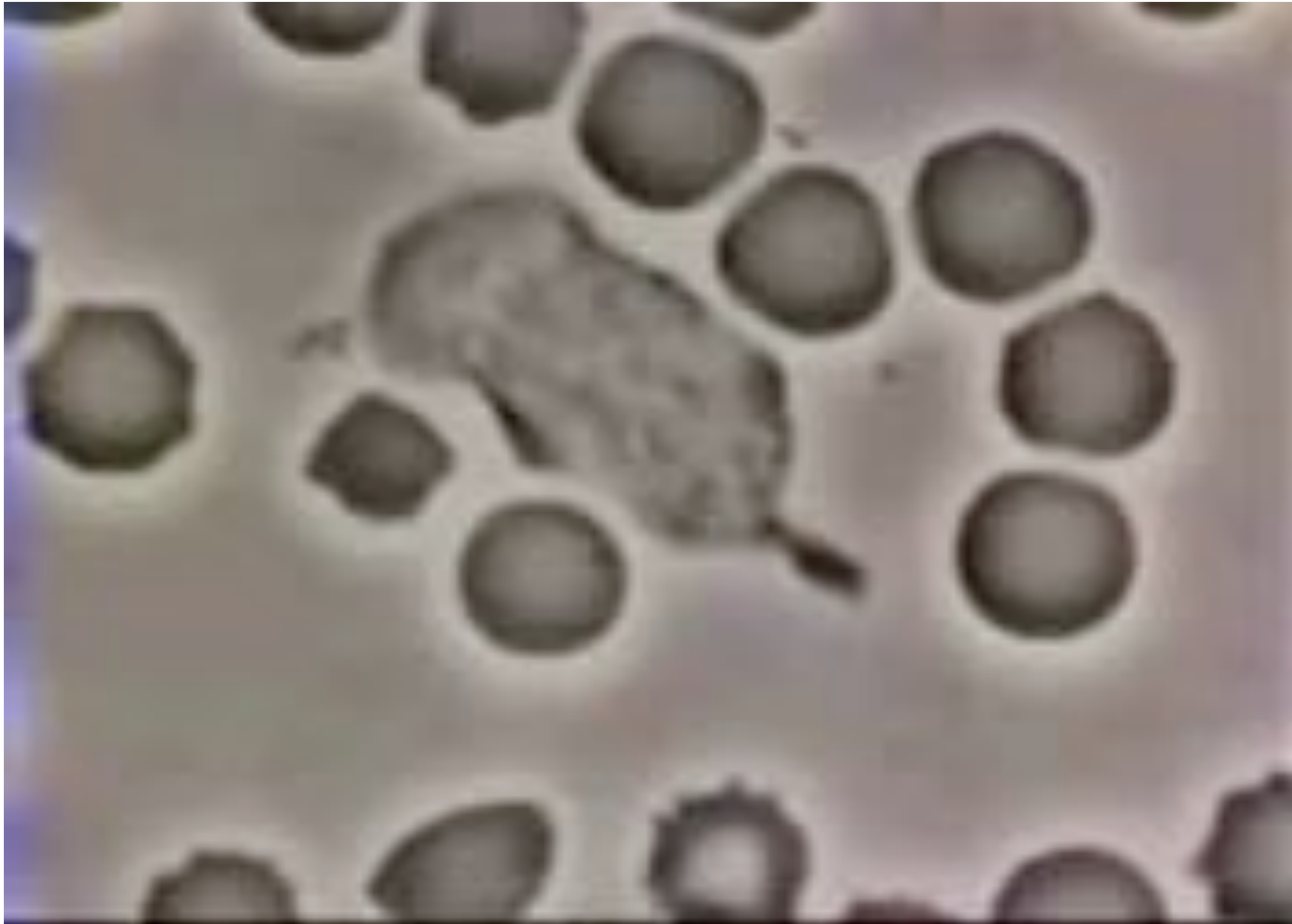
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- **THE PHAGOCYTOSIS!**



THE MAIN CYTOPLASMIC AND MEMBRANE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION ACTIVATE AND REGULATE:

- **THE PHAGOCYTOSIS!**



THE MAIN CYTOPLASMIC AND MEMBRANE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION ACTIVATE AND REGULATE:

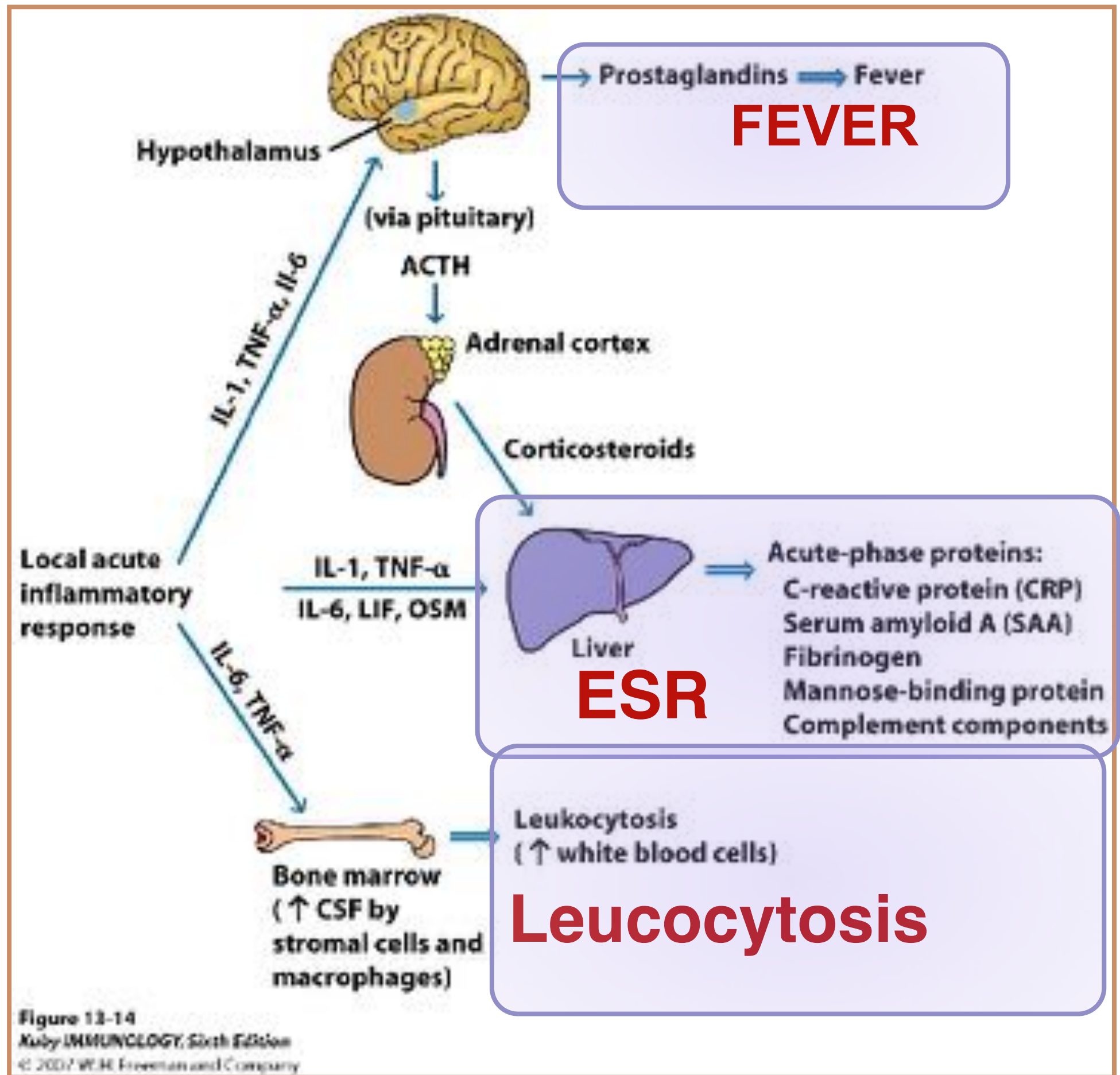
- **THE 5 CARDINAL SIGNS OF ACUTE INFLAMMATION**
(rubor, tumor, calor, dolor and functio lesa)!



**THE MAIN CYTOPLASMIC AND MEMBRANE DAMAGE
RECEPTORS OF NATURAL IMMUNITY AND
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**The acute phase
RESPONSE!**

The acute phase response and its the main signs!



**FEVER, ESR and
LEUCOCYTOSIS
ARE
THE MARKERS
OF INFLAMMATION!**

**APR (Acute Phase
Reactions) are fully
characterized:**

a) by neuroendocrine changes, fever, lethargy and anorexia, increased secretion of corticotropin-releasing hormone, cortisol, nitric oxide and decreased secretion of growth insuline-like factor;

**b) h e m a t o p o i e t i c
modifications such as anemia,
leukocytosis, thrombocytosis;**

**c) metabolic changes such
muscle loss and negative
nitrogen balance,
impaired gluconeogenesis,
osteoporosis, increased
hepatic lipogenesis,
increased lipolysis in
adipose tissue, cachexia;**

d) plasma modification of some metals such as calcium, iron and zinc, and vitamins, but mostly of some proteins and lipoproteins!

The acute phase proteins or APP!

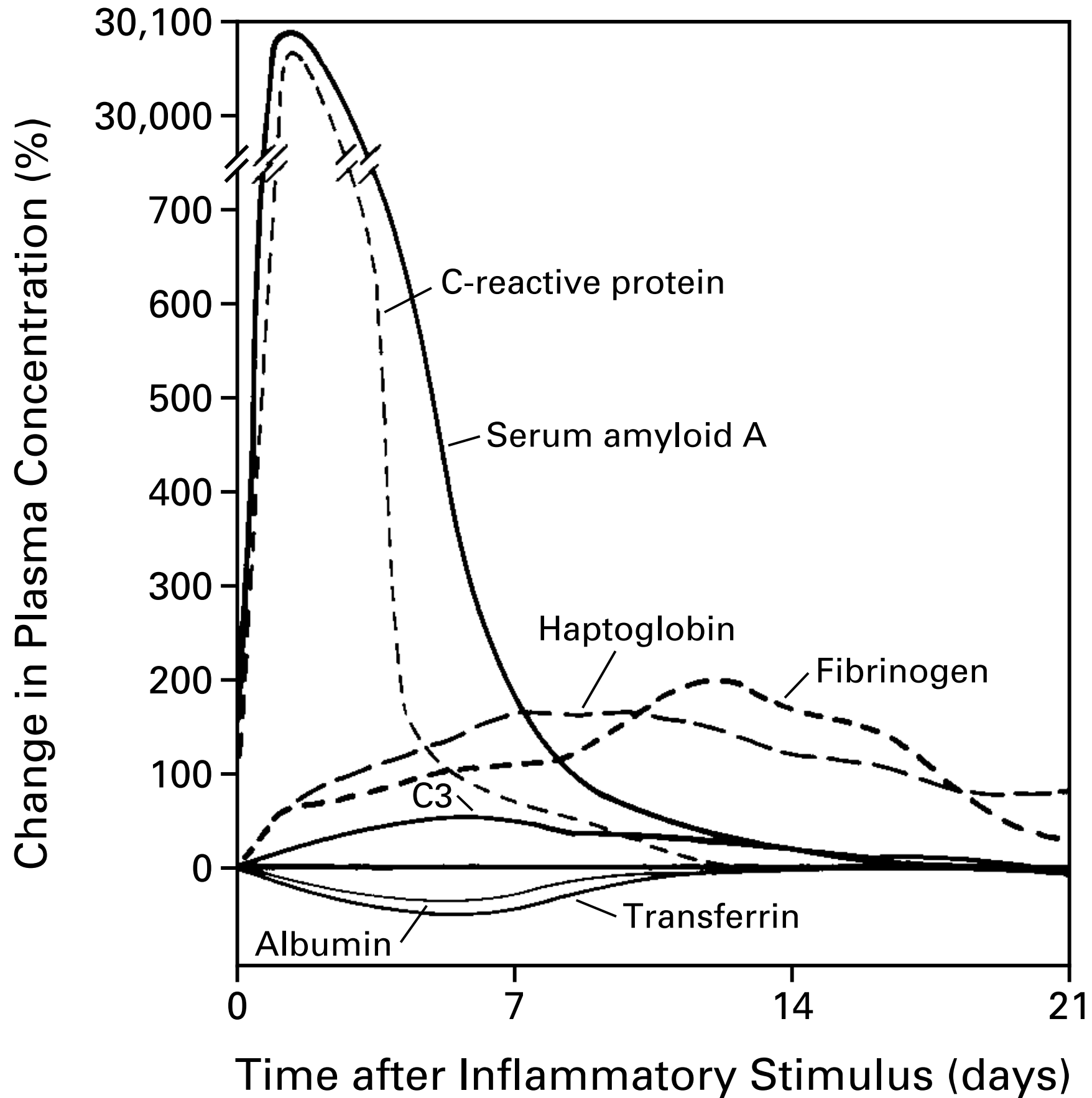
The study of APP was born with the identification of the first APP, the C-reactive protein, or CRP, with a fervent resurgence of research activities from the 90' until today. The discovery of PCR was made by Tillet and Francis in 1930 with the publication of a paper entitled "Serological Reactions In Pneumonia With A Somatic Nonprotein Fraction Of Pneumococcus" in the Journal of Experimental Medicine:

Tillett WS, Francis T: Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus.

J Exp Med 1930; 52: 561-71.

Later was observed, in addition to the CPR, the parallel increase in concentration of other plasma proteins including the Amyloid Protein A or SAA, the Fibrinogen, the C3 complement component, the α 1-antitrypsin and more proteins!

The APP!



Today we define as **acute phase protein (APP) that protein whose plasma concentration increases (**positive APP**) or decreases (**negative APP**) by at least 25% during the acute phase reaction!**

Classification of the positive and negative APP!

Positive acute phase reactants (concentrations increase with acute inflammation)

Immune-related

Complement (C')

Mannose-binding lectin (MBL)

C-reactive protein (CRP)

Orosomucoid (alpha-1 acid glycoprotein)

Antiproteases (anti-enzymes)

Alpha-1 antitrypsin (A1-AT)

Alpha-2 macroglobulin (A2M)

Anti-oxidants

Ceruloplasmin

Coagulation factors

Fibrinogen

Factor VIII

Others

Haptoglobin

Serum amyloid A (SAA)

Plasma fibronectin

Lipopolysaccharide-binding protein (LBP)

Ferritin

Negative acute phase reactants (concentrations decrease with acute inflammation)

Retinol-binding protein (RBP)

Transthyretin (TBPA)

Albumin

Transferrin

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Among the negative APP, are really important **albumin** and **transferrin!**

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The concentration of albumin in the blood (serum albumin) varies between 3.5 and 5.0 g/dl and its decrease during inflammation can be significant even if it is highly non-specific, as the hypoalbuminemia may occur in various physiopathological situations, such as **rheumatoid arthritis, cholecystitis acute ulcerative colitis, diabetes, pregnancy, hyperthyroidism, leukemia and Hodgkin's disease, SLE, malabsorption, peptic ulcers, malnutrition, liver and renal diseases and stress.**

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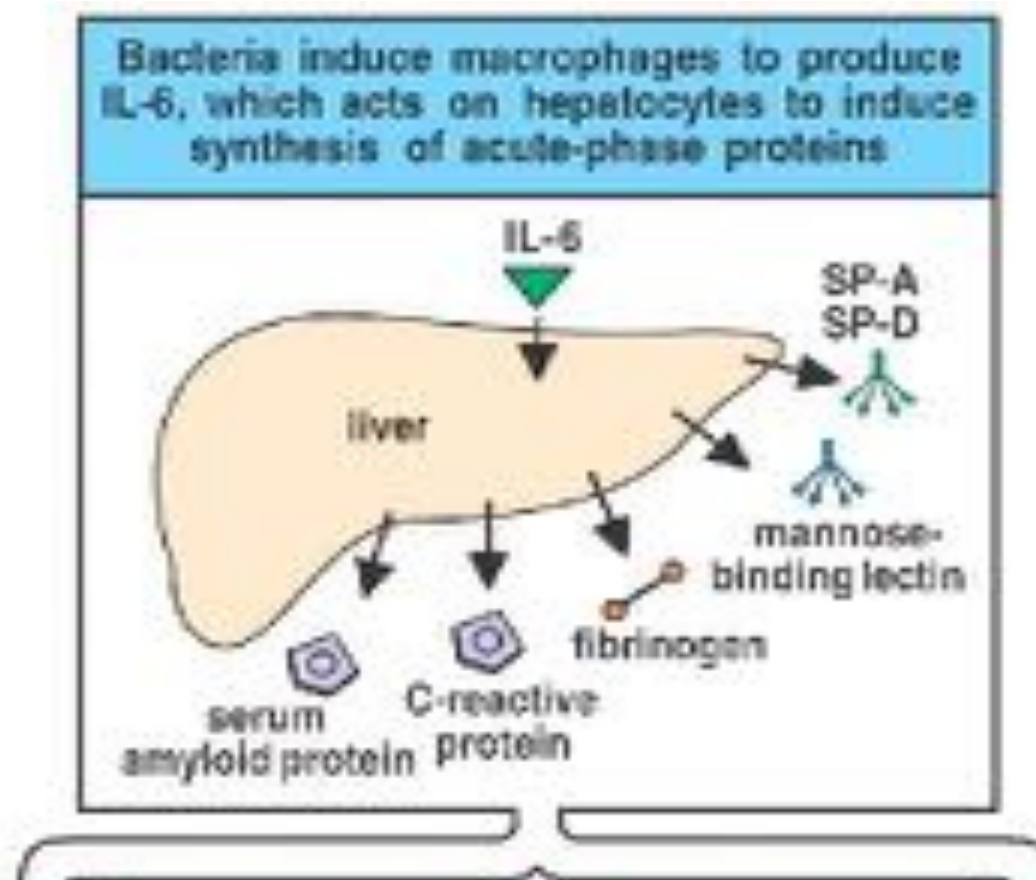
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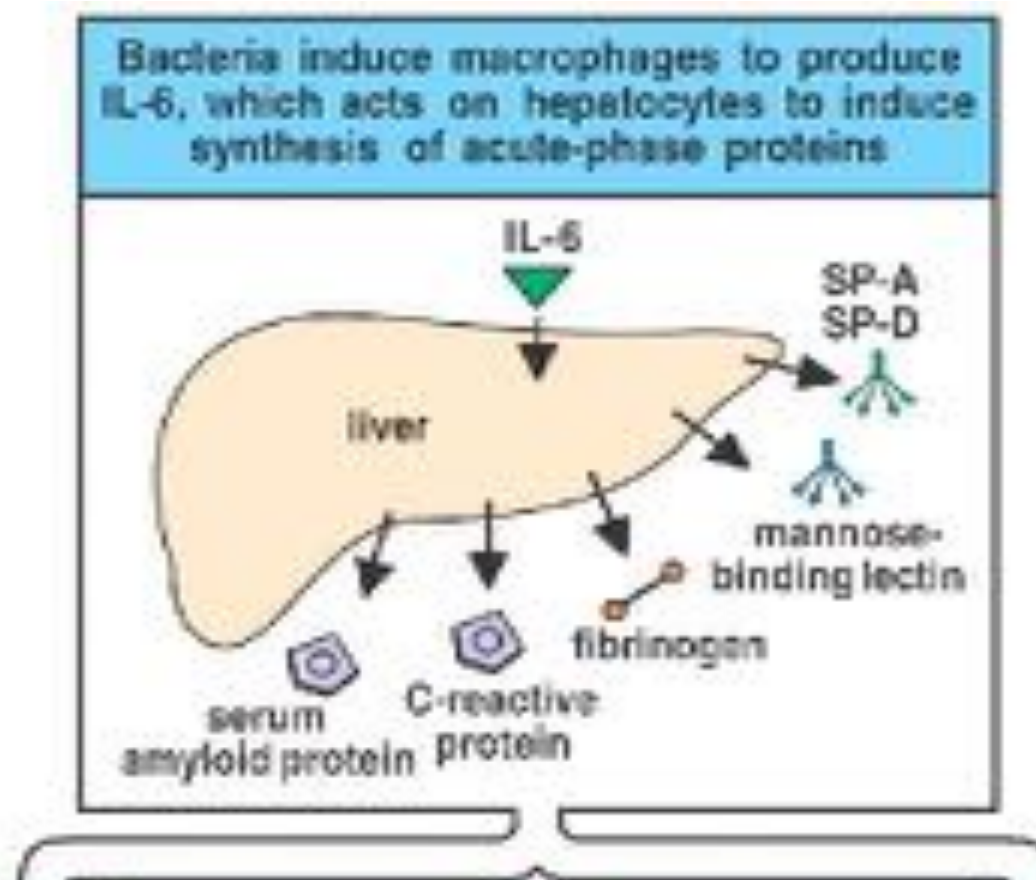
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- Electrophoretic variants of transferrin in serum are found occasionally due to changes in its amino acid structure but are not associated with functional deficits.

The positive APP! cytokine induction from the LIVER!



Inflammatory cytokines bind to specific receptors on hepatocytes and induce the activation of the **Janus family kinases (JAK)**, the **STAT (signal transducers and Activators of transcription)** and proinflammatory transcription factors such as **NF- κ B**. Transcription factors most characterized that regulate the synthesis of APP belong to the family of leucine zipper, C/EBPA (**CCAAT/inducer of protein binding**), C/EBPd and **NF-IL6 (nuclear factor associated with IL-6)**, that affect transcription by binding to a site called **bZIP1**. It is also been shown that the induction of acute phase proteins is correlated with a decreased synthesis of C/EBPA and an increase of that of **NF/IL-6**. Post-transcriptional modifications are observed also in the synthesis of APP, which are then secreted.

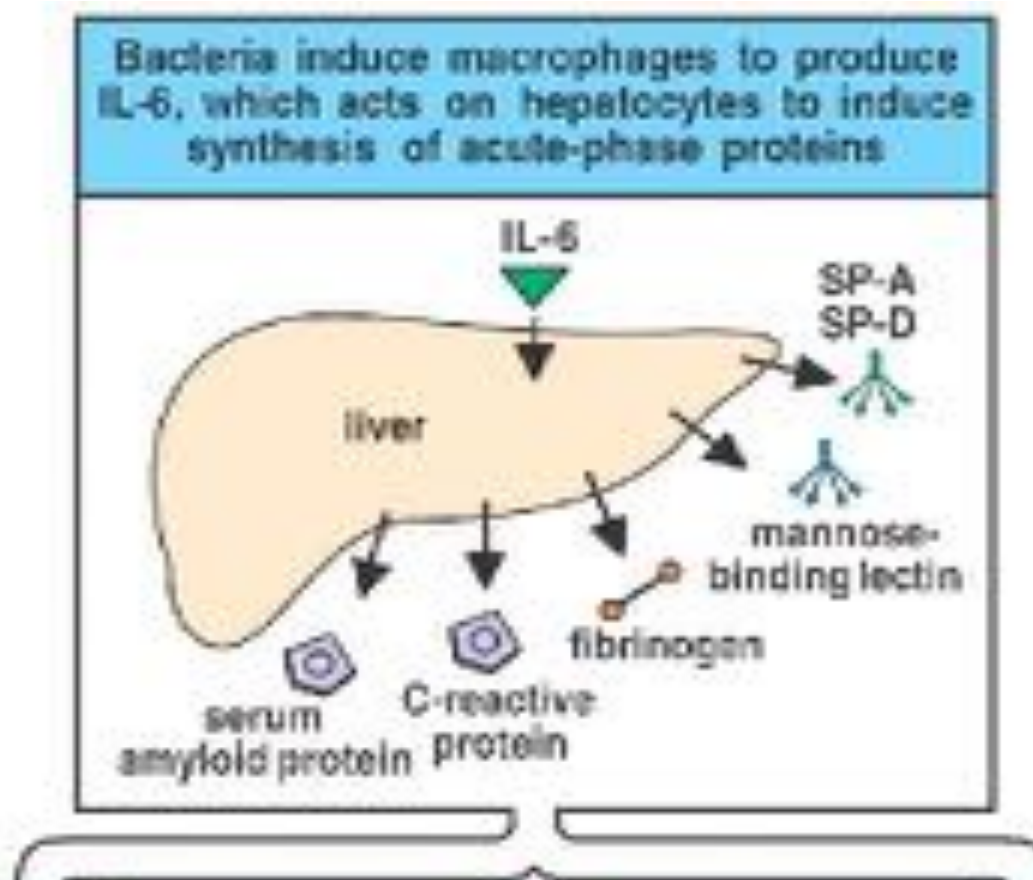
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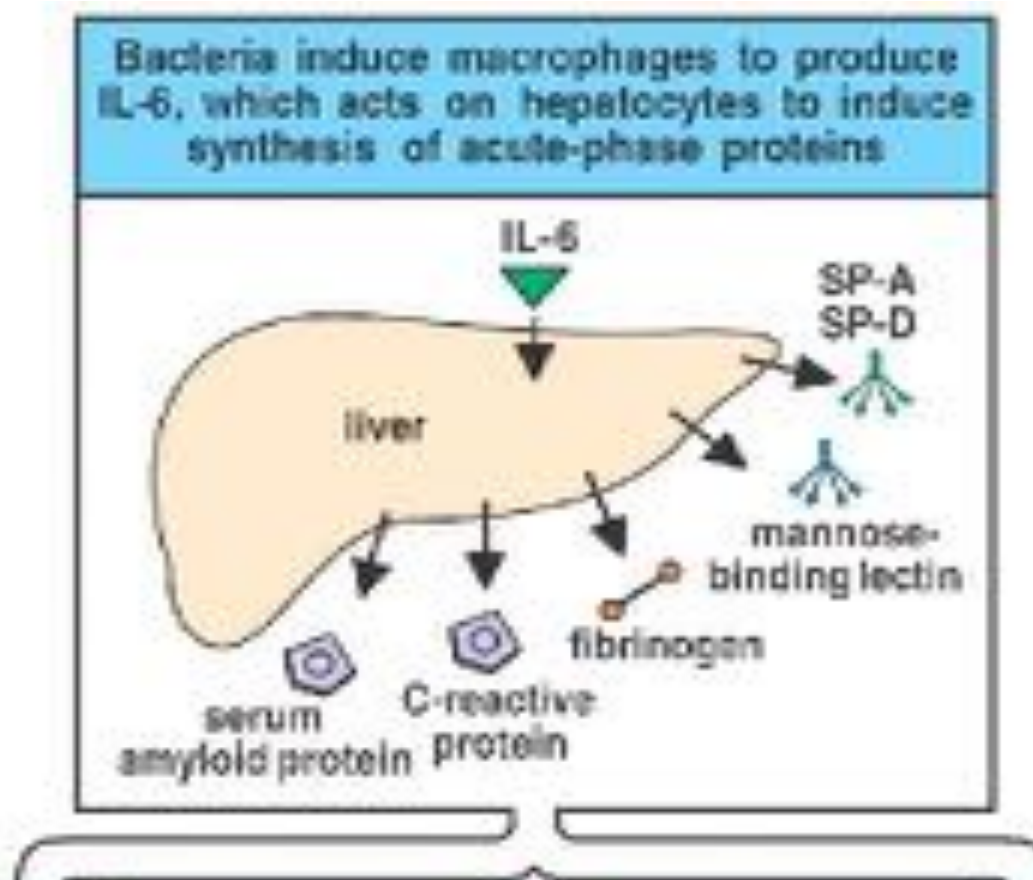
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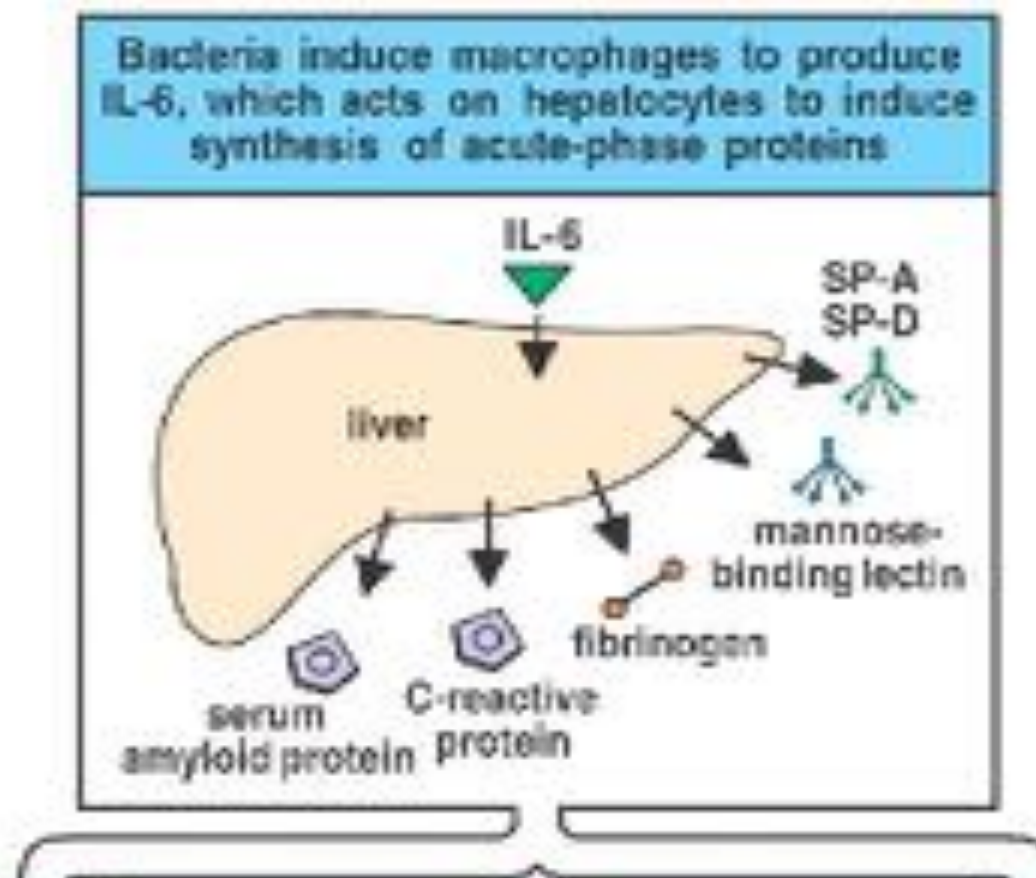
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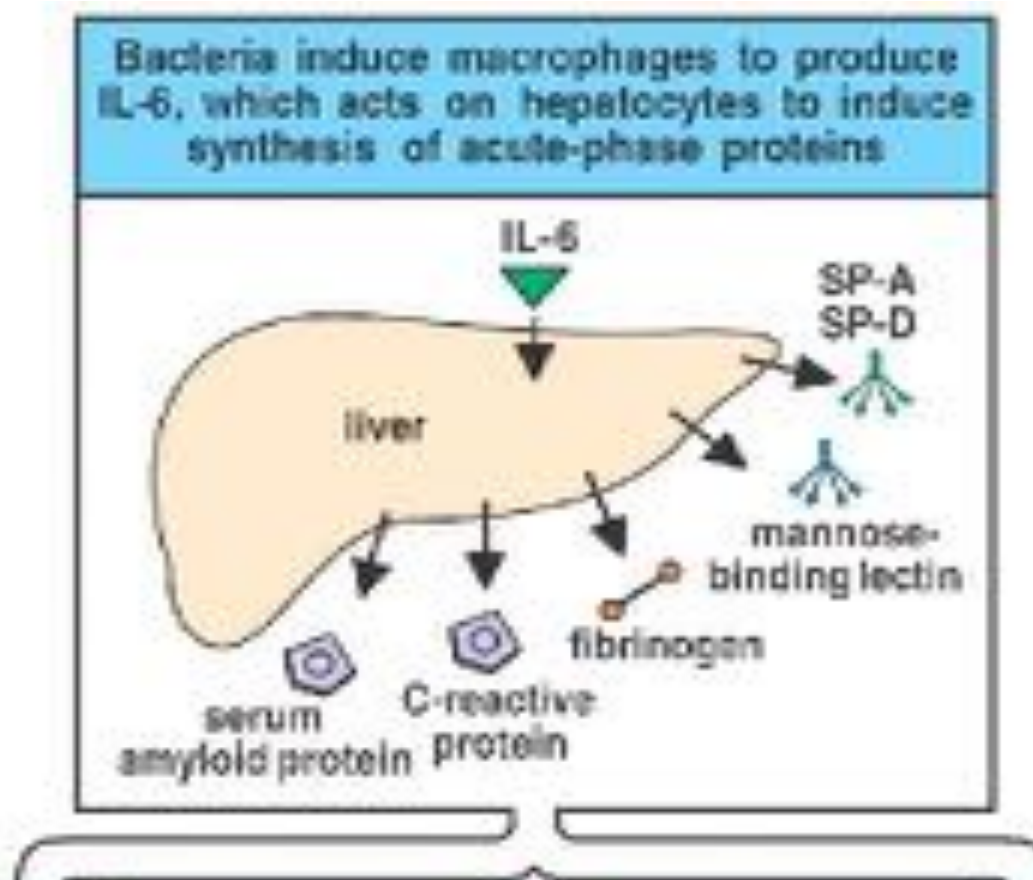
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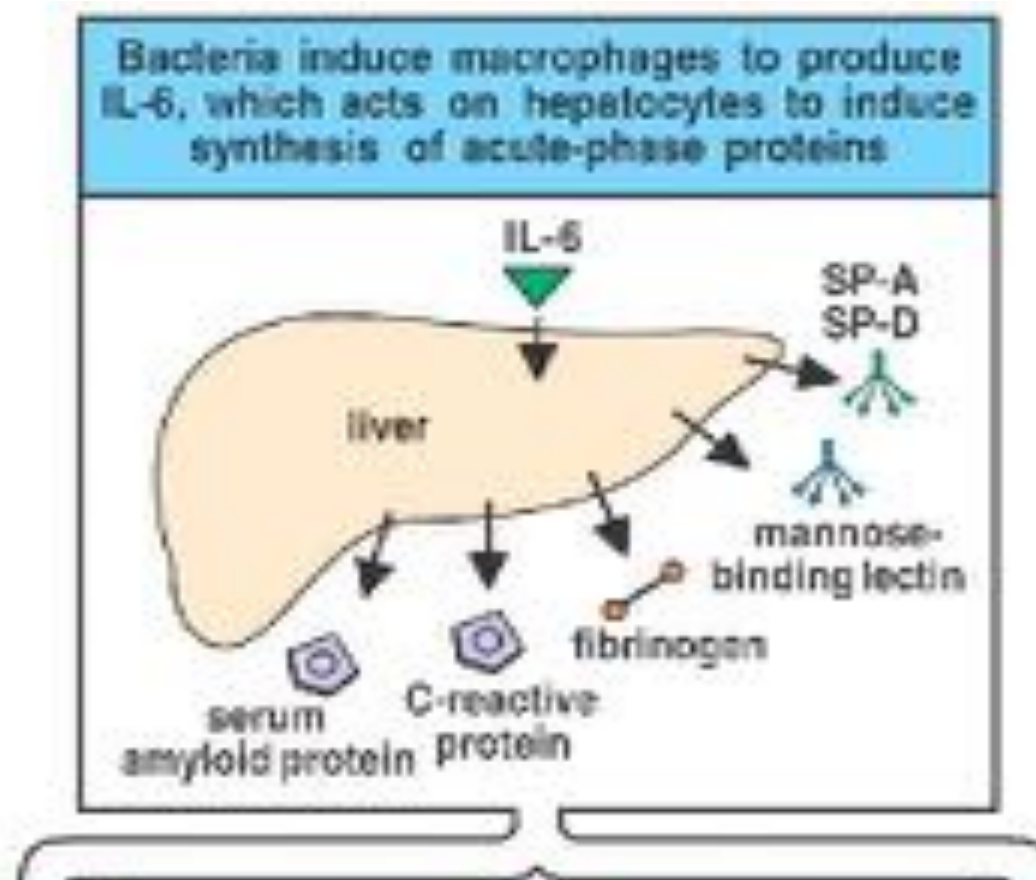
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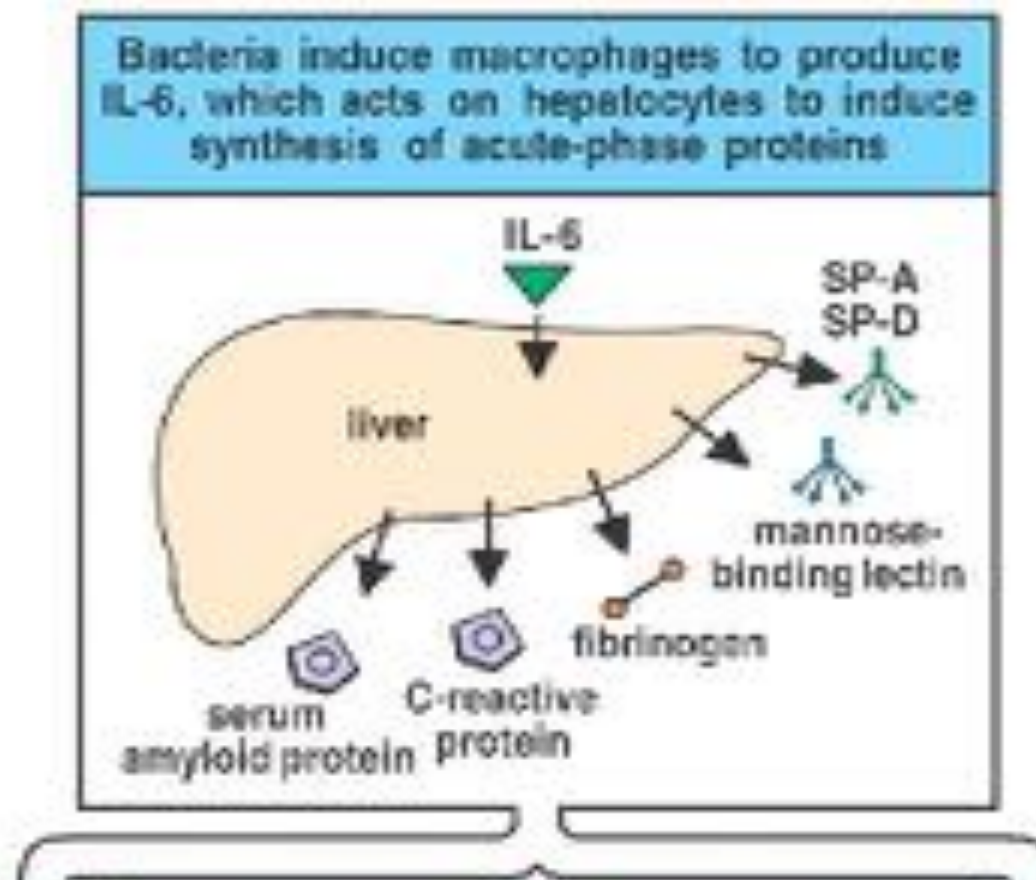


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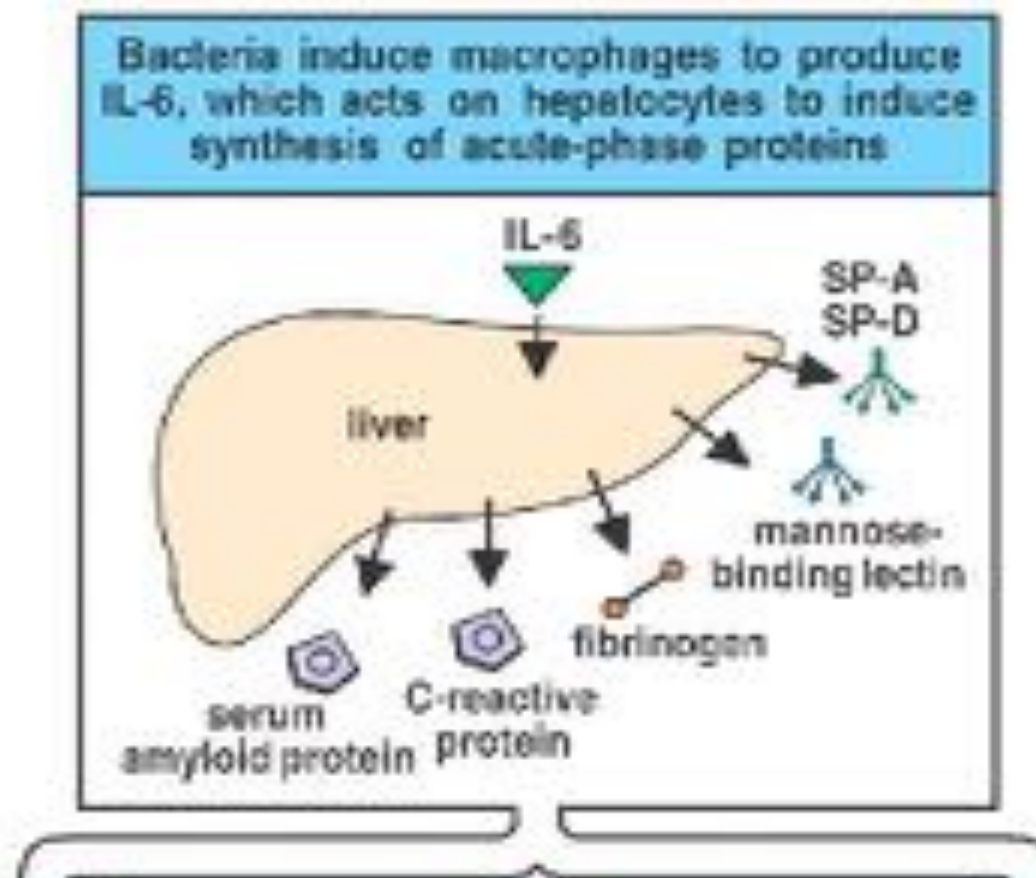


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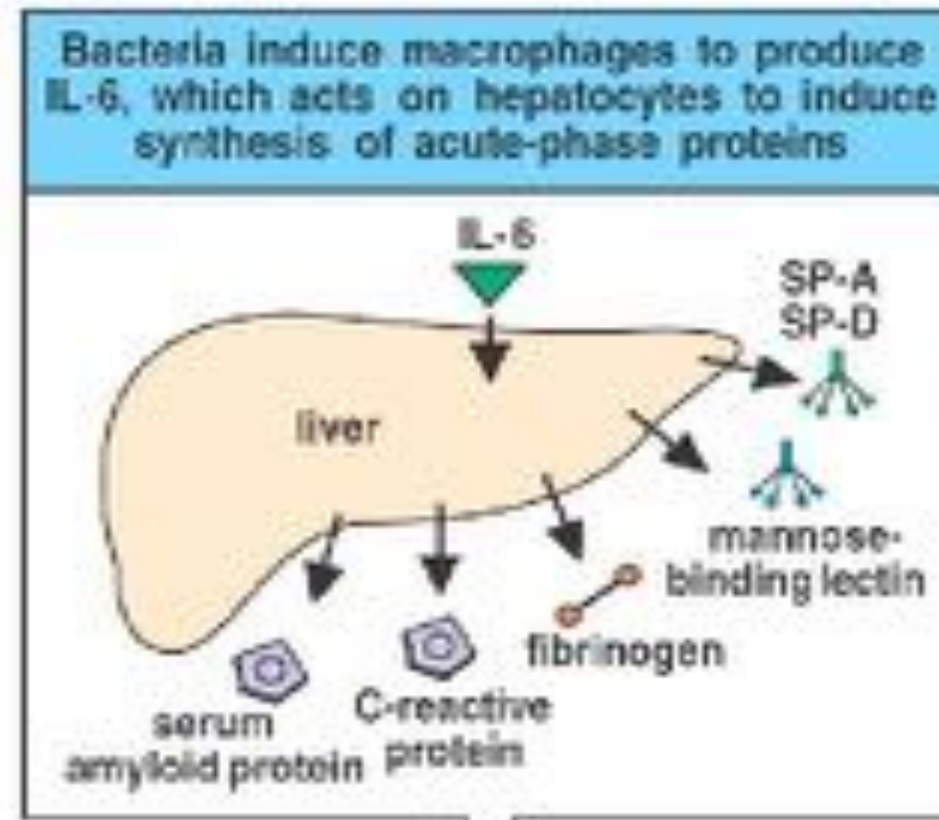
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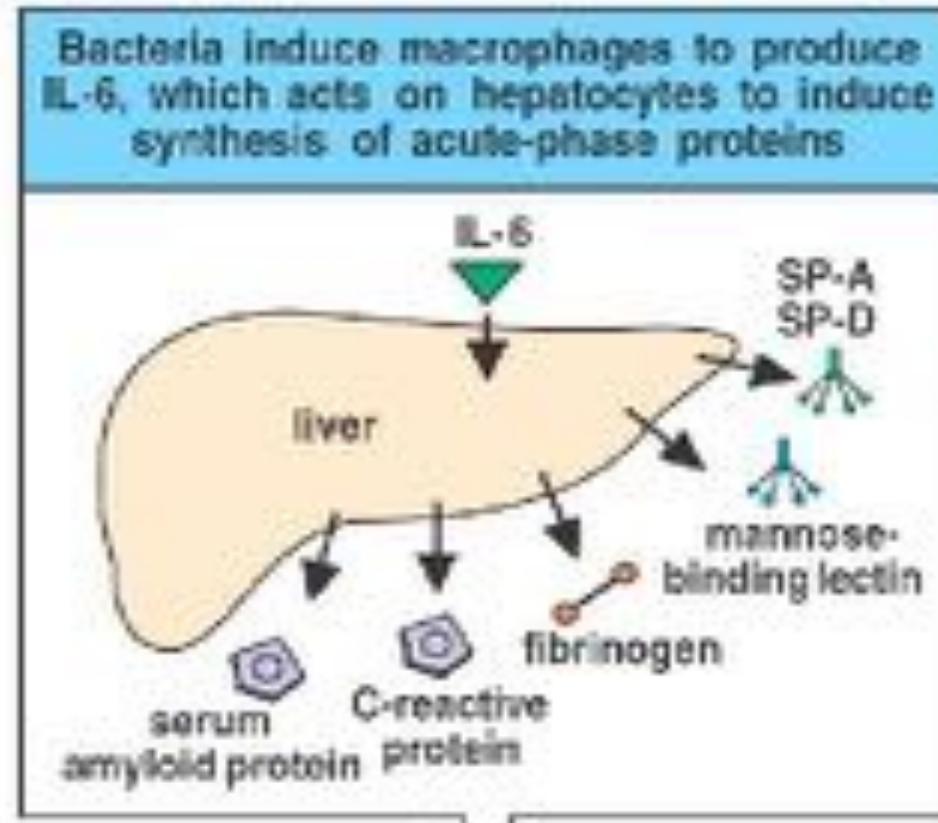


Inflammatory cytokines bind to specific receptors on hepatocytes and induce the activation of the **Janus family kinases (JAK)**, the **STAT (signal transducers and Activators of transcription)** and proinflammatory transcription factors such as **NF- κ B**. Transcription factors most characterized that regulate the synthesis of APP belong to the family of leucine zipper, C/EBPA (**CCAAT/inducer of protein binding**), C/EBPd and **NF-IL6 (nuclear factor associated with IL-6)**, that affect transcription by binding to a site called **bZIP1**. It is also been shown that the induction of acute phase proteins is correlated with a decreased synthesis of C/EBPA and an increase of that of **NF/IL-6**. Post-transcriptional modifications are observed also in the synthesis of APP, which are then secreted.

The positive APP!

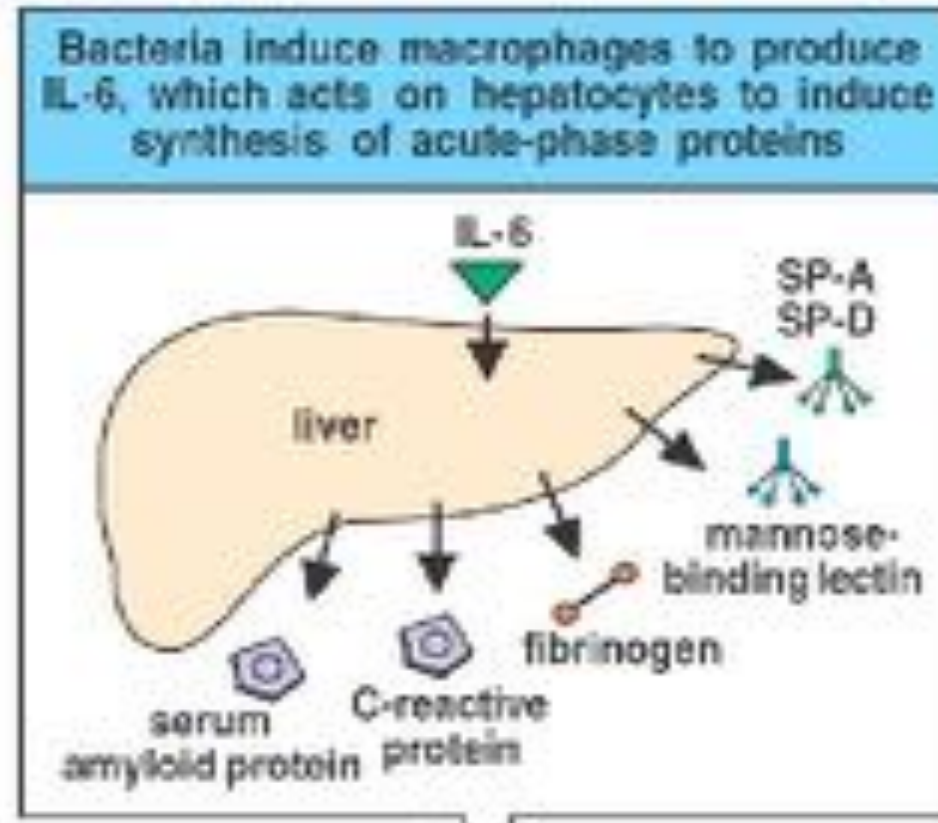


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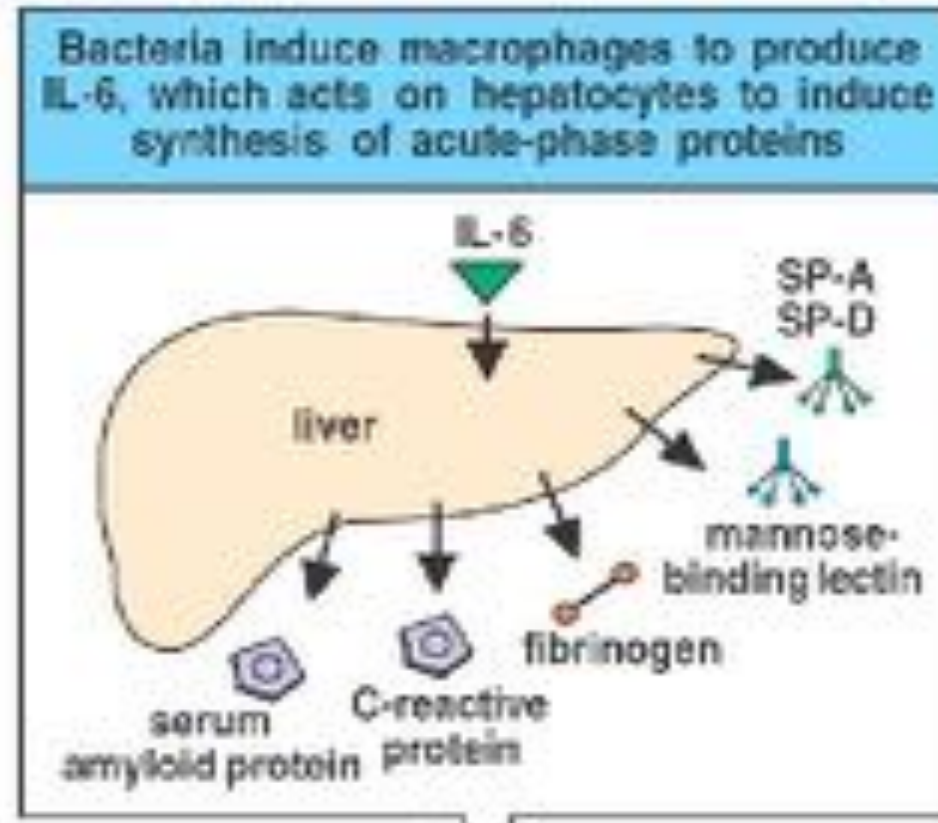
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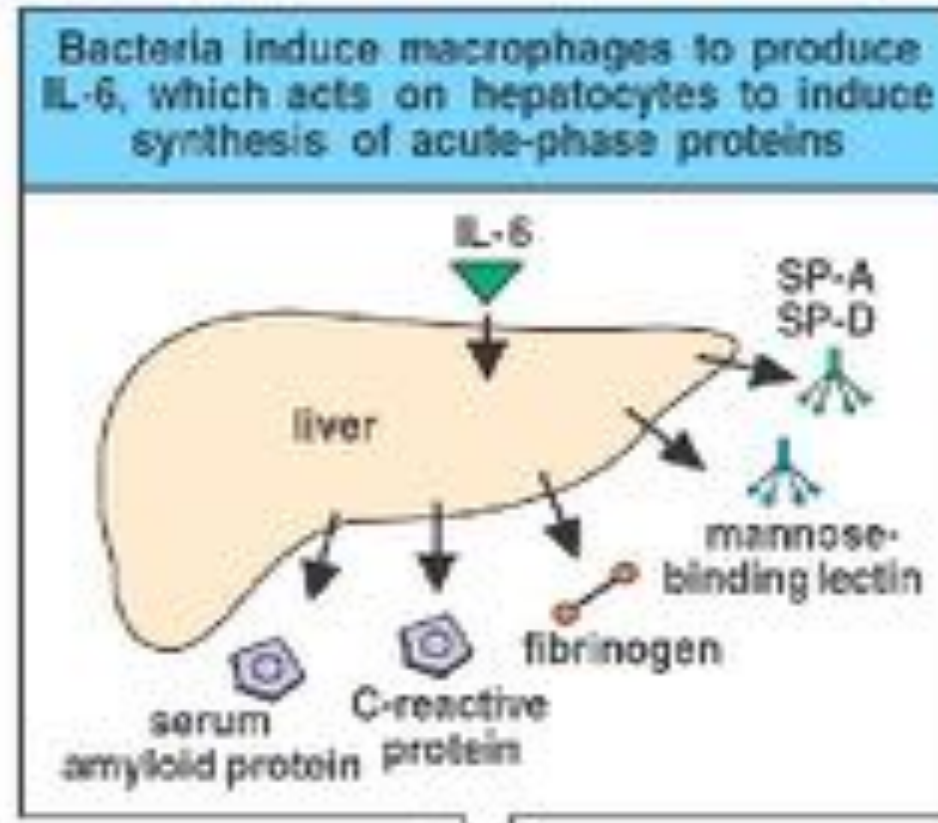
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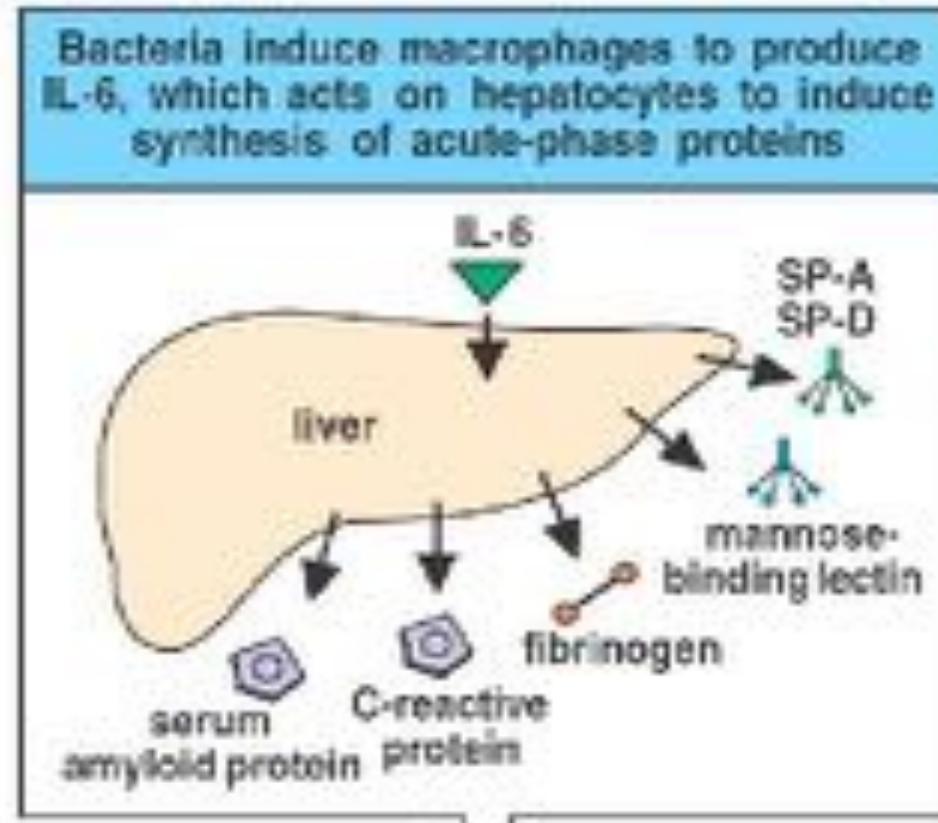
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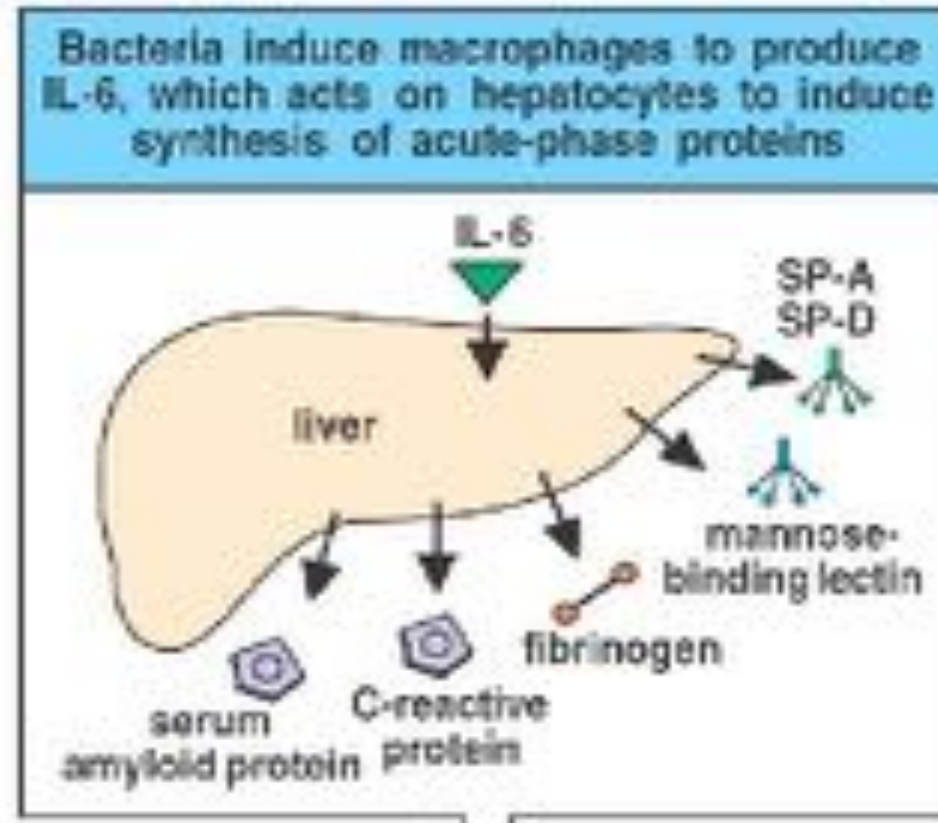


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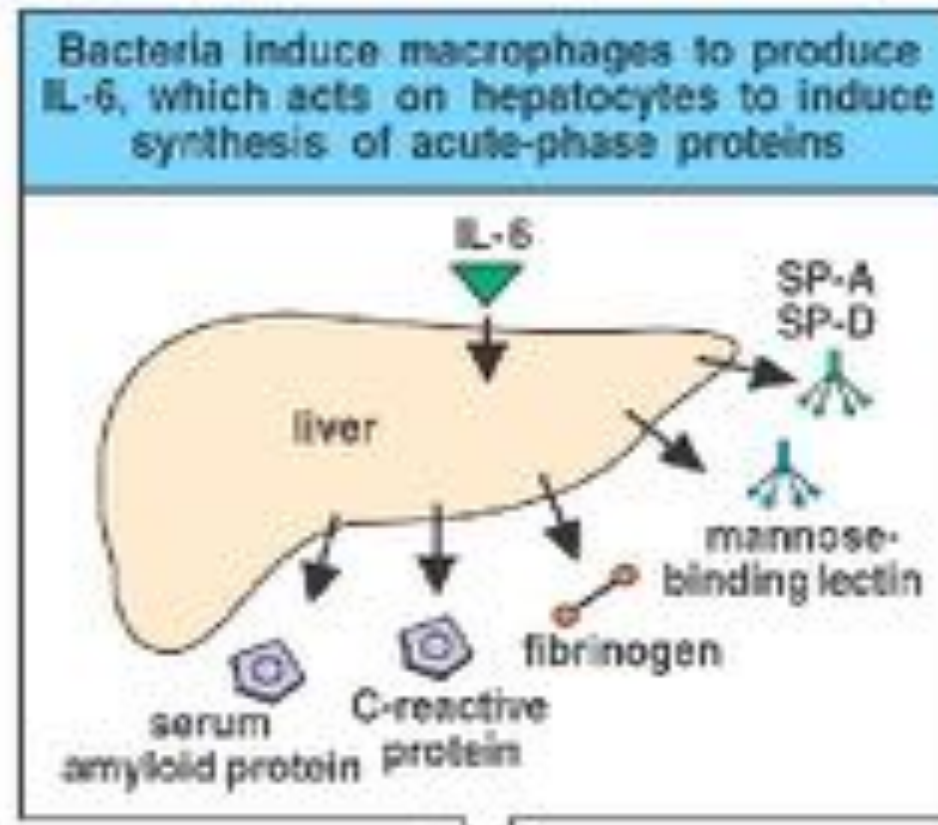


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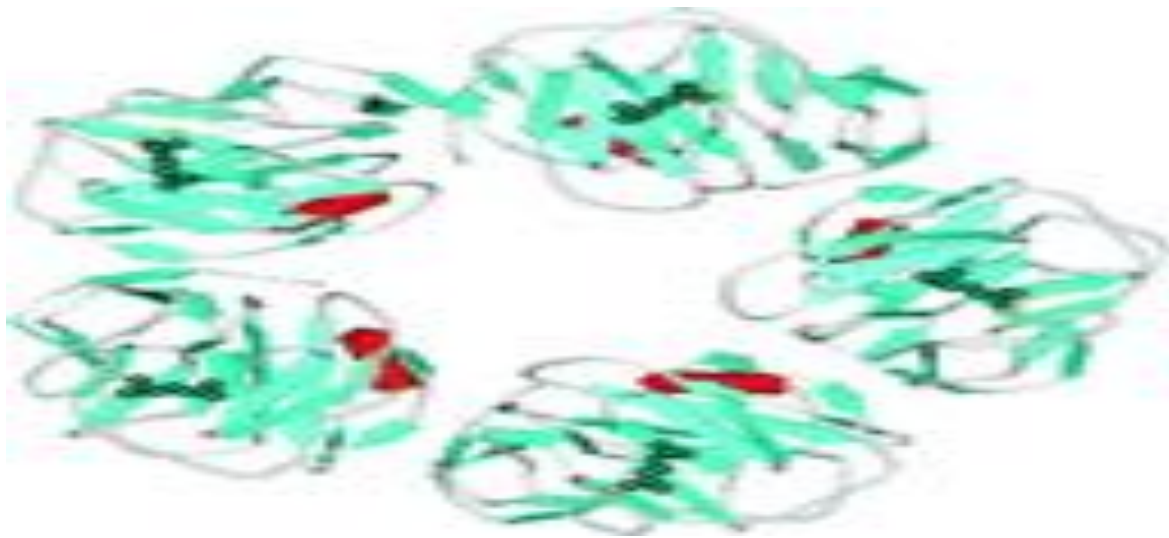
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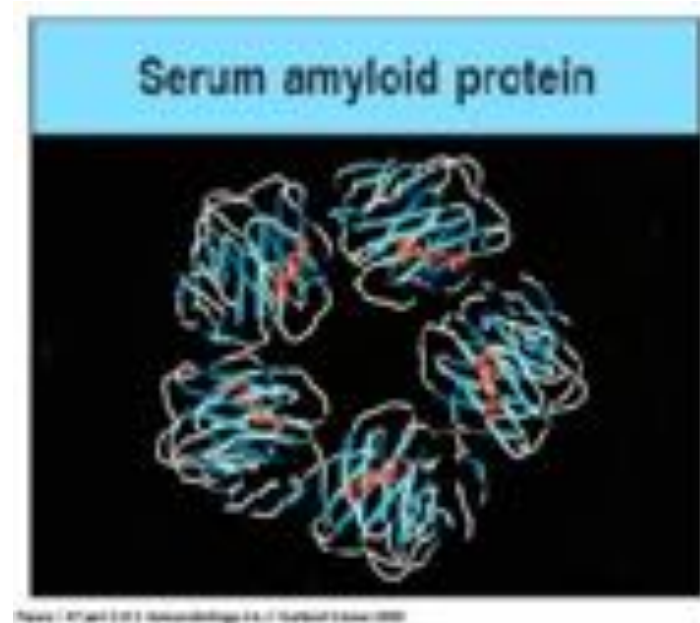
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- g) **Transport proteins**, such as ceruloplasmin, haptoglobin and hemopexin.

THE PENTRAXIN SUPERFAMILY!

- **SHORT PENTRAXINS:**

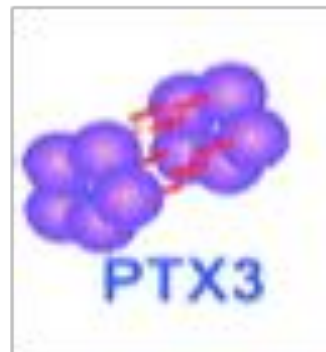


CPR

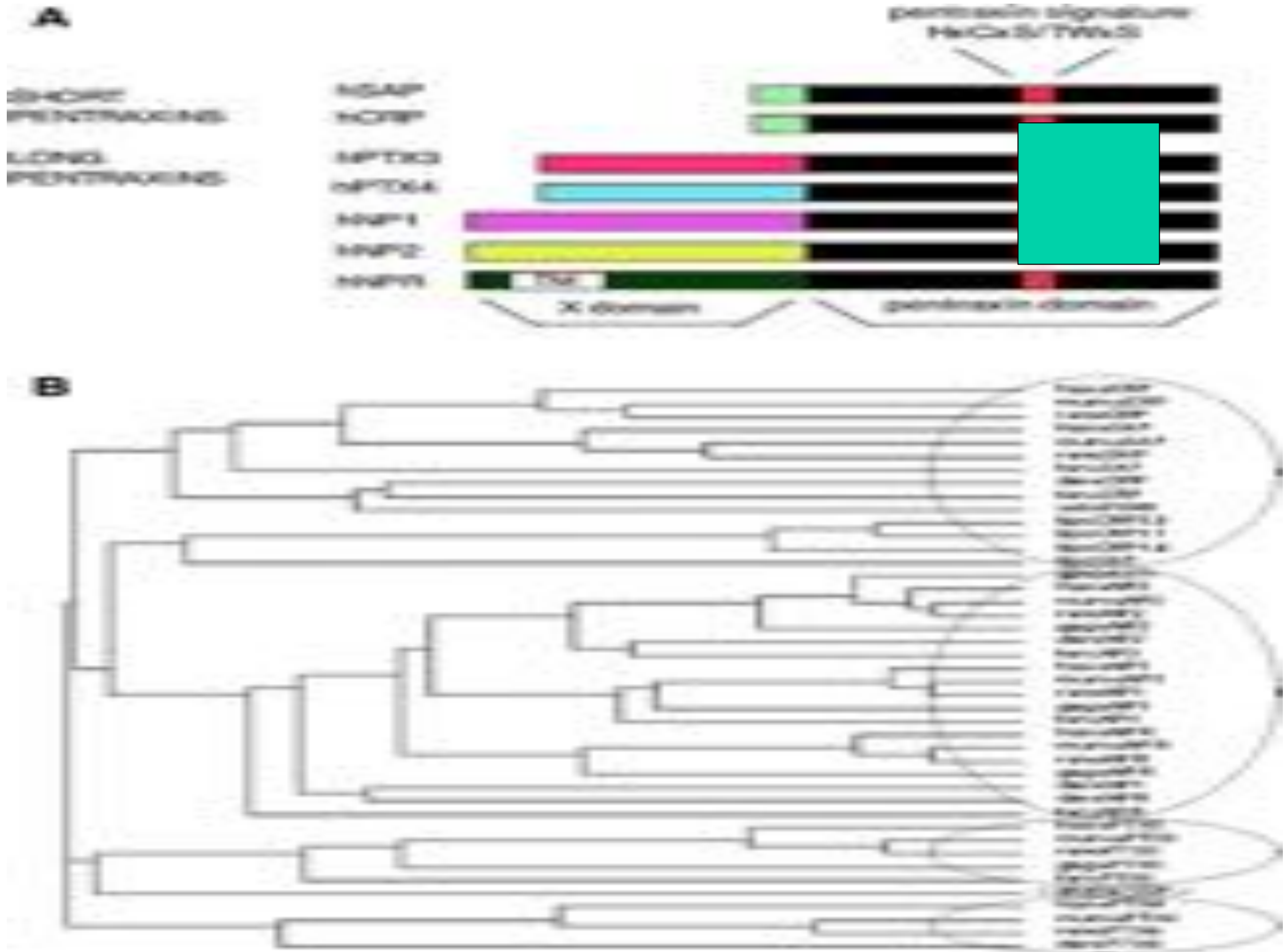


SAP

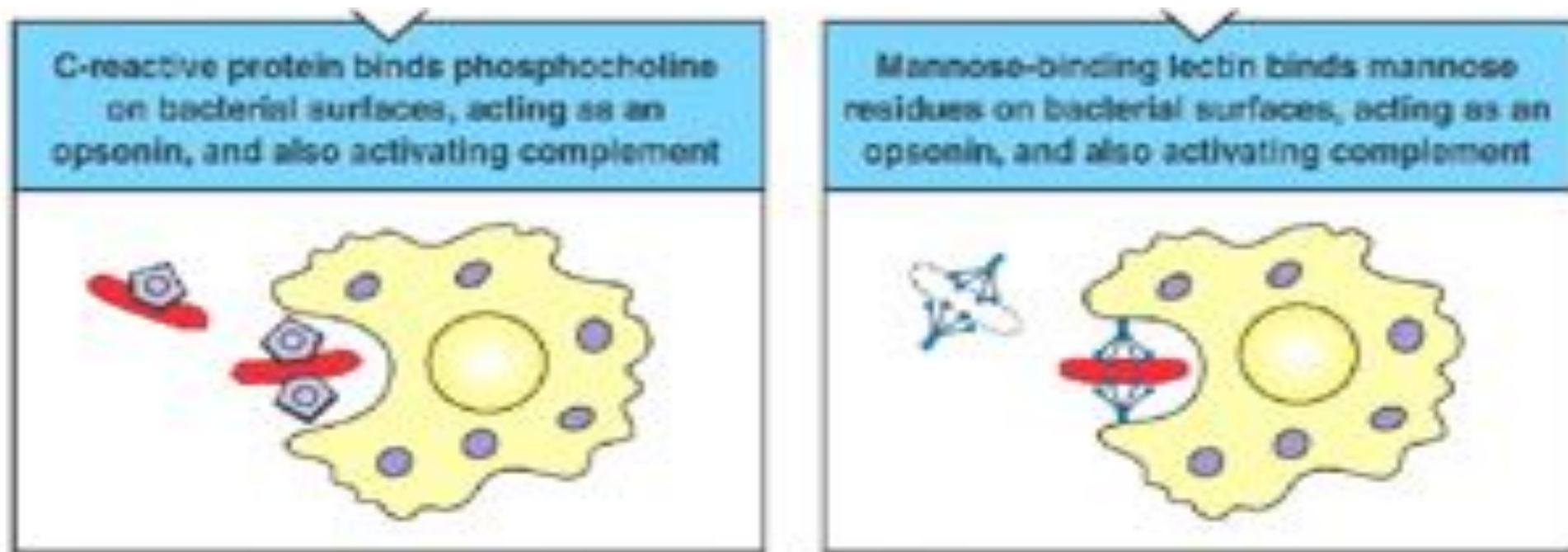
- **LONG PENTRAXINS: PTX3-PTX4**



The pentraxin superfamily!

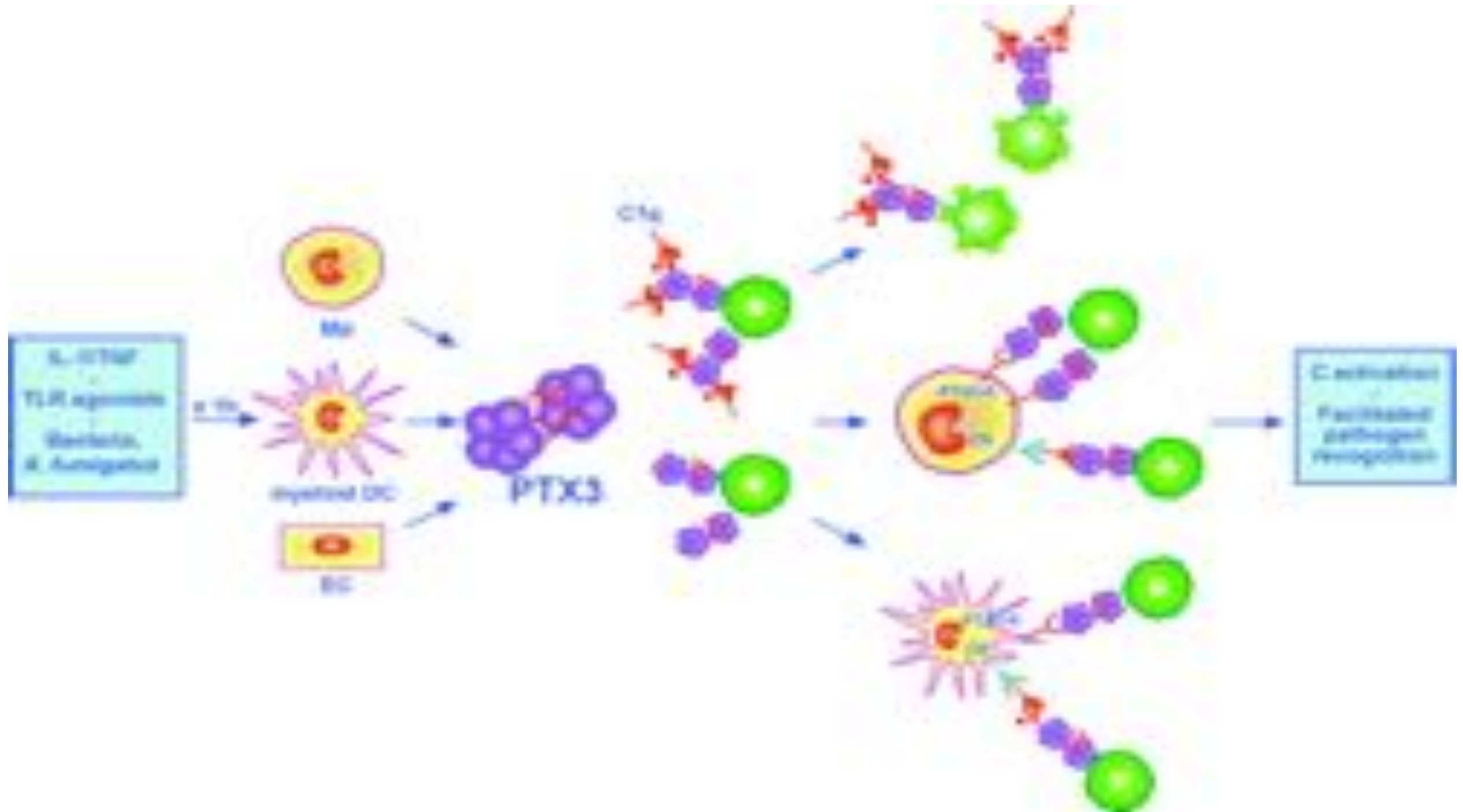


**CPR and SAA binds the phosphorylcholine
of bacteria
and phosphoethanolamine
of apoptotic cells
and activate complement and phagocytosis!**



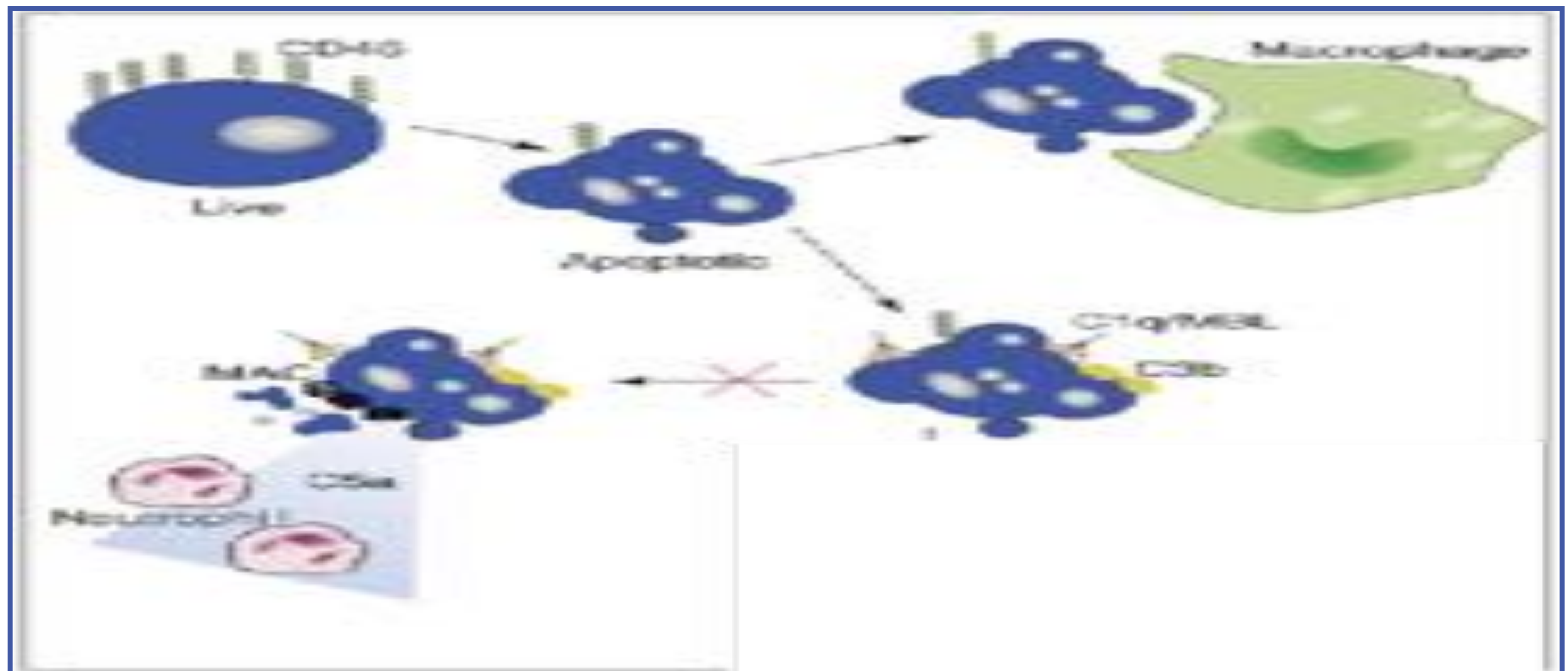
Like CPR and SAA, act PTX3!

Role of the long pentraxin PTX3 in antimicrobial resistance!

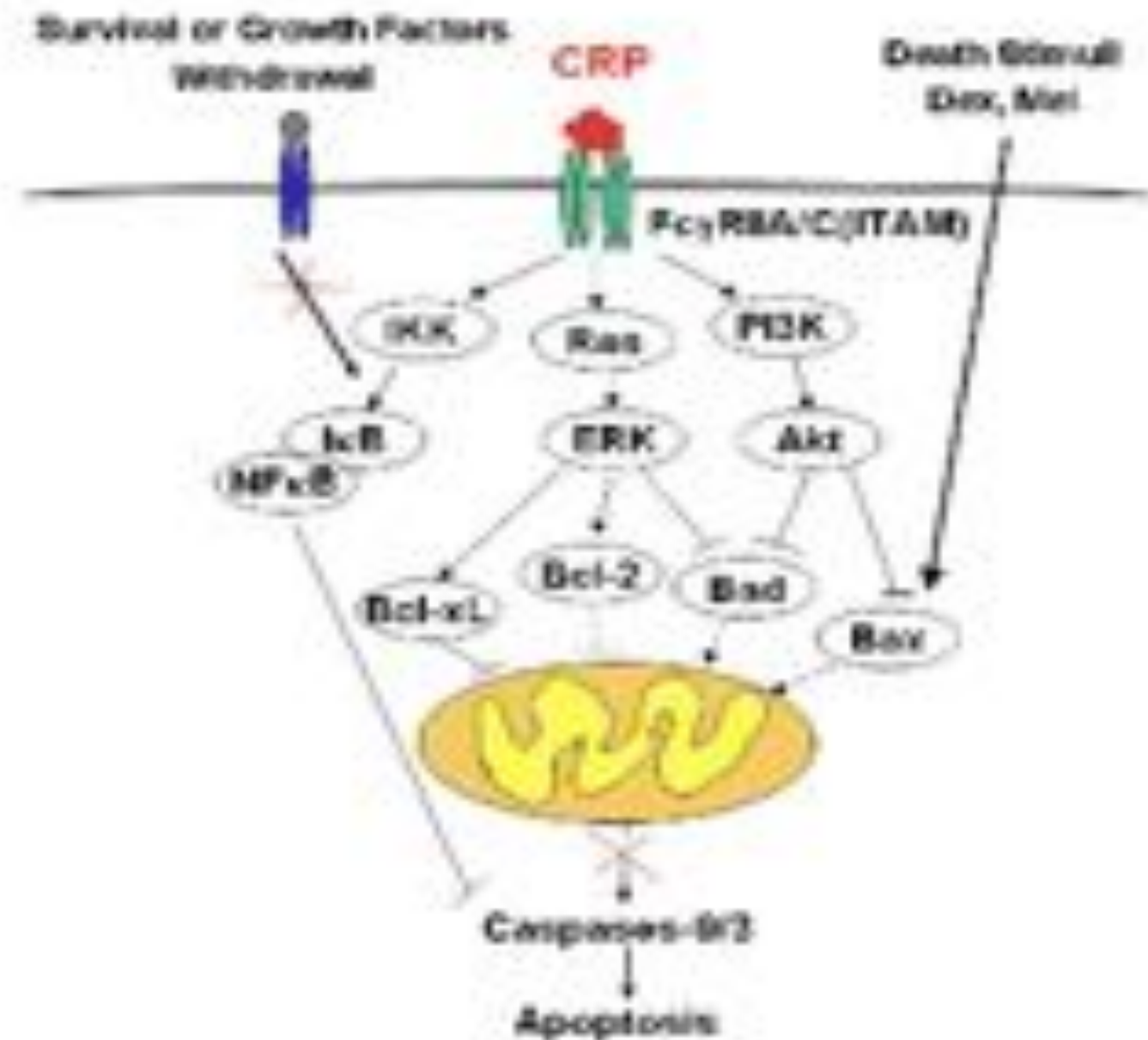
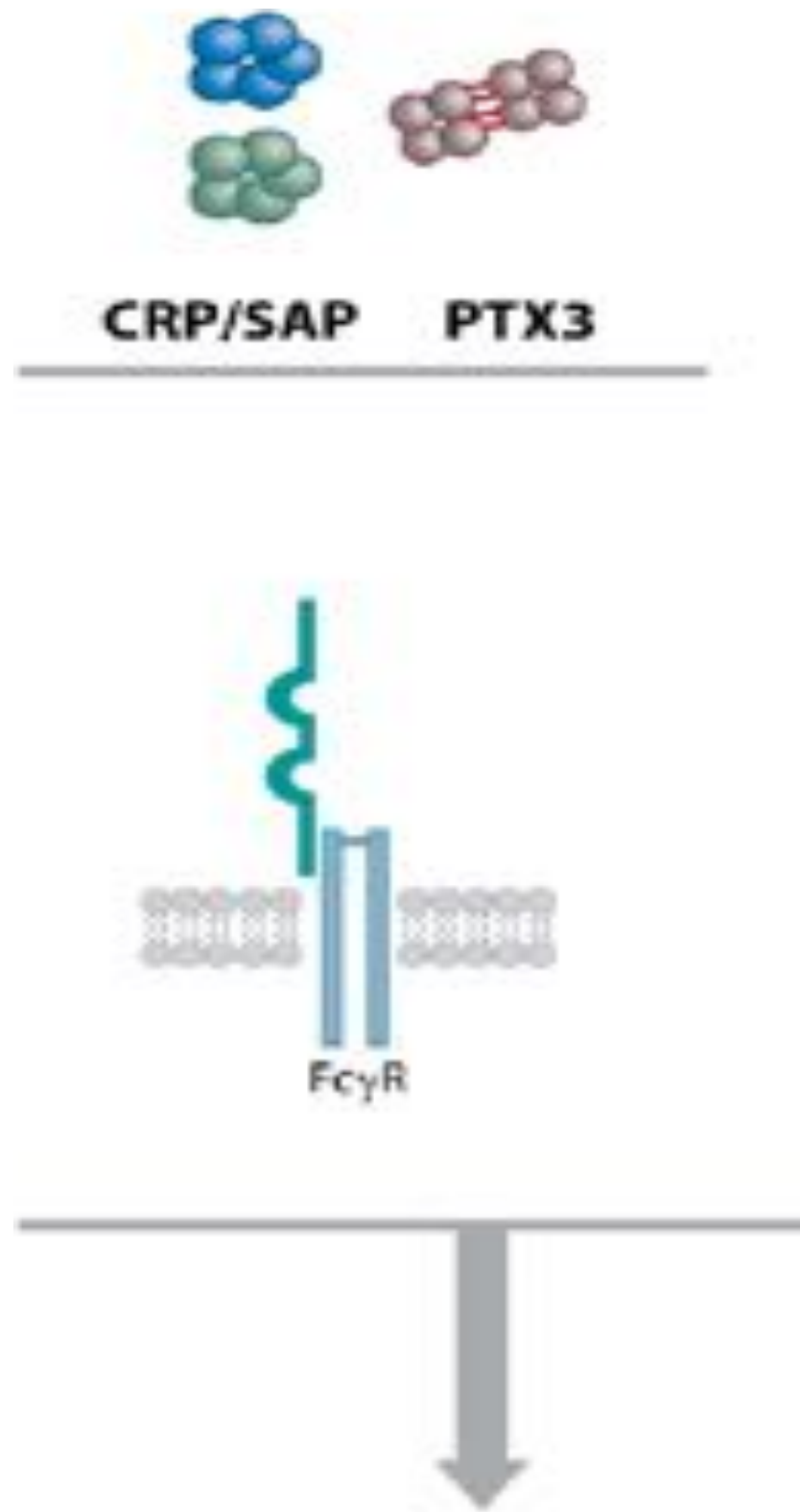


It has recently been shown that C1q, the 1st component of the classical pathway, as well as by antibodies, can be activated by PENTRAXINS:

CLASSICAL COMPLEMENT ACTIVATION IN THE NATURAL IMMUNITY AND INFLAMMATION!

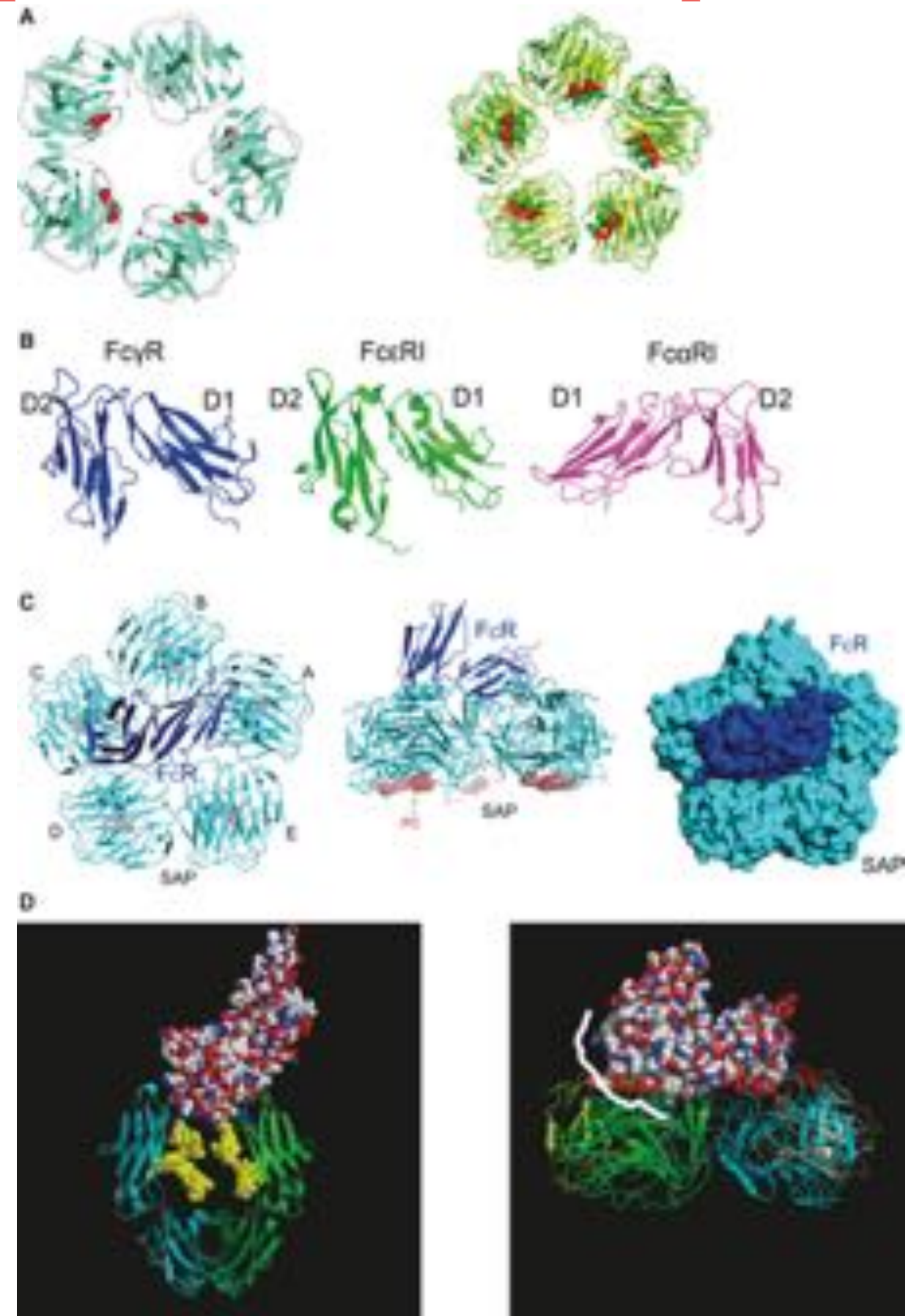


The pentraxins bind to Fc receptors for IgG antibodies and activate phagocytosis!!

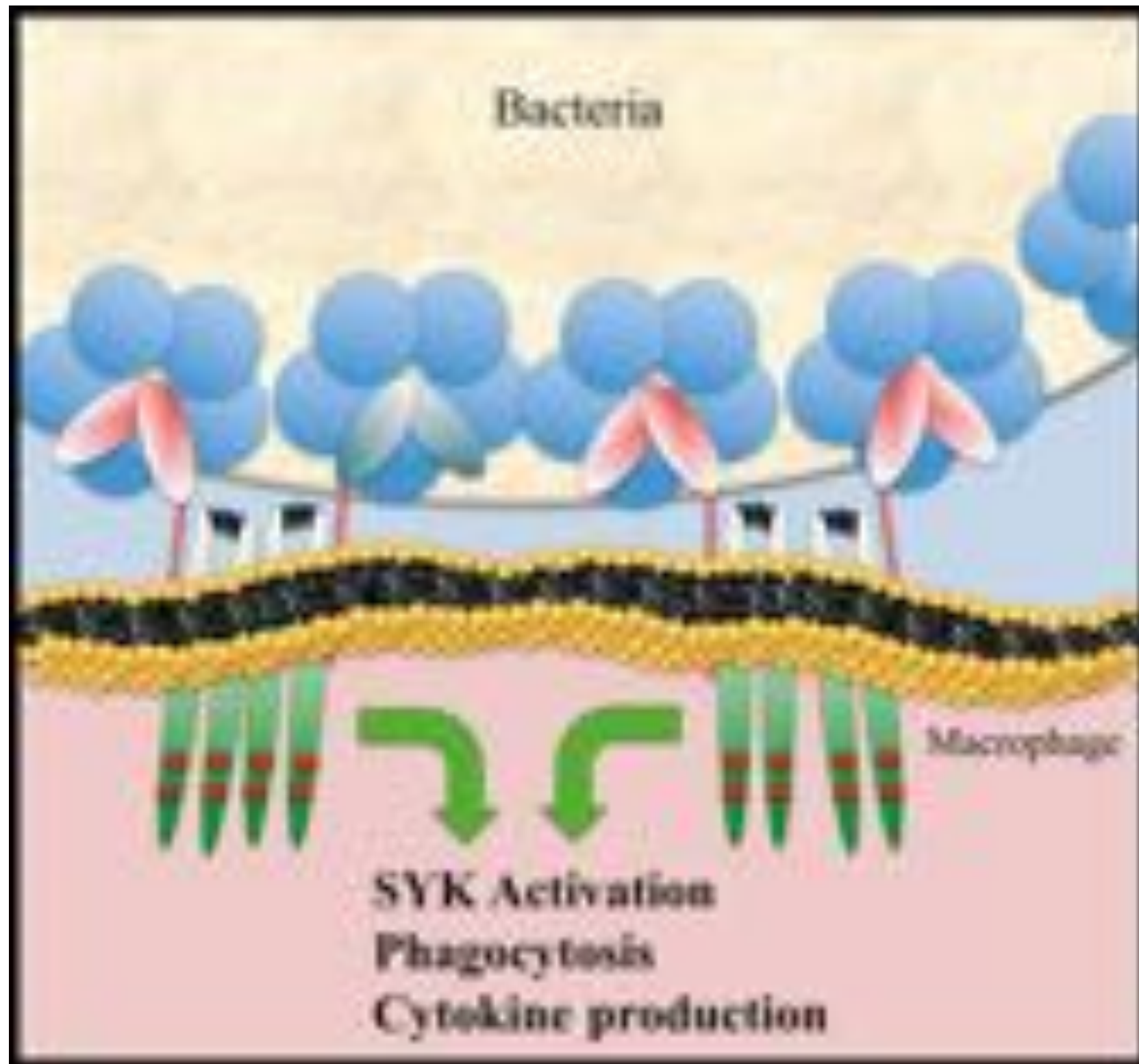


Structural recognition between pentraxins and Fc receptors!

- (A) Crystal structure of phosphocholine bound CRP (PDB entry 1B09, left) and the structural superposition between CRP and SAP (right).
- (B) Structures of FcγRIIA, FcεRI (PDB entry 1F2Q), and FcαRI (PDB entry 1QVZ).
- (C) Structural complex between human SAP (cyan) and FcγRIIA (blue) in two orthogonal views (left and middle panels) and in space filling model (right panel).
- (D) Binding mode of IgG–Fc on Fc receptor (left panel) partially overlap with that of SAP (right panel). The IgG–Fc interface region is highlighted in white line on the SAP complex structure.



Model of the binding of the pentraxin to FC receptor for IgG antibodies!

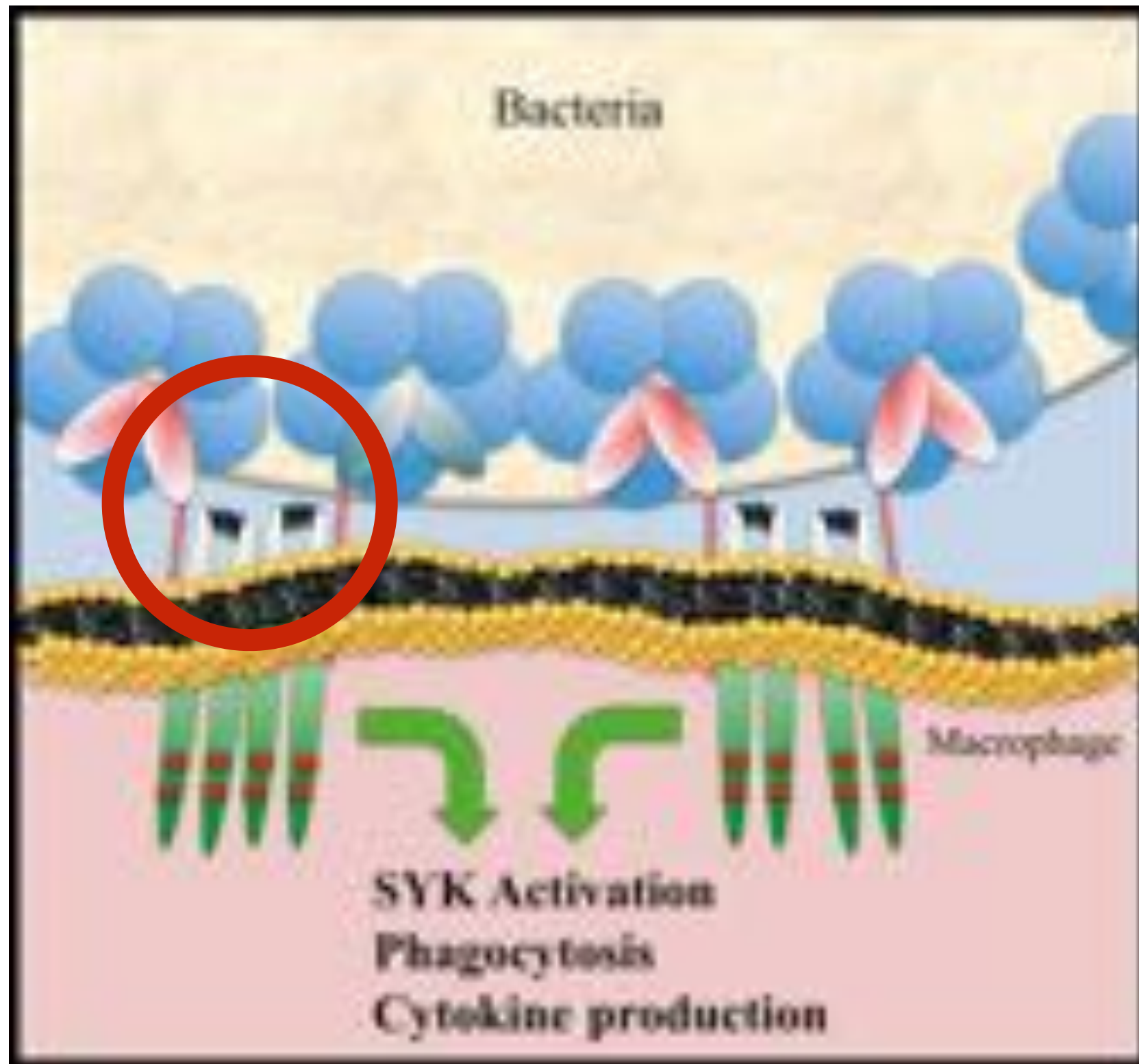


Immunological Reviews

Volume 250, Issue 1, pages 230-238, 10 OCT 2012 DOI: 10.1111/j.1600-065X.2012.01162.x

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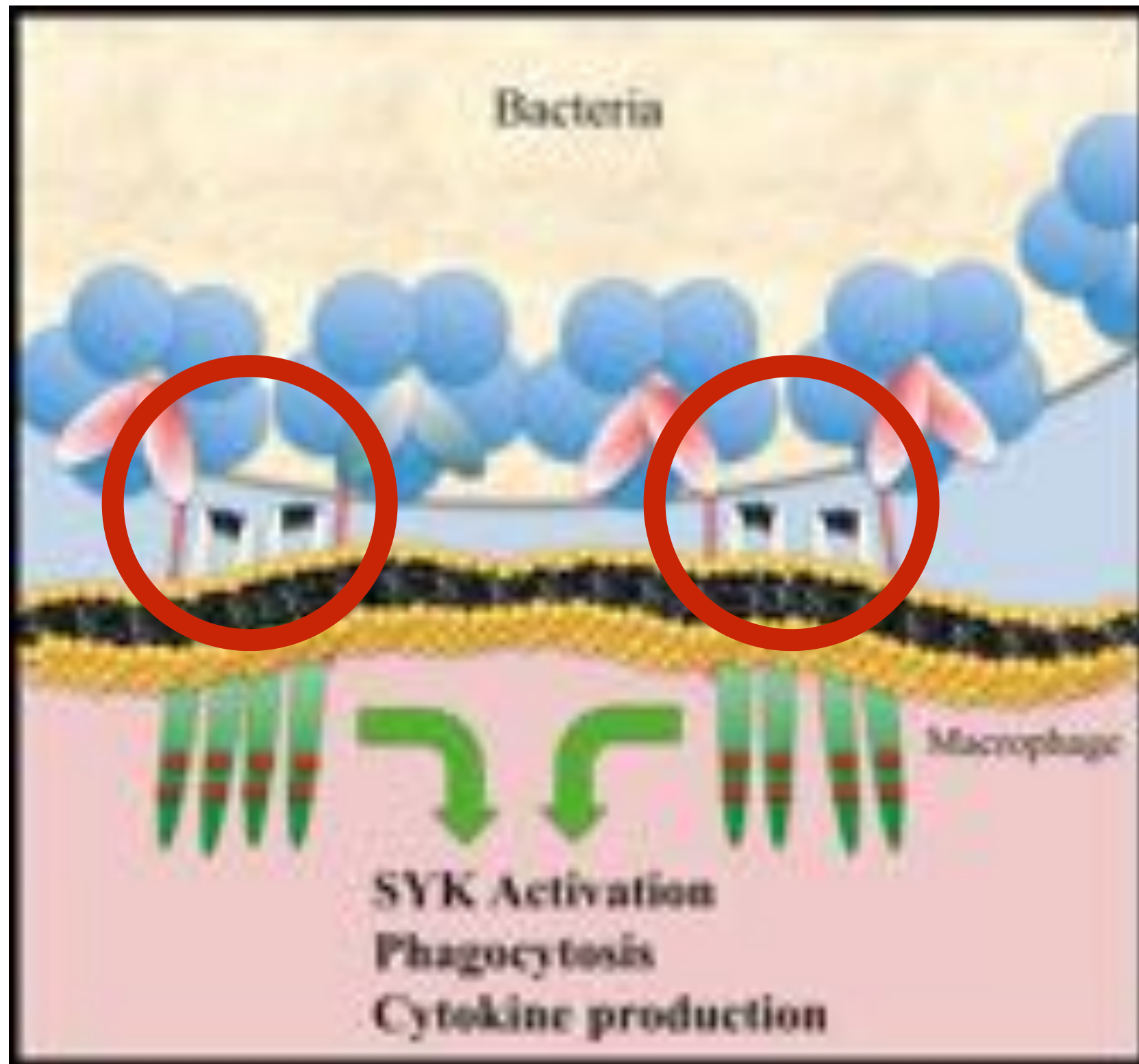


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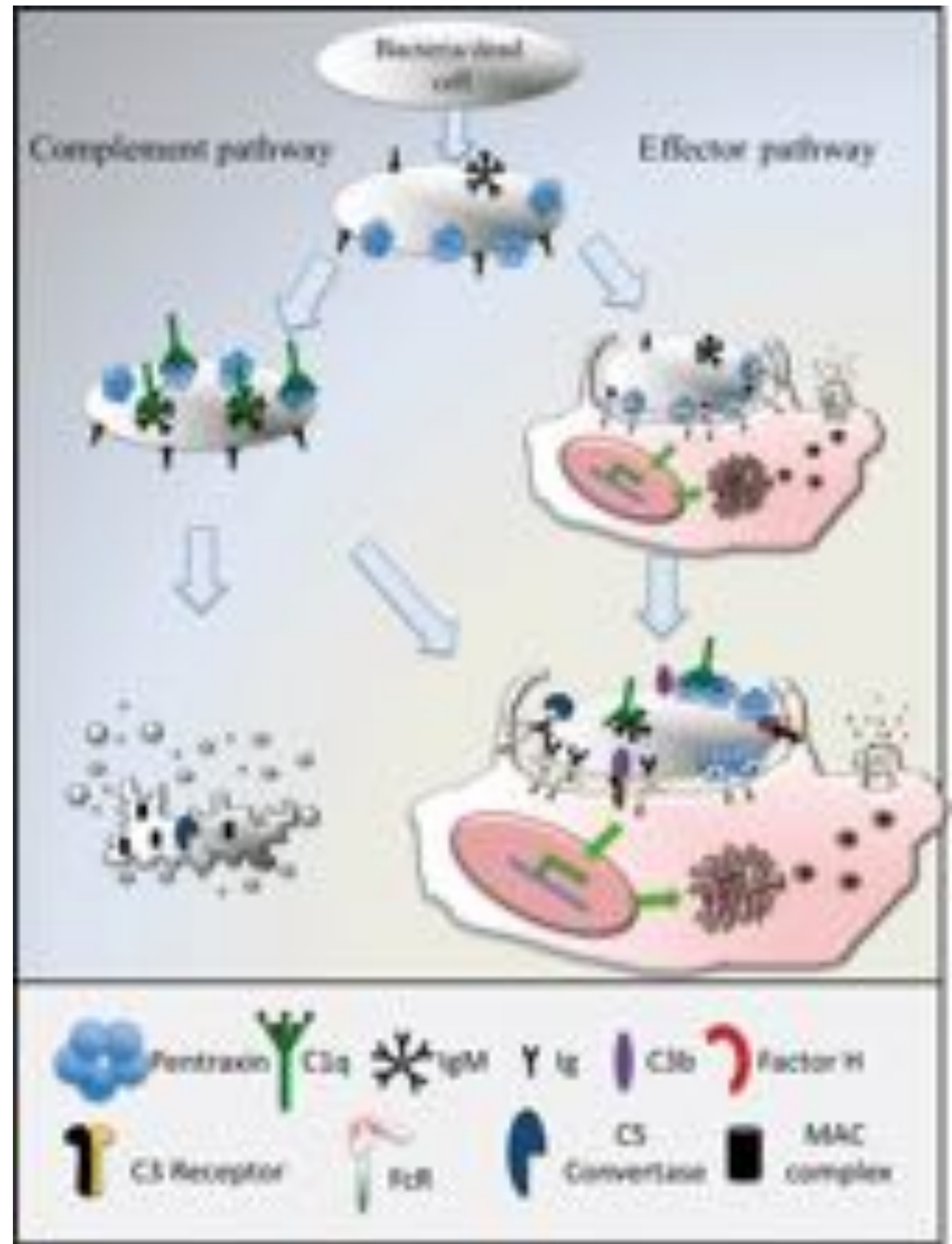
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Comparison of pentraxins and antibodies in complement and Fc receptor activation!



NATURAL IMMUNITY EVOLUTION!

Pentraxins and Fc receptors

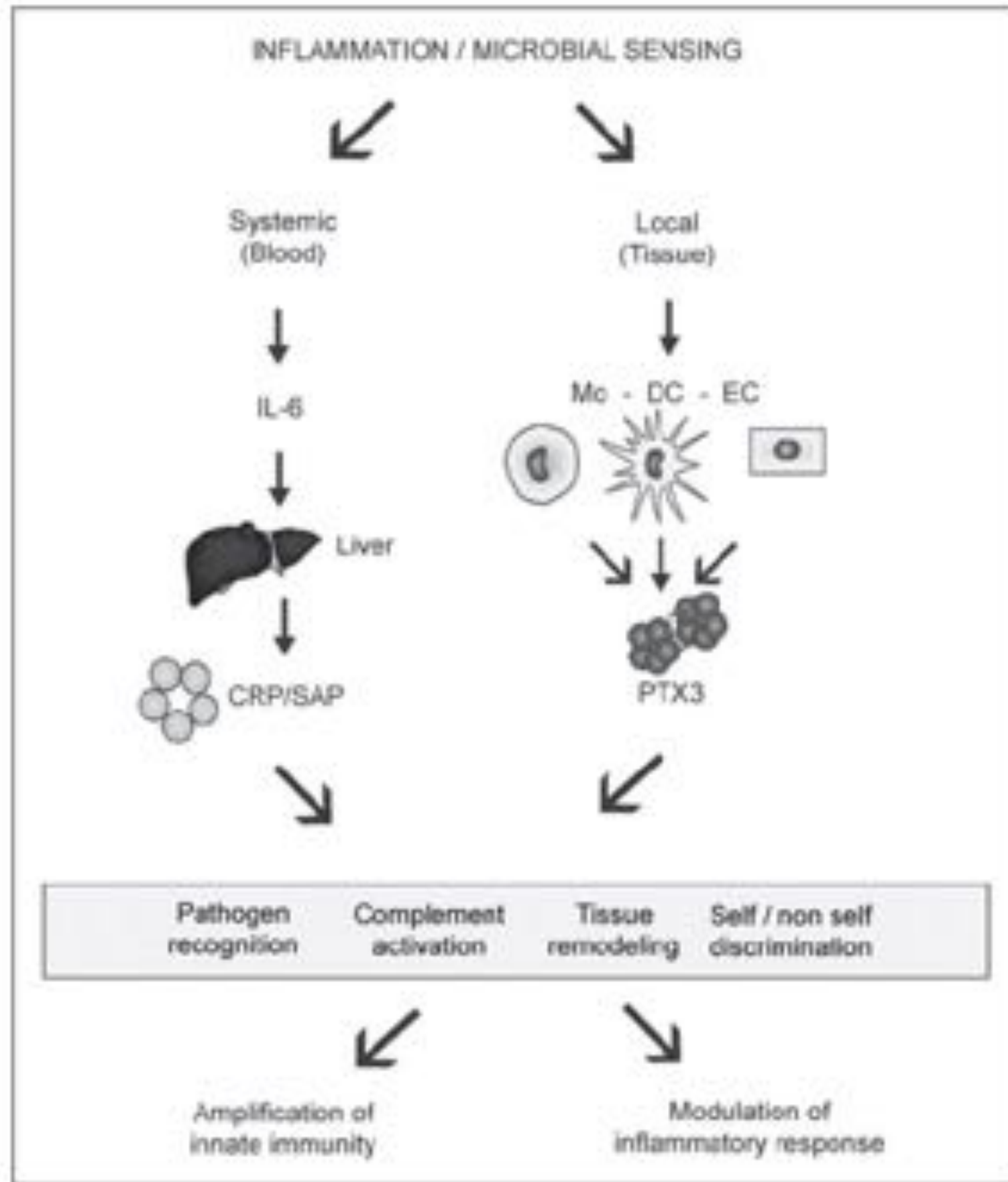


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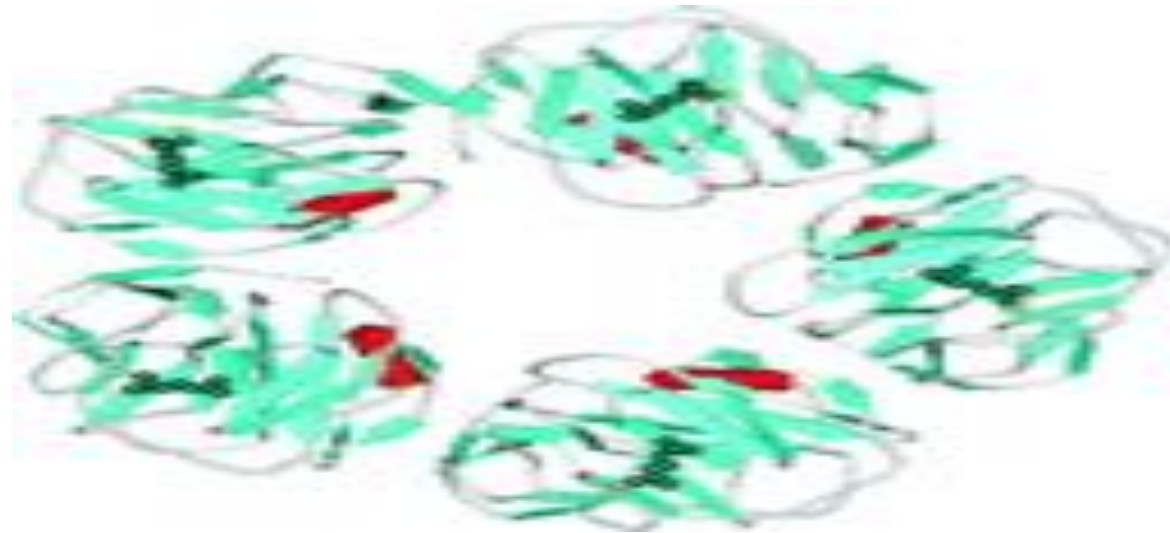
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THE SHORT AND LONG PENTRAXINs EXPRESS SIMILAR FUNCTIONS BUT DIFFERENT PRODUCTION AND LOCALIZATION!

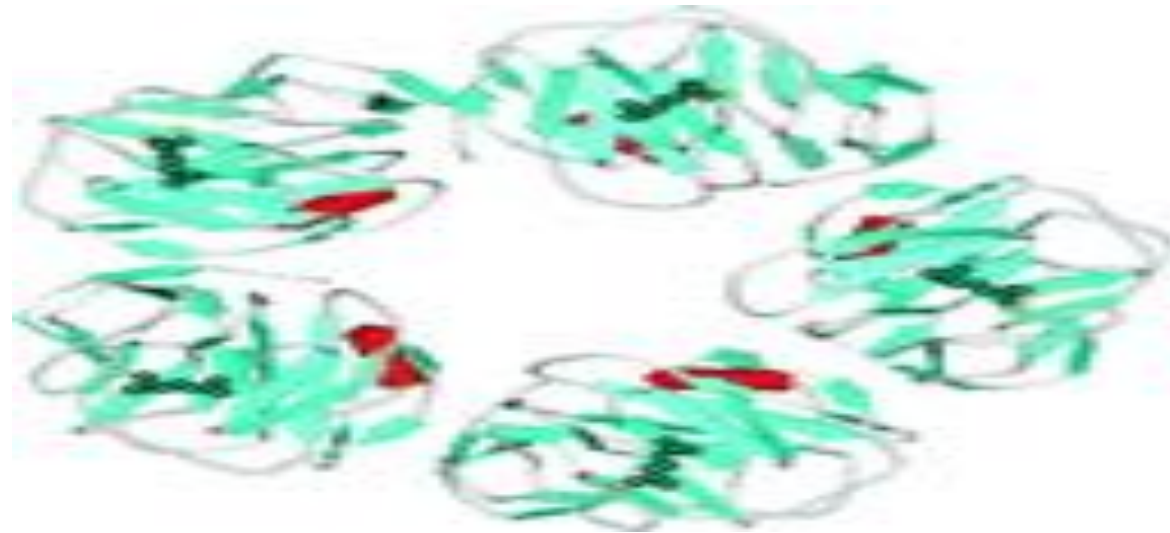


THE SHORT PENTRAXIN CPR IS THE MAJOR POSITIVE APP!



Its physiological concentration is less than 1 μ g/ml (100 ng/ml at birth, 170 ng/mL in children and from 470 to 1340 ng/mL in adults), but increases by 100-1000 times during inflammation.

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Although for a long time CRP levels have been used as a **quick test for the presumptive diagnosis of bacterial infection (high CPR) distinct from viral infection (low CPR)**, today a clear increase of the PCR can be observed in viral hepatitis, in bacterial acute flu-like syndromes, in active TB, gout, in burns, in peritonitis, in rheumatic fever, rheumatoid arthritis, and a less significant increase occurs in scarlet fever and Guillon-Barré syndrome. **CPR is often used by rheumatologists to follow the progress or remission of autoimmune diseases and by cardiologists and clinicians who use it to predict cardiovascular complications of atherosclerosis.**

Elevated serum C-reactive protein level predicts a poor prognosis for recurrent gastric cancer.

Kong F, Gao F, Chen J, Zheng R, Liu H, Li X, Yang P, Liu G, Jia Y.

BACKGROUNDS:

High serum C-reactive protein (CRP) was found to be associated with poor prognosis in kinds of solid tumors, however, its role in the recurrent gastric cancer (RGC) is unknown. The present study aimed to explore the prognostic value of serum CRP in RGC patients.

METHODS:

A total 72 RGC patients who underwent radical surgery from January 2005 to May 2008 were enrolled. The clinical, pathological and survival information were collected. The serum CRP level was measured when the recurrence was confirmed, and the association between serum CRP and clinicopathological characters was analyzed. The prognostic value of serum CRP for RGC was investigated.

RESULTS:

The serum CRP was elevated in 39 patients (H-CRP), while 33 patients were within the normal range (N-CRP). The elevated CRP was associated with Lymph node metastasis ($p = 0.003$) and tumor size ($p = 0.004$). The median survival time after recurrence was significantly worse in the H-CRP group than N-CRP group (6.5 months vs. 11.5 months, $p = 0.012$). Multivariate analyses identified that elevated CRP level ($HR=2.325$, $p < 0.001$), time to recurrence ($HR = 0.466$, $p=0.033$), and the follow-up treatment ($HR = 2.650$, $p=0.001$) were independent prognostic factors.

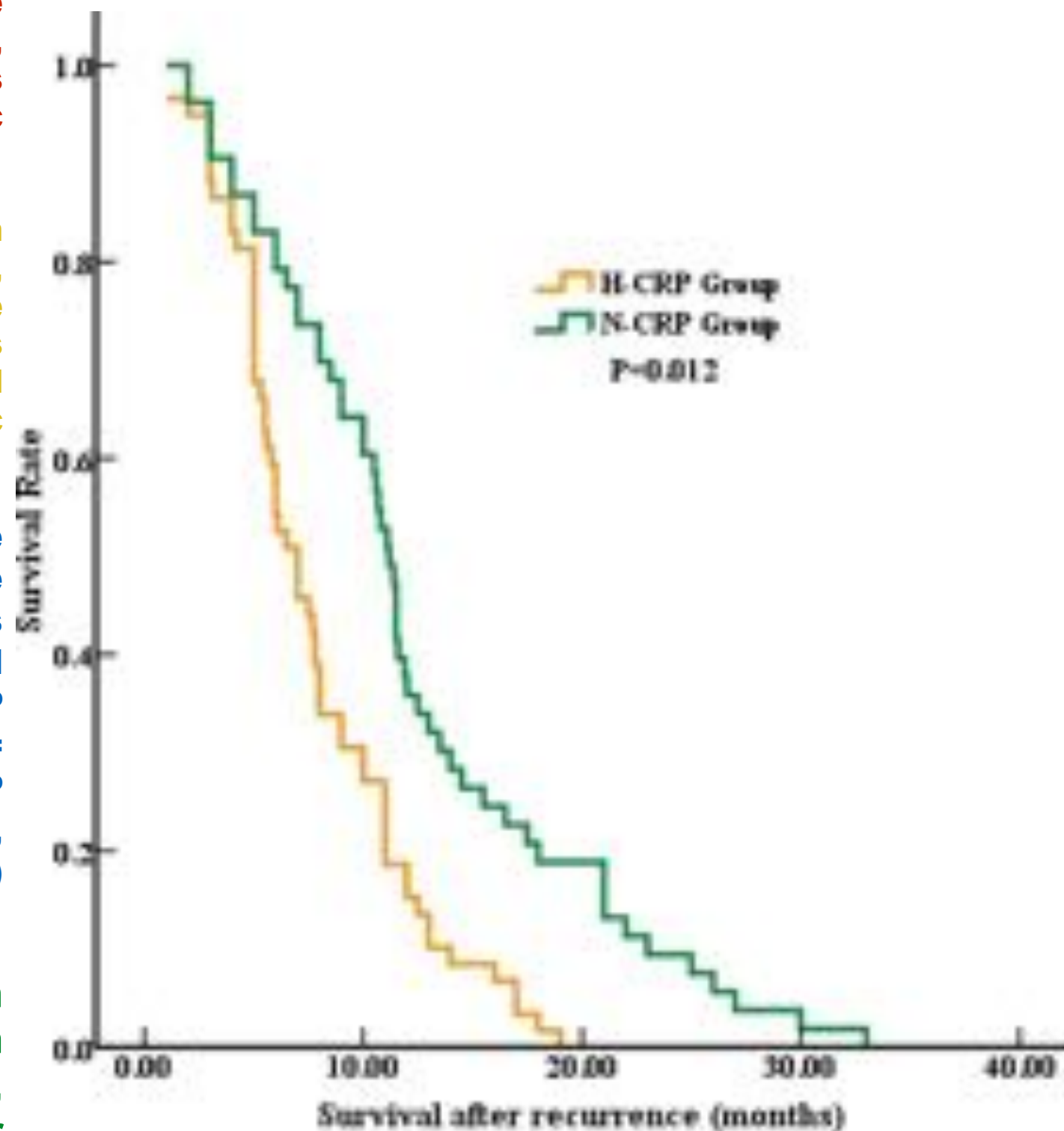
CONCLUSIONS:

High serum CRP level was associated with aggressive pathological features, was an independent poor prognostic factors for RGC, which might be a potential prognostic marker for RGC patients.

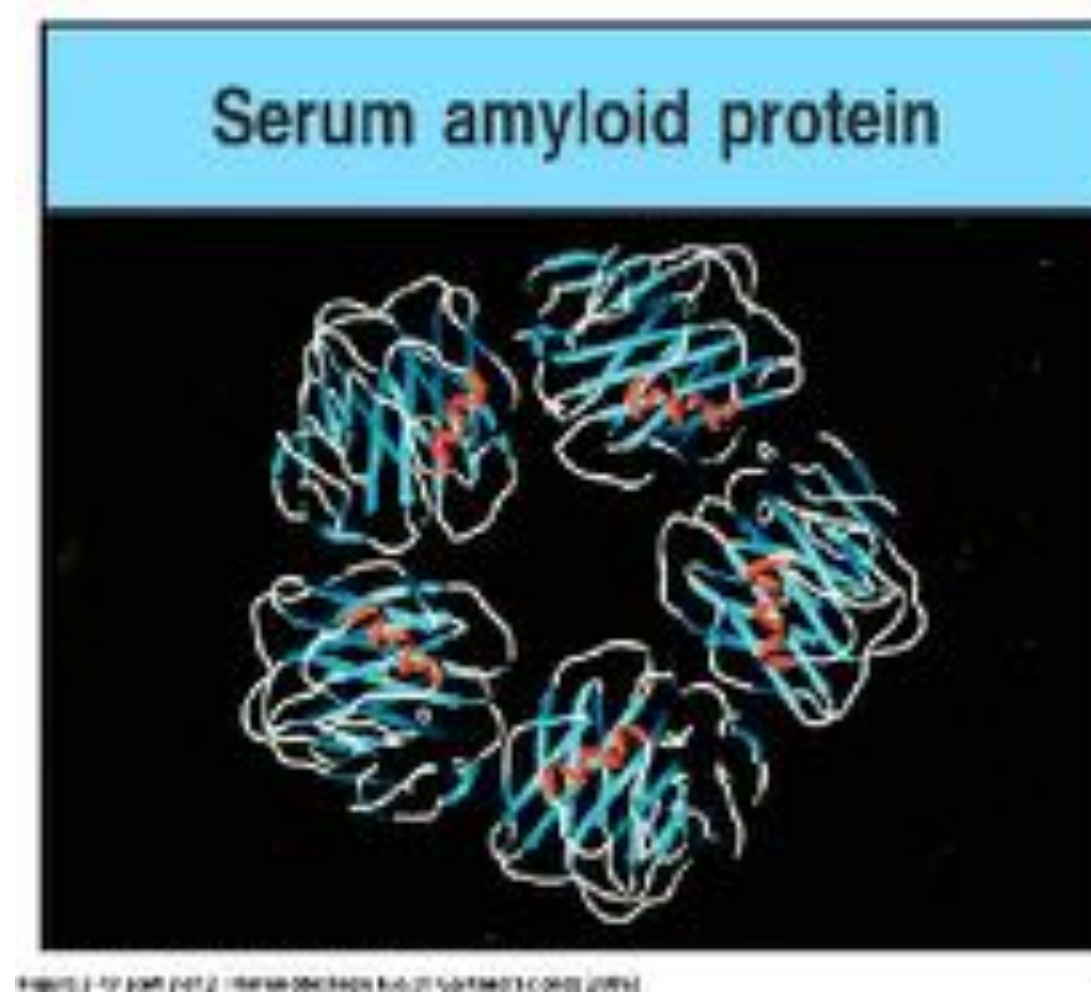
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C-reactive protein; prognosis; recurrent gastric cancer

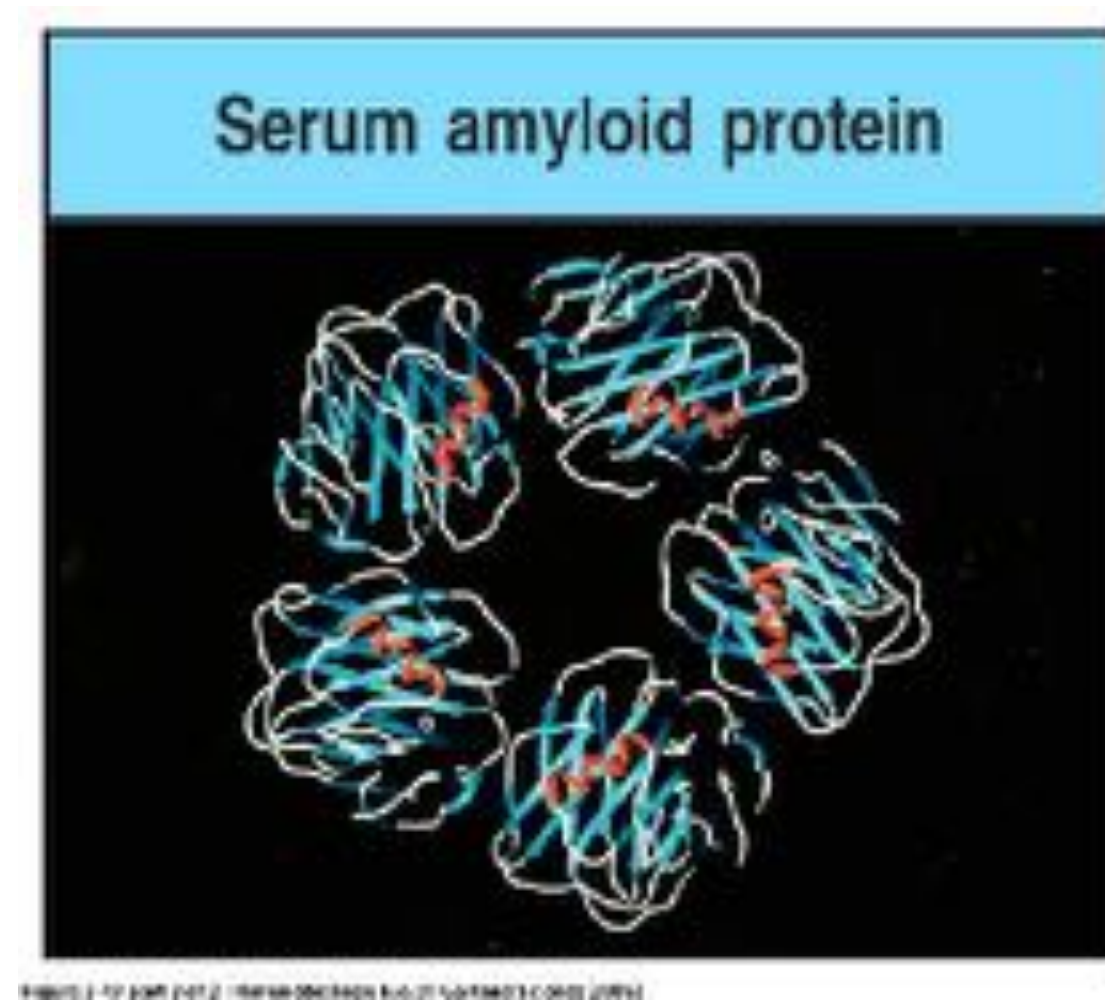
[Oncotarget. 2016 Aug 23; 7\(34\): 55765–55770.](#)



THE SHORT PENTRAXIN SAP or SAA IS ANOTHER POSITIVE APP!



**THE SHORT PENTRAXIN SAP or SAA IS ANOTHER
POSITIVE APP!**



Source: U.S. Census Bureau, *Marriage, Divorce, Remarriage in the 1990s*, Washington, D.C., 1995.

Many authors have reported the existence of various types of SAA that can be classified in: CSAA (constitutive SAA) and ASAA (Acute phase SAA).

These latter have:

- immunological functions, such as promoting the lysis of apoptotic cells; promoting phagocytosis and adhesion and chemotaxis of leukocytes; inducing ECM-degrading enzymes (collagenase, stromalysin, MMP2 and 3) and inflammatory cytokines (IL-6 , TNF- α);
- functions associated with lipids, as transport lipids to the cells to increase their metabolism during tissue regeneration and their removal in the sites of damage.

Clinical importance of determination of serum amyloid A!

Clinical importance

1

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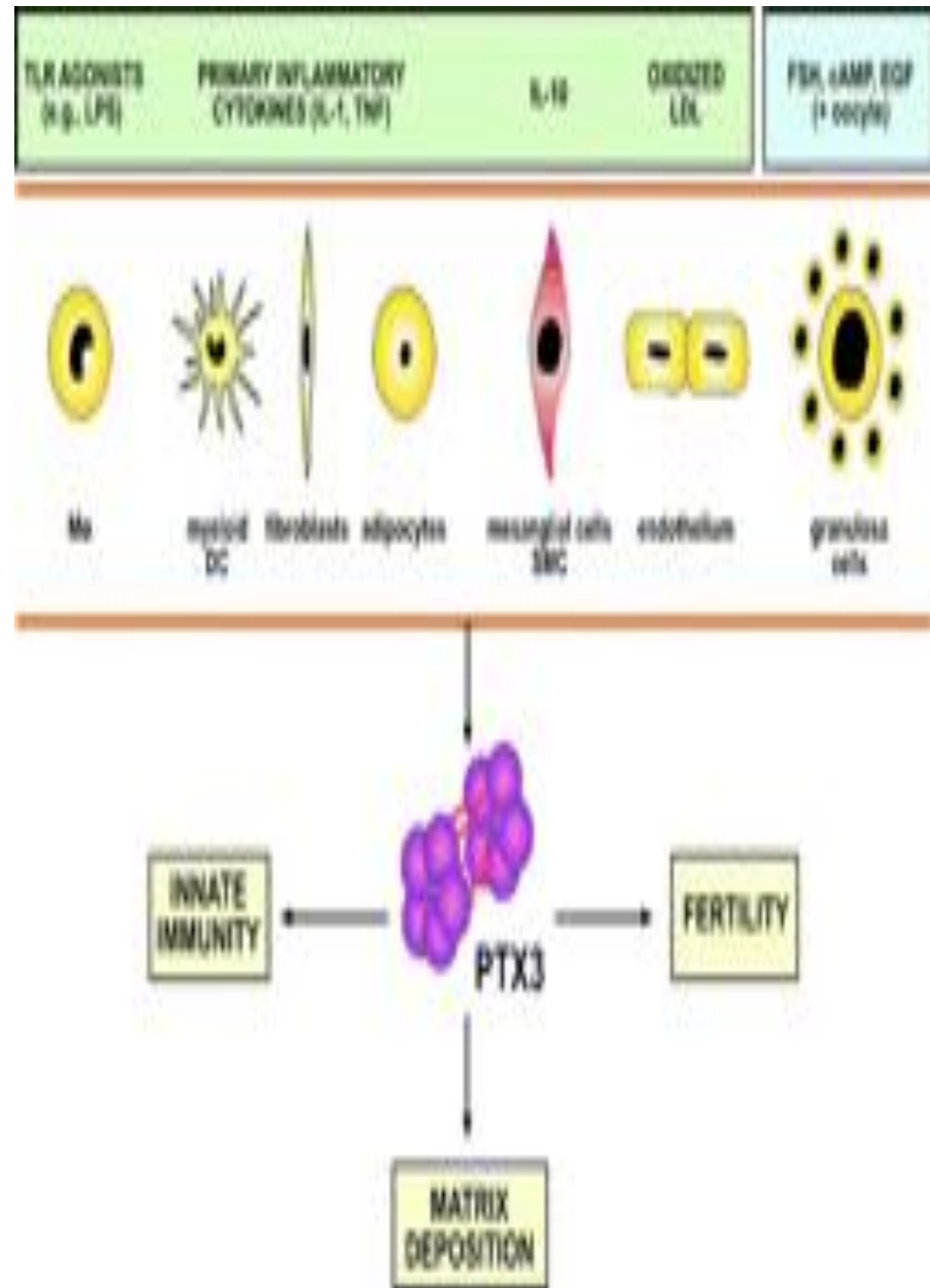
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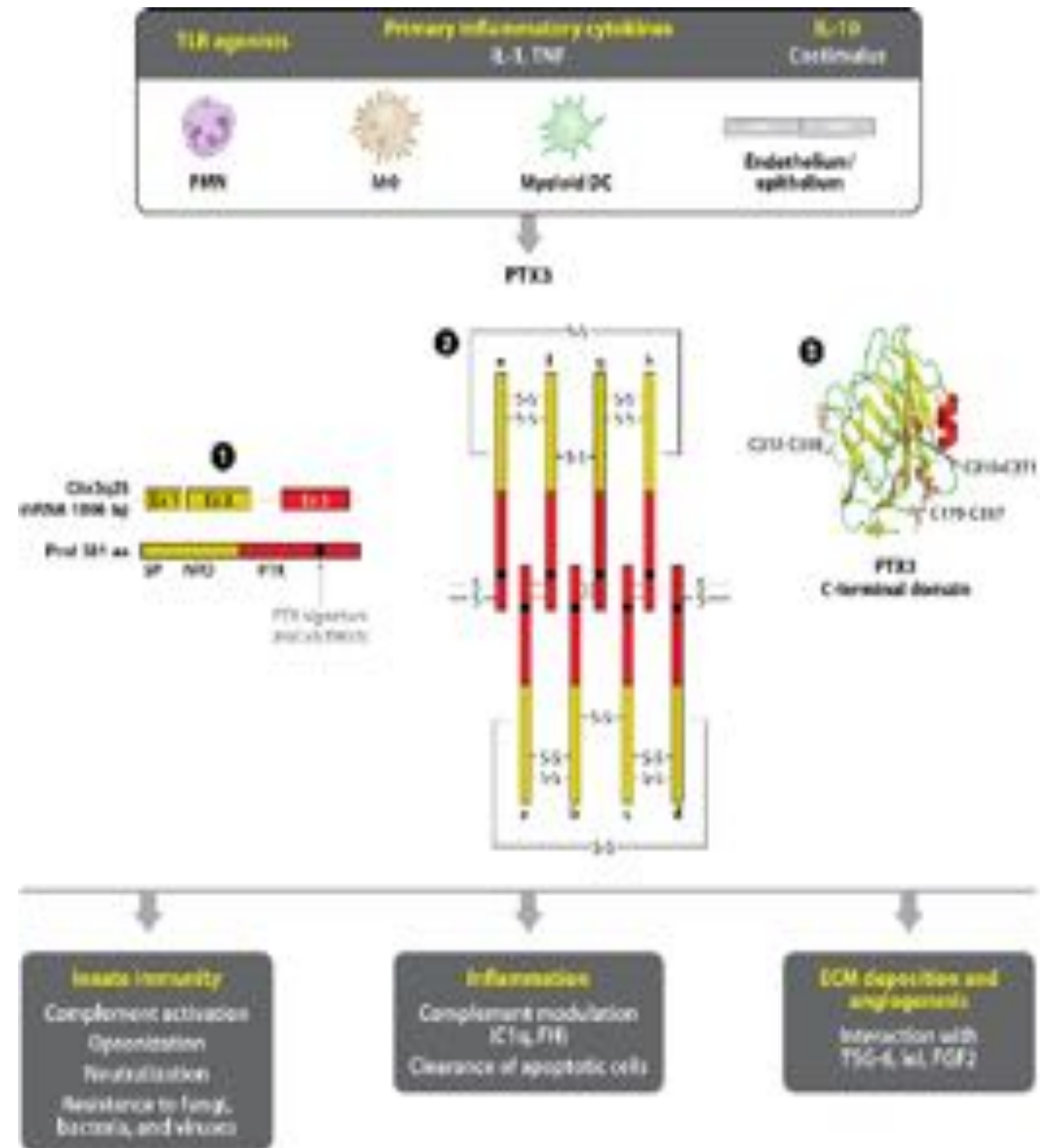
THE LONG PENTRAXINS PTX3/PTX4 ARE NOT APP SINCE THEY HAVE EXTRAHEPATIC PRODUCTION!

**Cellular
sources
and
inducers
of the long
pentraxin
PTX3!**

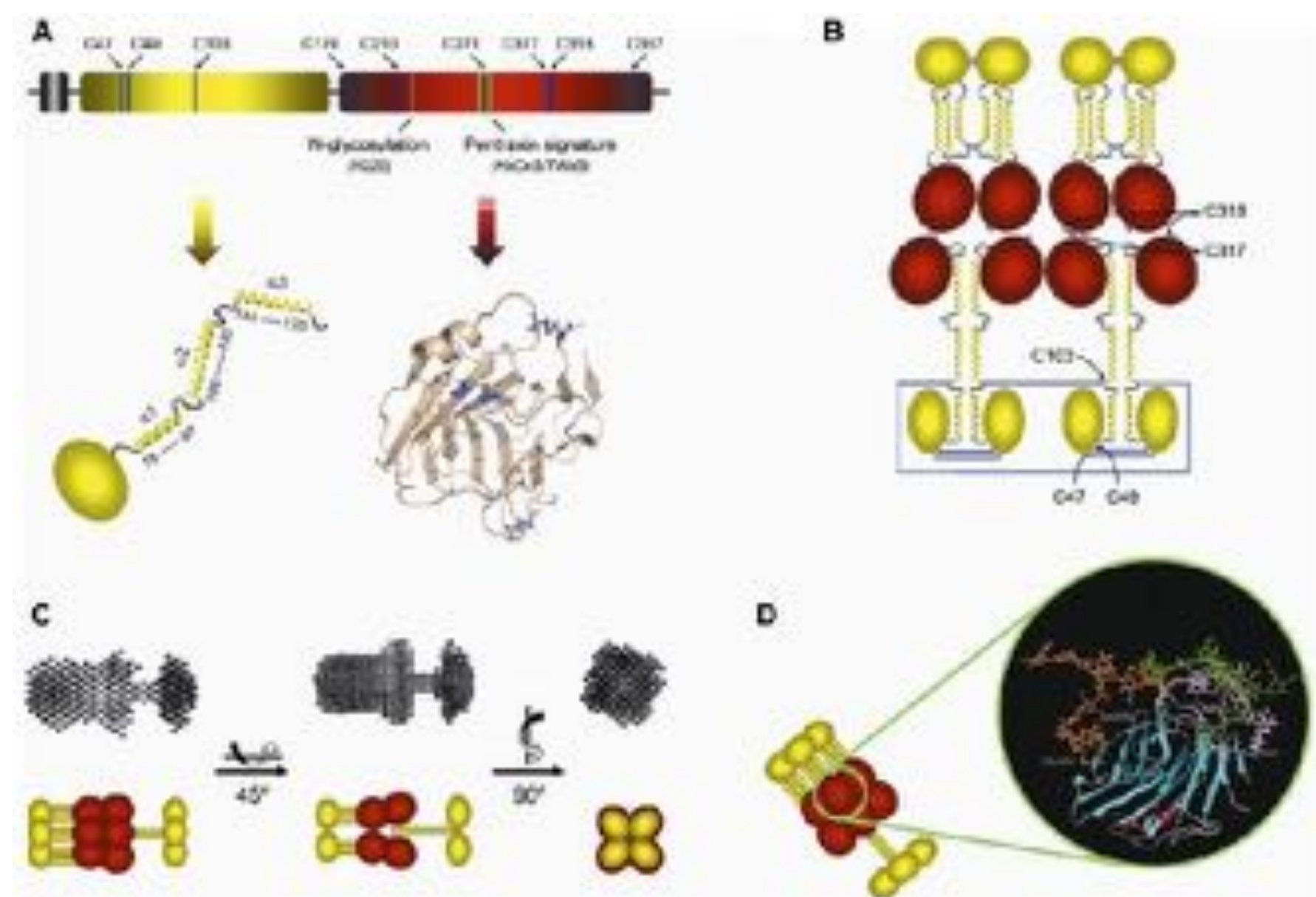


THE LONG PENTRAXINS PTX3/PTX4 ARE NOT APP SINCE THEY HAVE EXTRAHEPATIC PRODUCTION!

**Cellular
sources
and
inducers
of the long
pentraxin
PTX3!**



**The
pentraxins
LONG PTX3/
PTX4, after
their
extrahepatic
production
can be
glycosylated!**



(A) Schematic representation of the PTX3 protomer subunit showing the N-terminal domain in yellow, followed by the globular pentraxin domain in red. Positions of the Cys residues, the N-glycosylation site at Asn220 and the pentraxin signature motif are indicated.

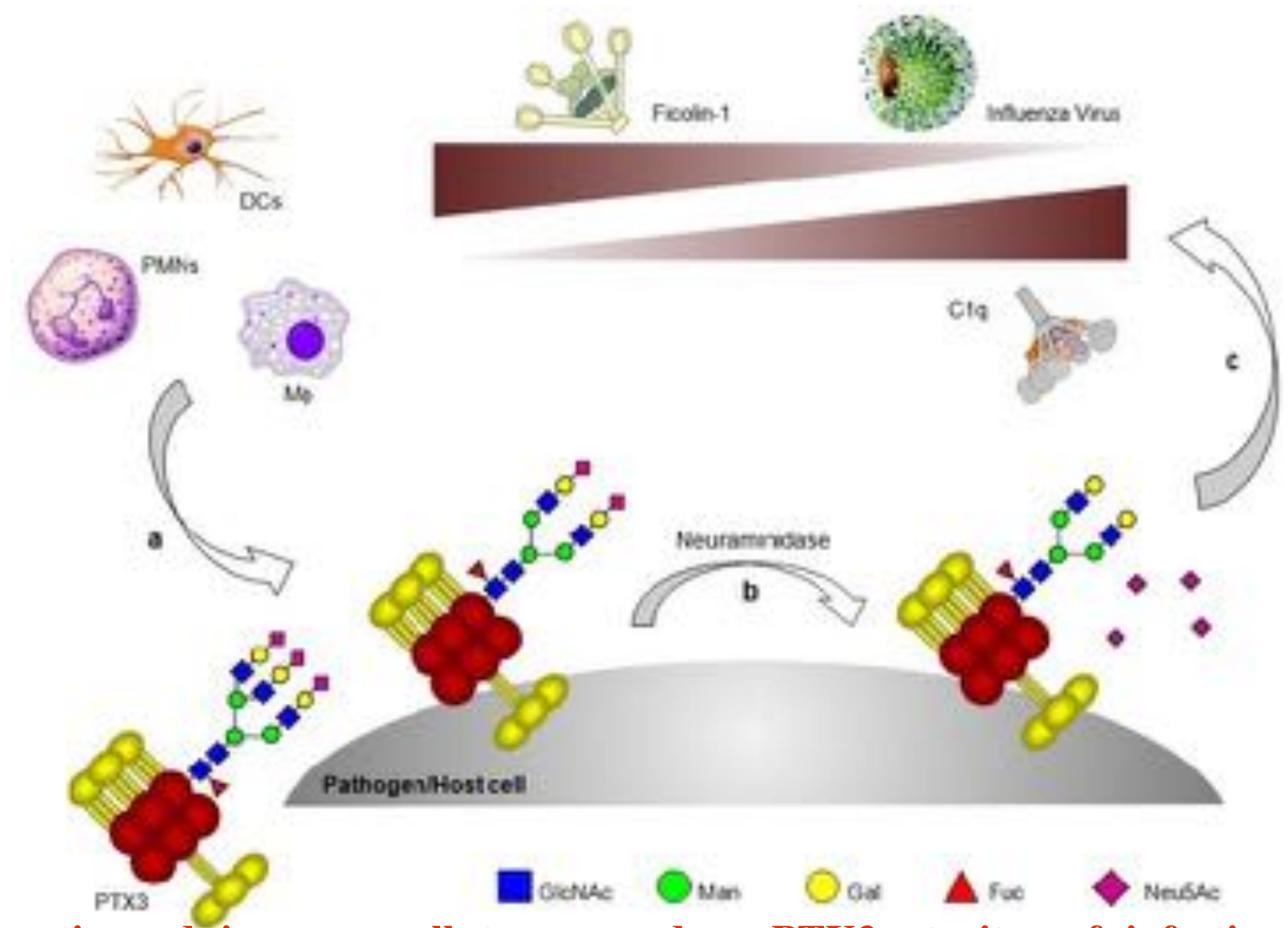
(B) Disulfide bond organization of the PTX3 octamer.

(C) Schematic model of PTX3 based on the two different structural arrangements proposed for the N-terminal domain. The α -helical segments of the N-terminal domain are depicted as yellow rods. The C-terminal pentraxin domains are in red.

(D) Molecular dynamics simulations indicate that the PTX3 oligosaccharides, here represented by a core monofucosylated and desialylated biantennary glycan, can adopt different conformations (orange, green, and purple), where terminal residues of sialic acid can contact specific amino acids (ball-and-stick) at the protein surface.

Glycosylation as a tuner of PTX3 functions in innate immunity!

Front
Immunol.
2012; 3: 407. 7



A number of both somatic and immune cell types produce PTX3 at sites of infection/inflammation. The glycosylation status of PTX3 (e.g., branching and sialylation) might change depending on cellular source and inducing stimuli (a). In addition, the protein oligosaccharides might undergo processing by glycosidases, including neuraminidase, which are expressed or mobilized on the surface of both pathogens and host cells (e.g., neutrophils) (b). Desialylated PTX3 has higher affinity for C1q but loses recognition of ficolin-1 and influenza virus (c).

The pentraxin PTX3 in stroke, pre-eclampsia and fertility!

PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization.

Garlanda C. Et al., 2004, Development 131, 1577-1586.

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The PTX3 is related to the CPR, whose levels in case of infection rise dramatically in a matter of hours. Compared to PCR , PTX3 is the fact of being produced from any tissue subject to inflammation. In this way its concentration increases much more quickly, enabling an early diagnosis. For example, in the case of infarction was found that when the patient arrives in the ER, PTX3 levels are already very high, while those of CPR change after a few hours. It can also give important information about prognosis.

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A condition in which the evaluation of the levels of PTX3 could be very useful, it is preeclampsia. This is a very serious complication that may occur during pregnancy, with an increase of blood pressure. In these cases the raising of PTX3 concentration occurs early, while the clinical manifestations due to altered vascularization of the placenta are found much later. Even in this case PTX3 could become important for an early diagnosis.

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The absence of PTX3 corresponds to a condition of infertility, since this protein is a key component of the structure of cells (the cumulus oophorus) surrounding the oocyte when ovulation occurs. Without it, the egg cell remains virtually 'naked', deprived of external elements that have the function of guiding the sperm in the right direction and, thus, fertilization can not occur.

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The long pentraxin PTX3: a paradigm for humoral pattern recognition molecules

Alberto Mantovani,^{1,2} Sonia Valentino,¹ Stefania Gentile,¹ Antonio Inforzato,¹ Barbara Bottazzi,¹ and Cecilia Garlanda¹

¹Humanitas Clinical and Research Center, Rozzano, Milan, Italy. ²Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

Address for correspondence: Alberto Mantovani, MD, Scientific Director, Istituto Clinico Humanitas, Via Manzoni 113, 20089 Rozzano, Milano, Italy. alberto.mantovani@humanitasresearch.it

Pattern recognition molecules (PRMs) are components of the humoral arm of innate immunity; they recognize pathogen-associated molecular patterns (PAMP) and are functional ancestors of antibodies, promoting complement activation, opsonization, and agglutination. In addition, several PRMs have a regulatory function on inflammation. Pentraxins are a family of evolutionarily conserved PRMs characterized by a cyclic multimeric structure. On the basis of structure, pentraxins have been operationally divided into short and long families. C-reactive protein (CRP) and serum amyloid P component are prototypes of the short pentraxin family, while pentraxin 3 (PTX3) is a prototype of the long pentraxins. PTX3 is produced by somatic and immune cells in response to proinflammatory stimuli and Toll-like receptor engagement, and it interacts with several ligands and exerts multifunctional properties. Unlike CRP, PTX3 gene organization and regulation have been conserved in evolution, thus allowing its pathophysiological roles to be evaluated in genetically modified animals. Here we will briefly review the general properties of CRP and PTX3 as prototypes of short and long pentraxins, respectively, emphasizing in particular the functional role of PTX3 as a prototypic PRM with antibody-like properties.

Keywords: innate immunity; pentraxins; PTX3; pattern recognition molecules

Table 1. Ligand specificity of CRP, SAP, and PTX3

| Ligand | CRP | SAP | PTX3 |
|---|-----------------|-----|------|
| Microorganisms | | | |
| Bacteria | | | |
| <i>Pseudomonas aeruginosa</i> | NT ^a | NT | + |
| <i>Klebsiella pneumoniae</i> | NT | NT | + |
| <i>Salmonella typhimurium</i> | – | + | + |
| Fungi and yeasts | | | |
| <i>Aspergillus fumigatus</i> | + | NT | + |
| <i>Saccharomyces cerevisiae</i> (zymosan) | + | + | + |
| <i>Paracoccidioides brasiliensis</i> | NT | NT | + |
| Viruses | | | |
| Influenza virus | – | + | + |
| Human cytomegalovirus (HCMV) | NT | NT | + |
| Membrane moieties | | | |
| Phosphocholine (PC) | + | – | – |
| Phosphoethanolamine (PE) | – | + | – |
| LPS | – | + | – |
| Outer membrane protein A from <i>Klebsiella pneumoniae</i> (KpOmpA) | NT | NT | + |
| Complement components | | | |
| C1q | + | + | + |
| Factor H | + | NT | + |
| C4BP | + | + | + |
| M-, L-ficolin | + | – | + |
| MBL | – | + | + |
| Extracellular matrix proteins | | | |
| TNF-stimulated gene-6 (TSG-6) | NT | NT | + |
| Inter- α -trypsin-inhibitor (I α I) | – | NT | + |
| Hyaluronan | NT | NT | – |
| Laminin | + | + | – |
| Collagen IV | NT | + | – |
| Fibronectin | + | + | – |
| Growth factors | | | |
| FGF2 | +/– | NT | + |
| FGF1 and FGF4 | NT | NT | – |
| Adhesion molecules | | | |
| P-selectin | – | NT | + |

^aNT: not tested.

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| Phosphoethanolamine (PE) | – | + | – |
| LPS | – | + | – |
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| FGF1 and FGF4 | NT | NT | – |
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| P-selectin | – | NT | + |

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PTX3 Is an Extrinsic Oncosuppressor Regulating Complement-Dependent Inflammation in Cancer.

Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, Greco C, Feruglio F, Molgora M, Laface I, Tartari S, Doni A, Pasqualini F, Barbatì E, Basso G, Galdiero MR, Nebuloni M, Roncalli M, Colombo P, Laghi L, Lambris JD, Jaillon S, Garlanda C, Mantovani A.

Abstract

PTX3 is an essential component of the humoral arm of innate immunity, playing a nonredundant role in resistance against selected microbes and in the regulation of inflammation. PTX3 activates and regulates the Complement cascade by interacting with C1q and with Factor H. PTX3 deficiency was associated with increased susceptibility to mesenchymal and epithelial carcinogenesis. Increased susceptibility of Ptx3(-/-) mice was associated with enhanced macrophage infiltration, cytokine production, angiogenesis, and Trp53 mutations. Correlative evidence, gene-targeted mice, and pharmacological blocking experiments indicated that PTX3 deficiency resulted in amplification of Complement activation, CCL2 production, and tumor-promoting macrophage recruitment. PTX3 expression was epigenetically regulated in selected human tumors (e.g., leiomyosarcomas and colorectal cancer) by methylation of the promoter region and of a putative enhancer. Thus, PTX3, an effector molecule belonging to the humoral arm of innate immunity, acts as an extrinsic oncosuppressor gene in mouse and man by regulating Complement-dependent, macrophage-sustained, tumor-promoting inflammation.

THE COMPLEMENT SYSTEM HAS A DUAL ACTION IN CANCER!

Experimental data support the idea that complement is activated by tumors. However, some studies also suggest that malignant cells evade the harmful effects of complement and make use of some complement effector molecules to promote cancer growth. Unfortunately, the exact mechanisms and consequences of this duality are not very well known!



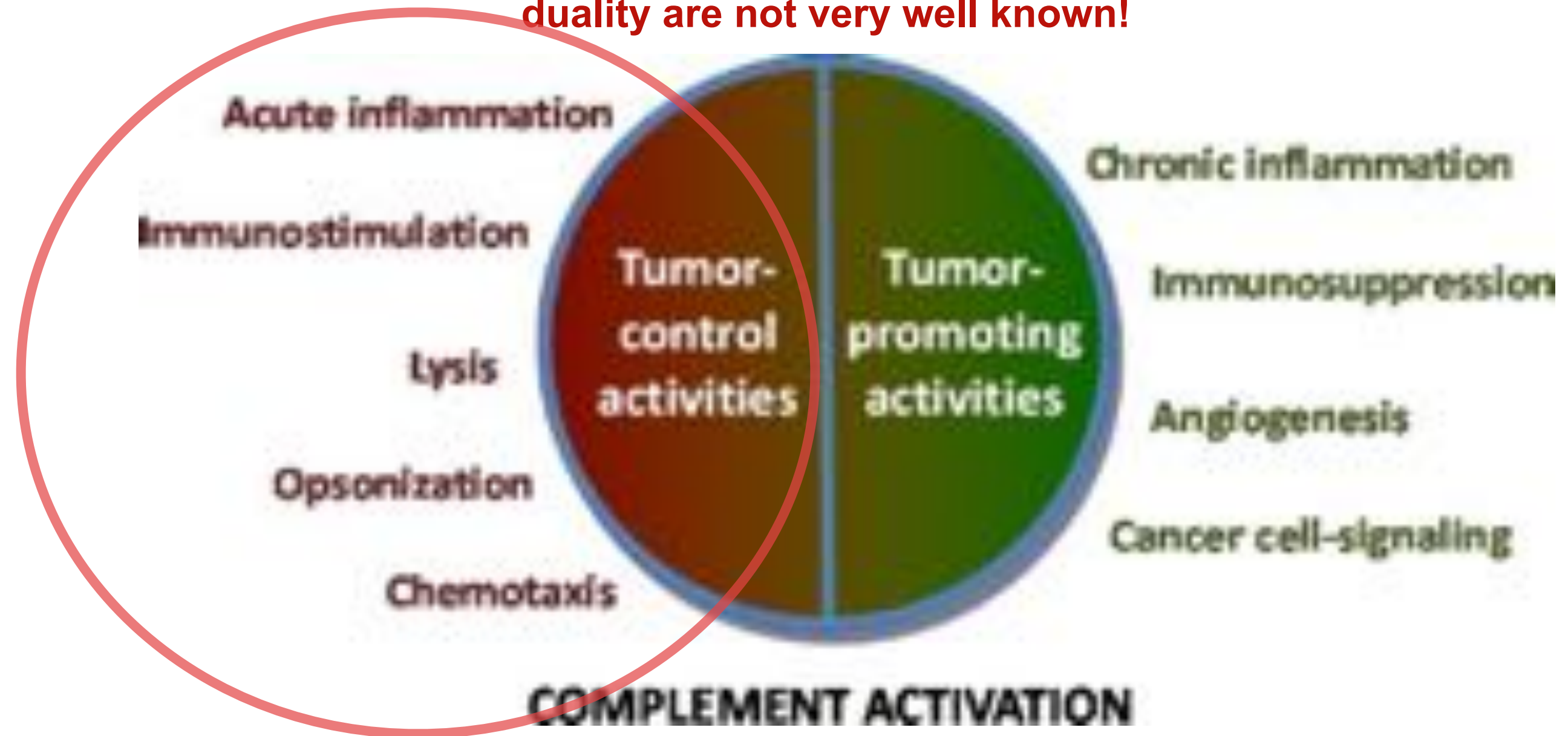
Semin Immunol. 2013 Feb;25(1):54-64

Complement inhibition in cancer therapy.

Pio R, Ajona D, Lambris JD.

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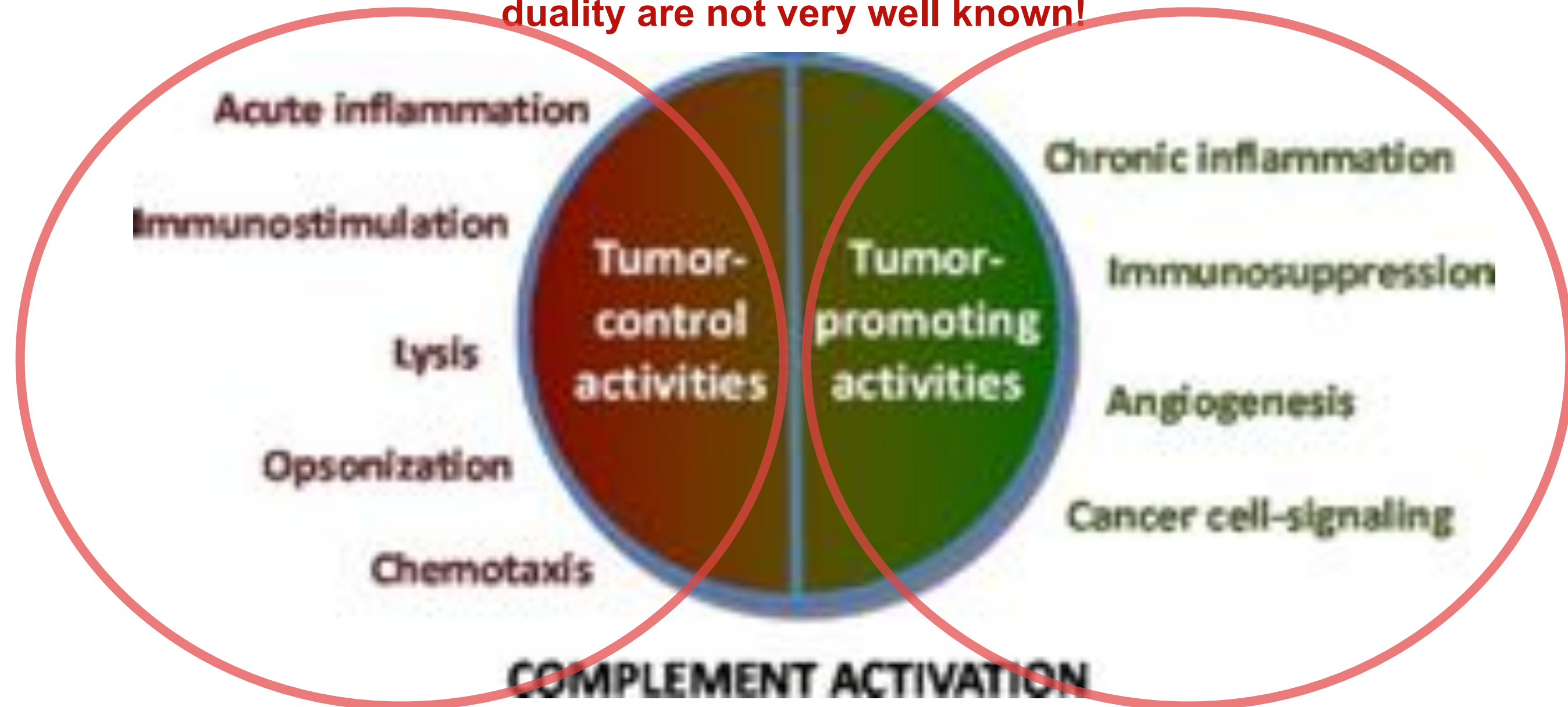
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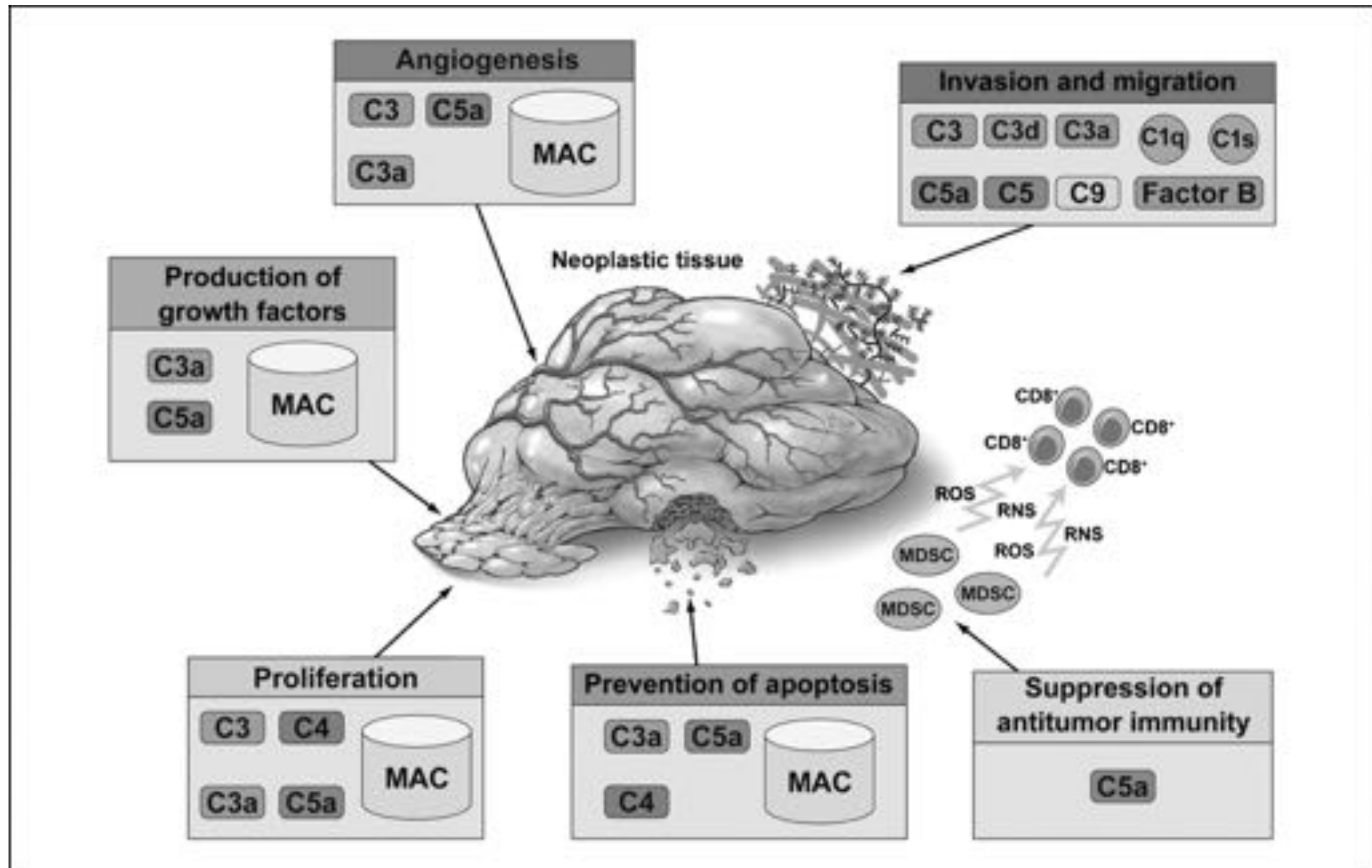


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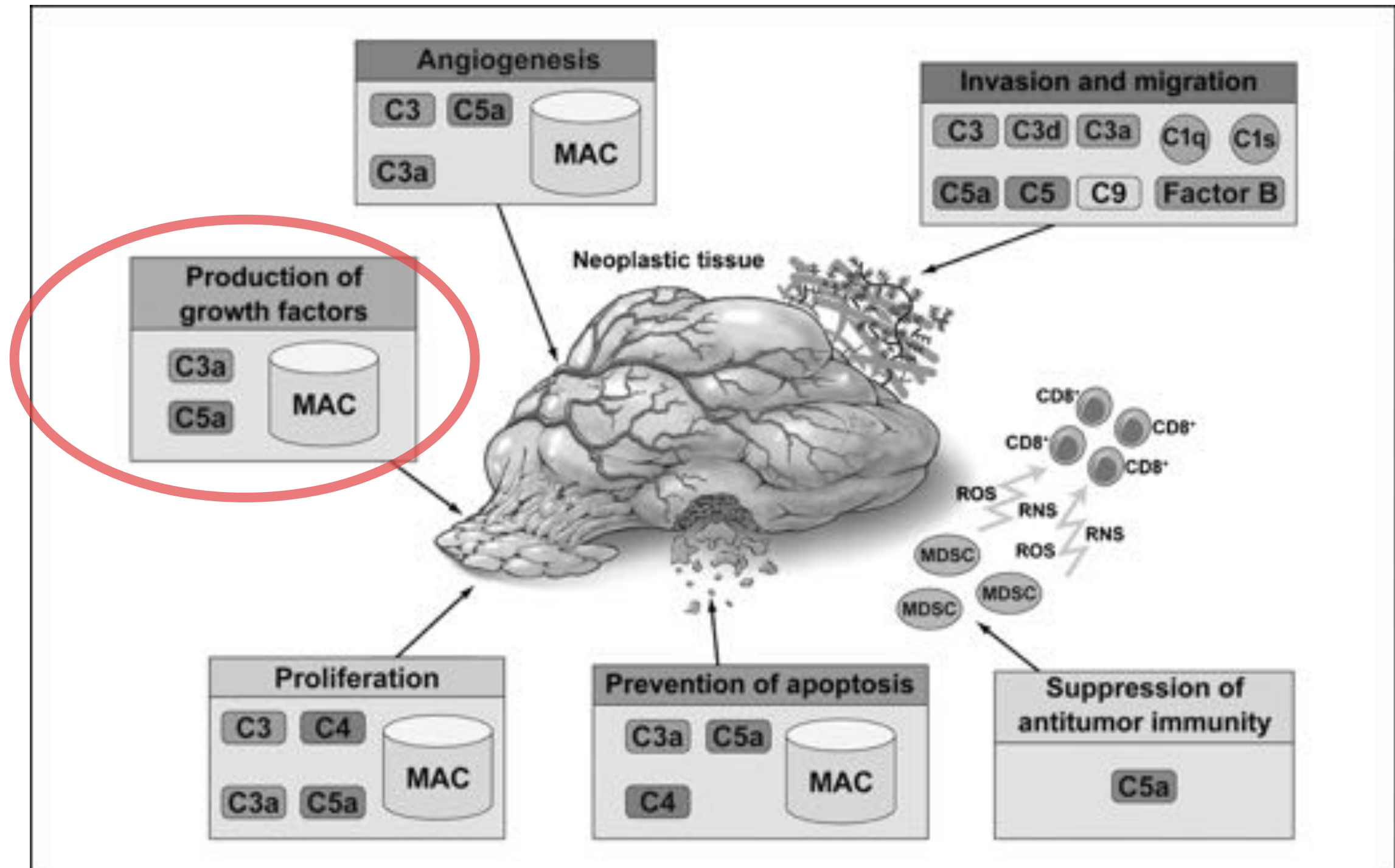
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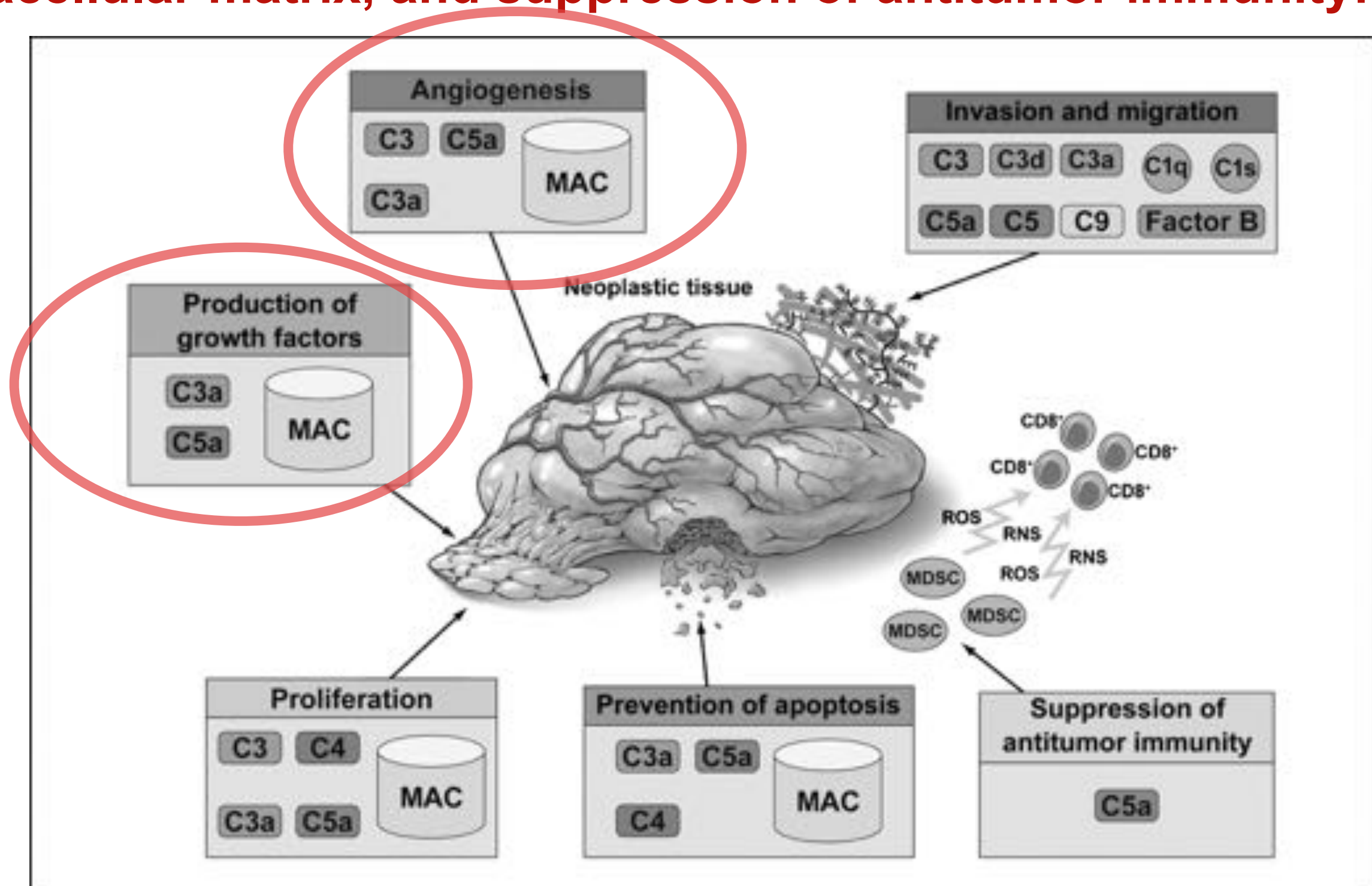
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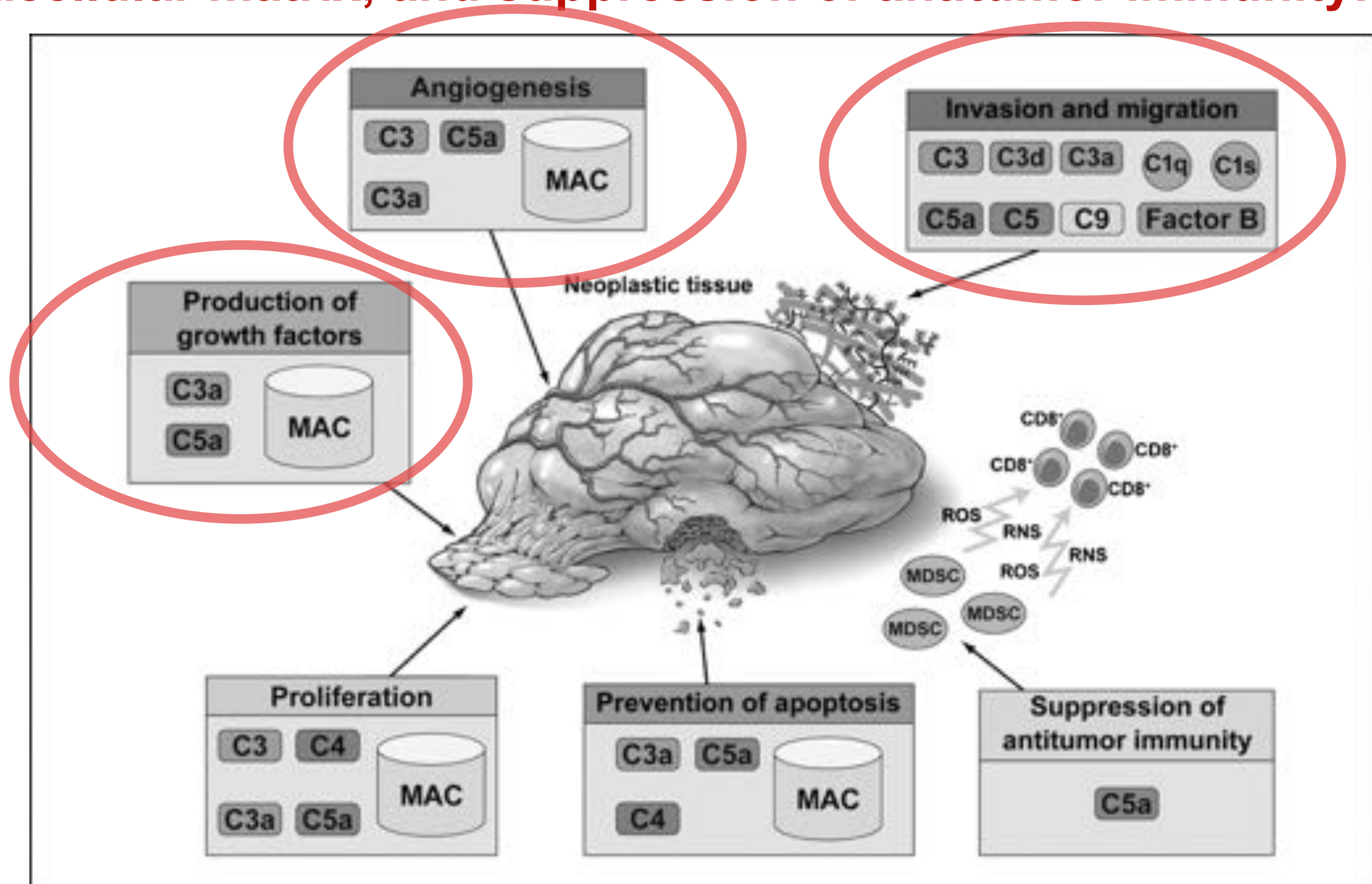
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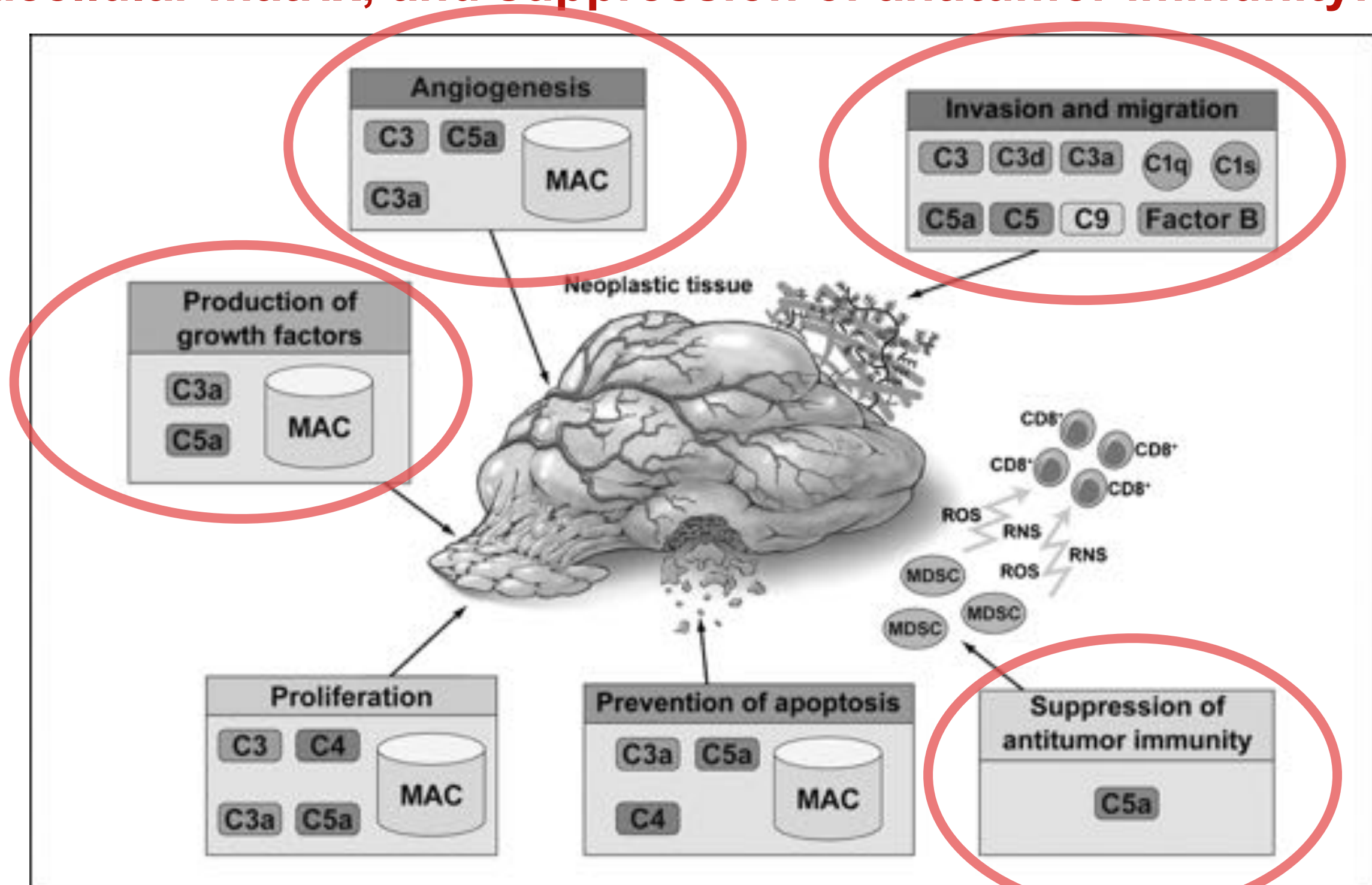
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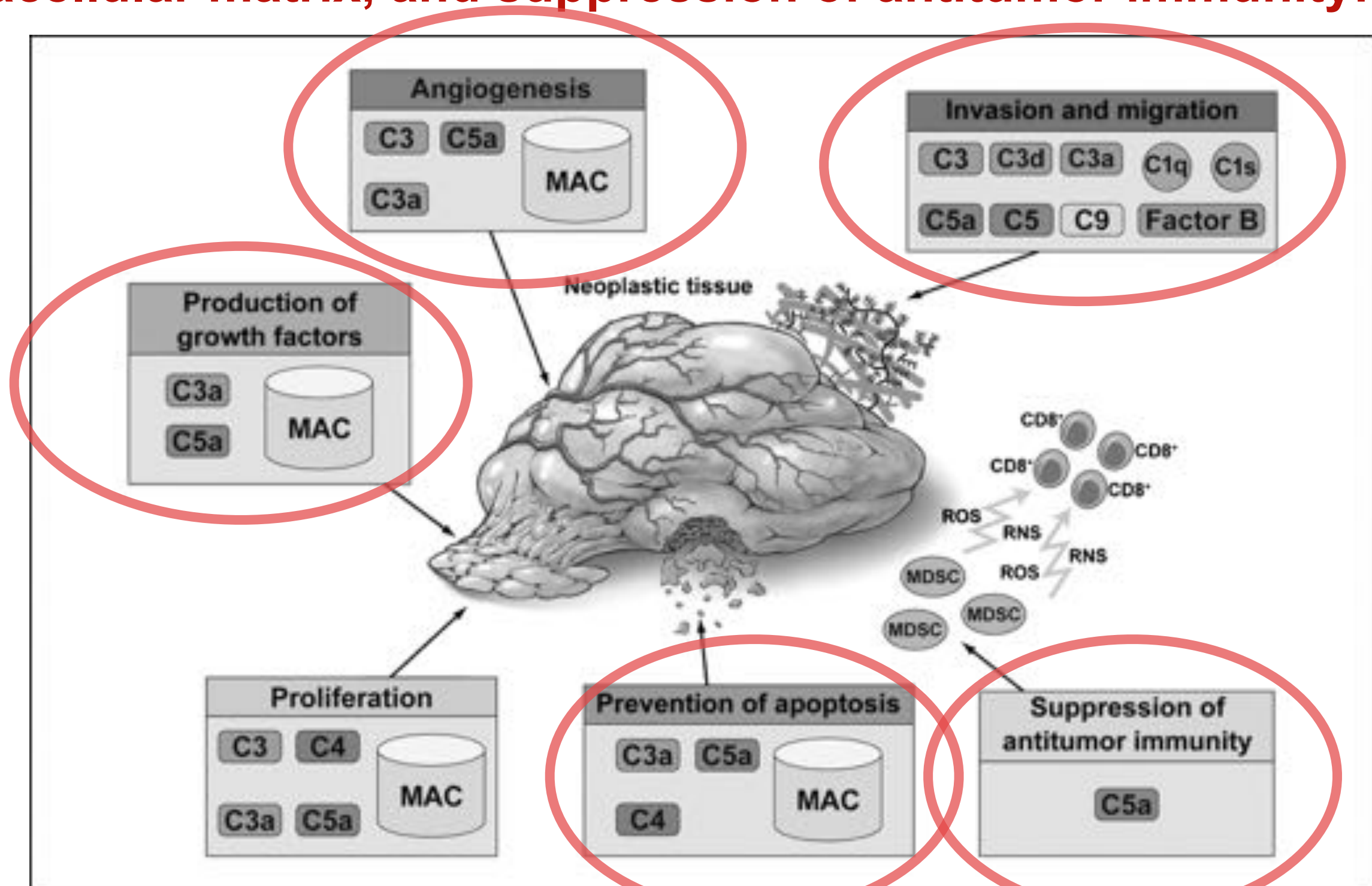
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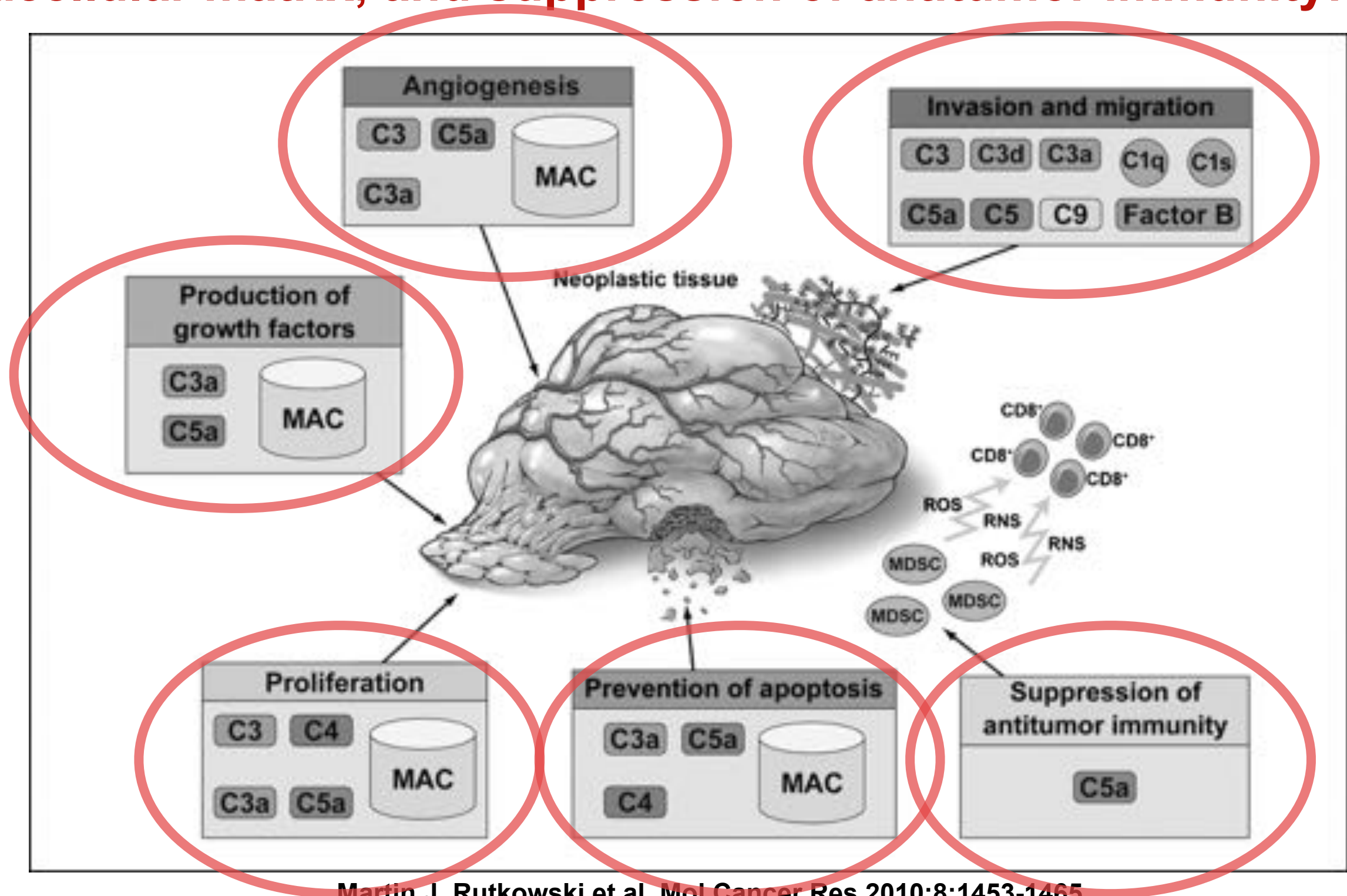
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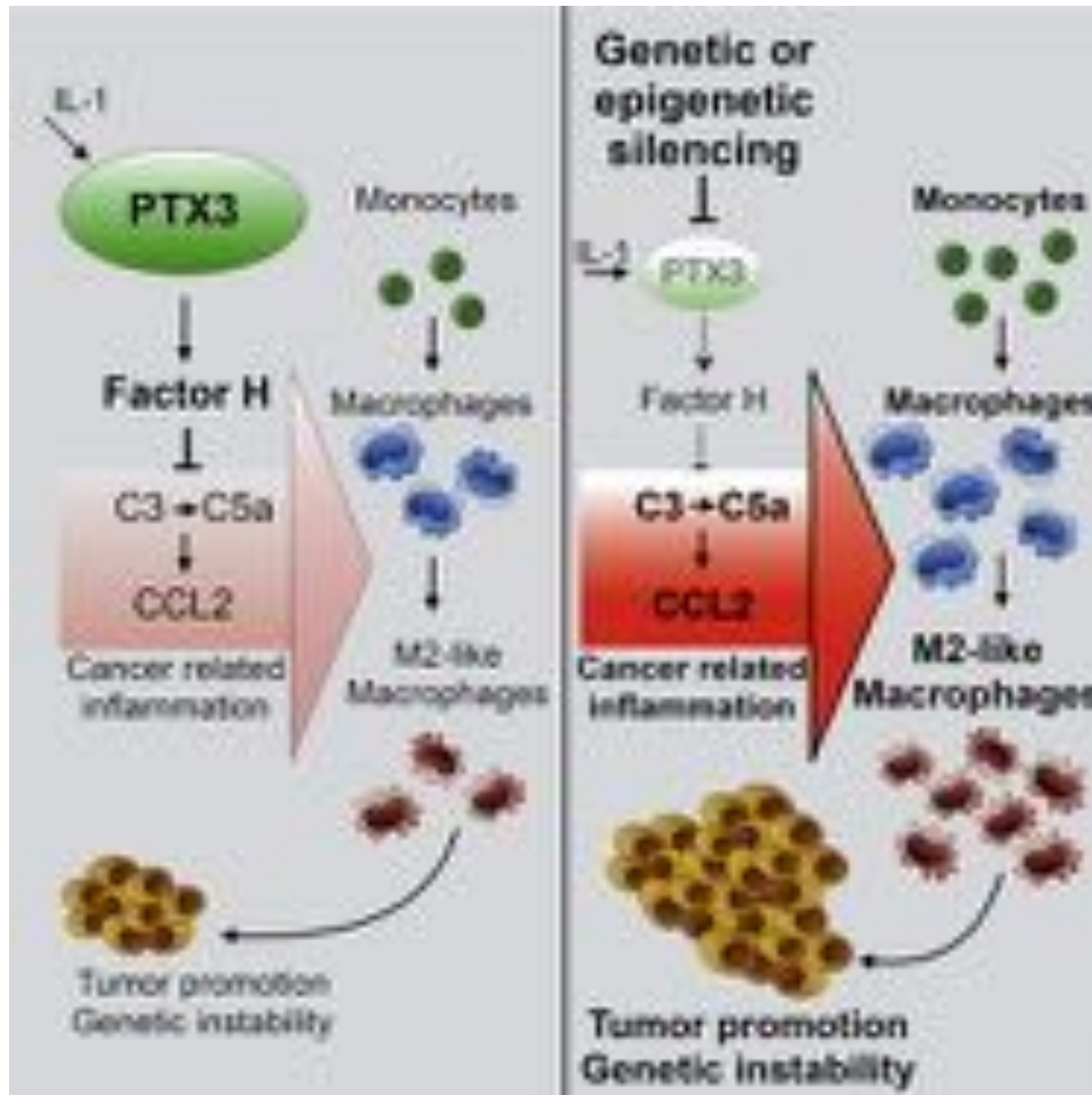
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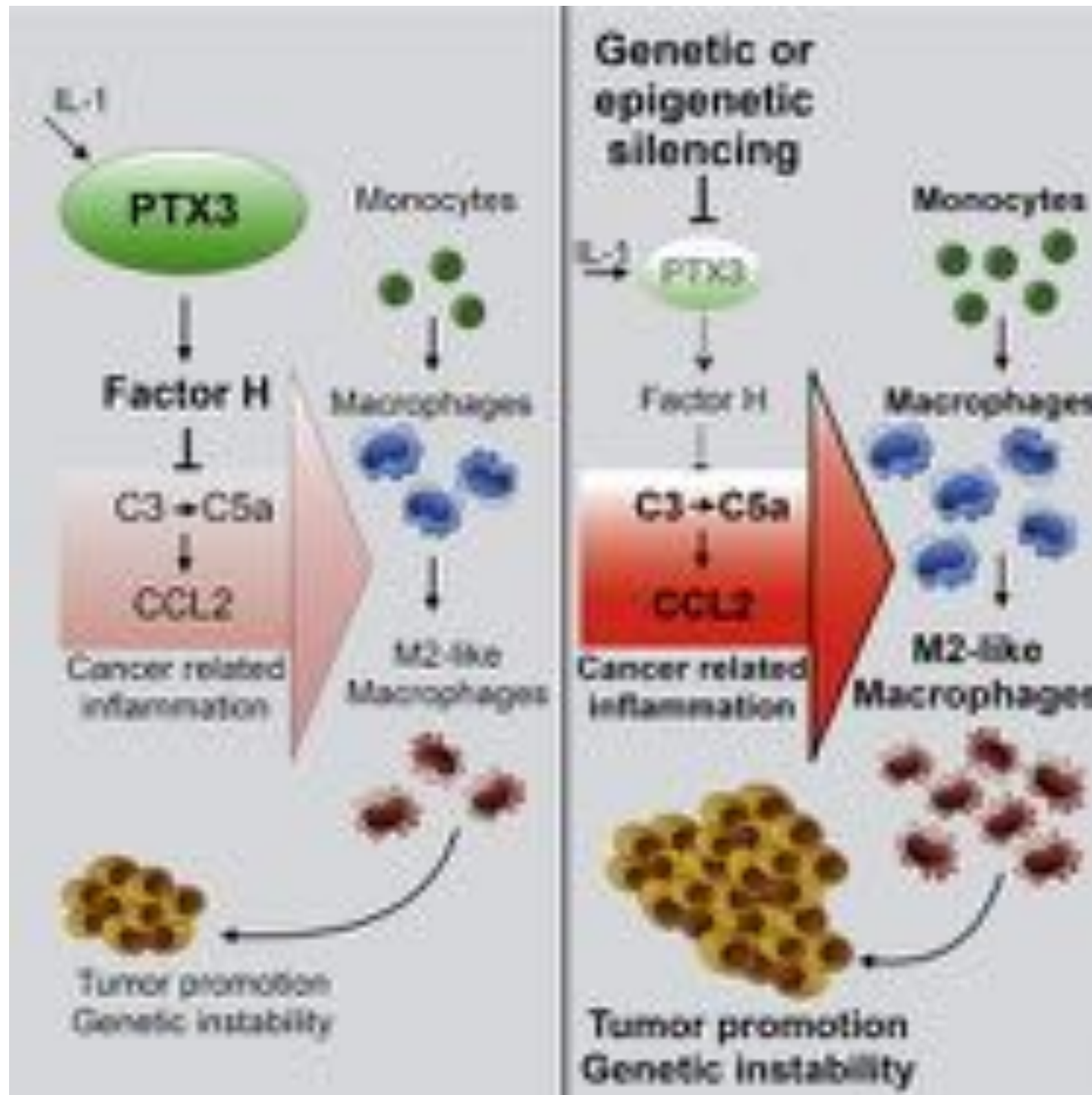
Highlights

PTX3 deficiency unleashes Complement-dependent tumor-promoting inflammation!

Tumors developed in a PTX3-deficient context have higher frequency of mutated Trp53!

Complement is an essential component of tumor-promoting inflammation!

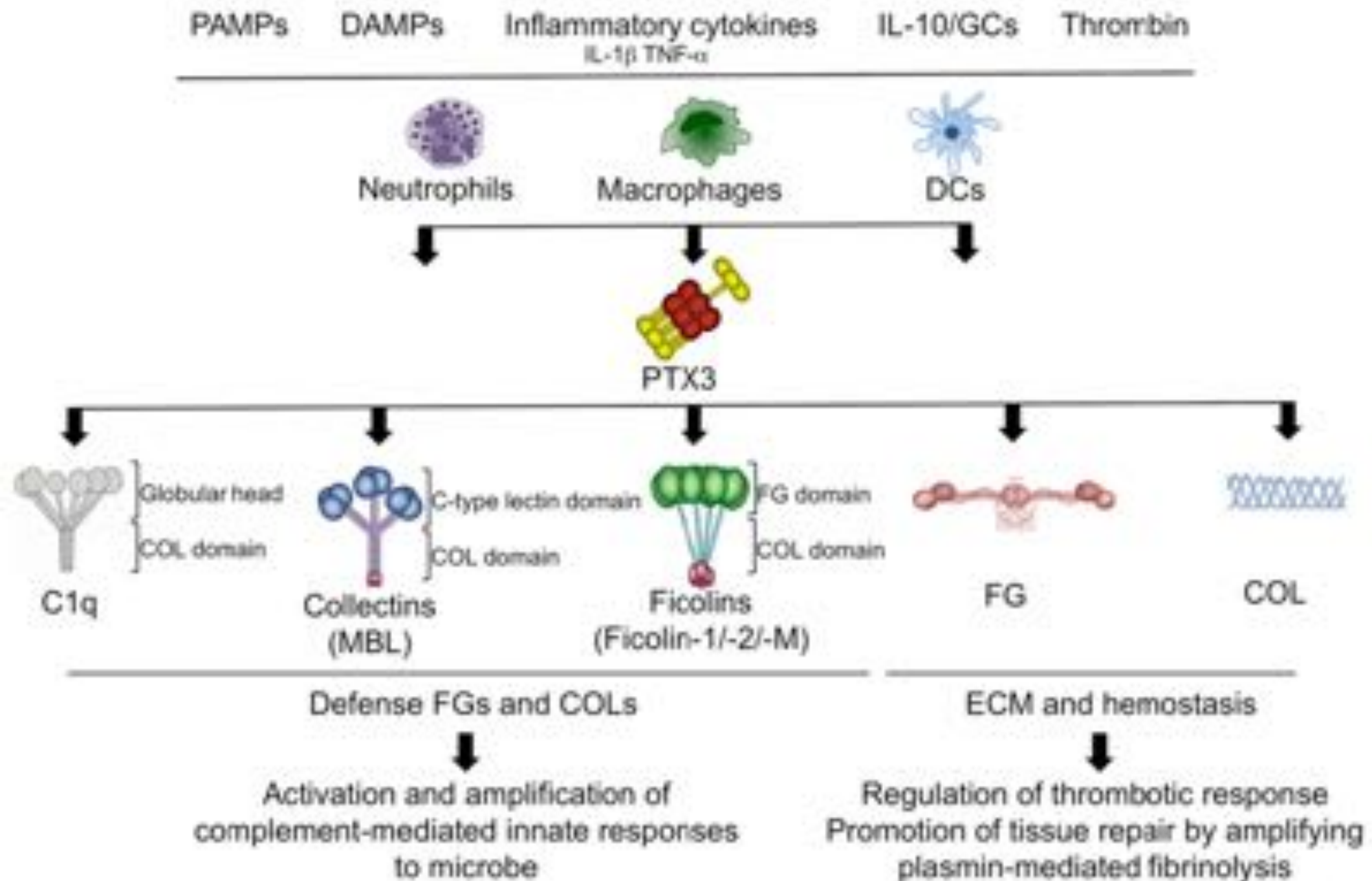
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PTX3 gene is silenced by hypermethylation in selected human tumors including colorectal cancer (CRC) and this event occurs early in progression already at the level of adenomas!

NEW PTX3 FUNCTIONS!

Recently has been demonstrated that PTX3 by interacting with defense collagens and fibrinogens amplifies other effector functions of the innate immune system. At wound sites, PTX3 regulates the injury-induced thrombotic response and promotes wound healing by favoring timely fibrinolysis. Therefore, PTX3 interacts with ancestral domains conserved in innate immunity, hemostasis and extracellular matrix and exerts functions related to both antimicrobial resistance and tissue repair.



Semin Immunol. 2016 Dec; 28(6): 570–577.

The COLLECTIN FAMILY!

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- **MBL**

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- MBL
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The COLLECTIN FAMILY!

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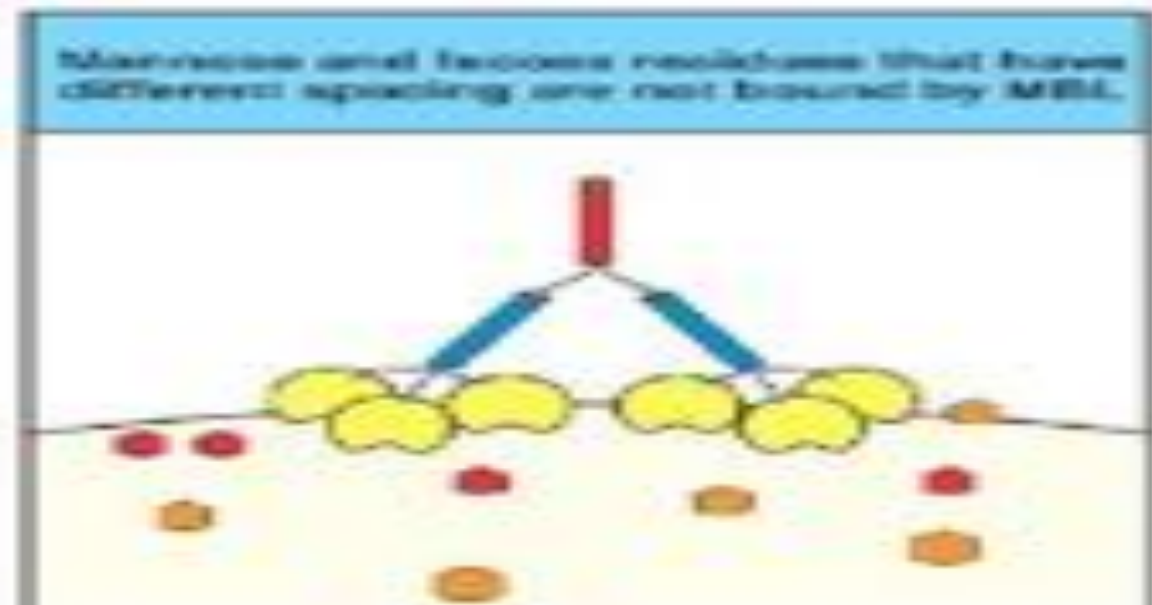
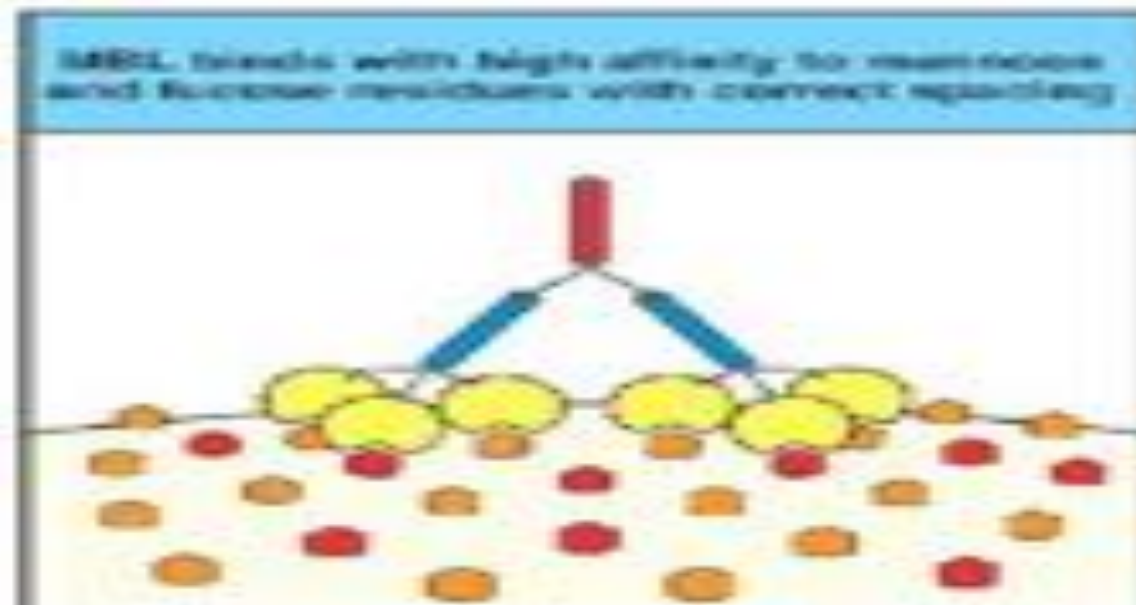
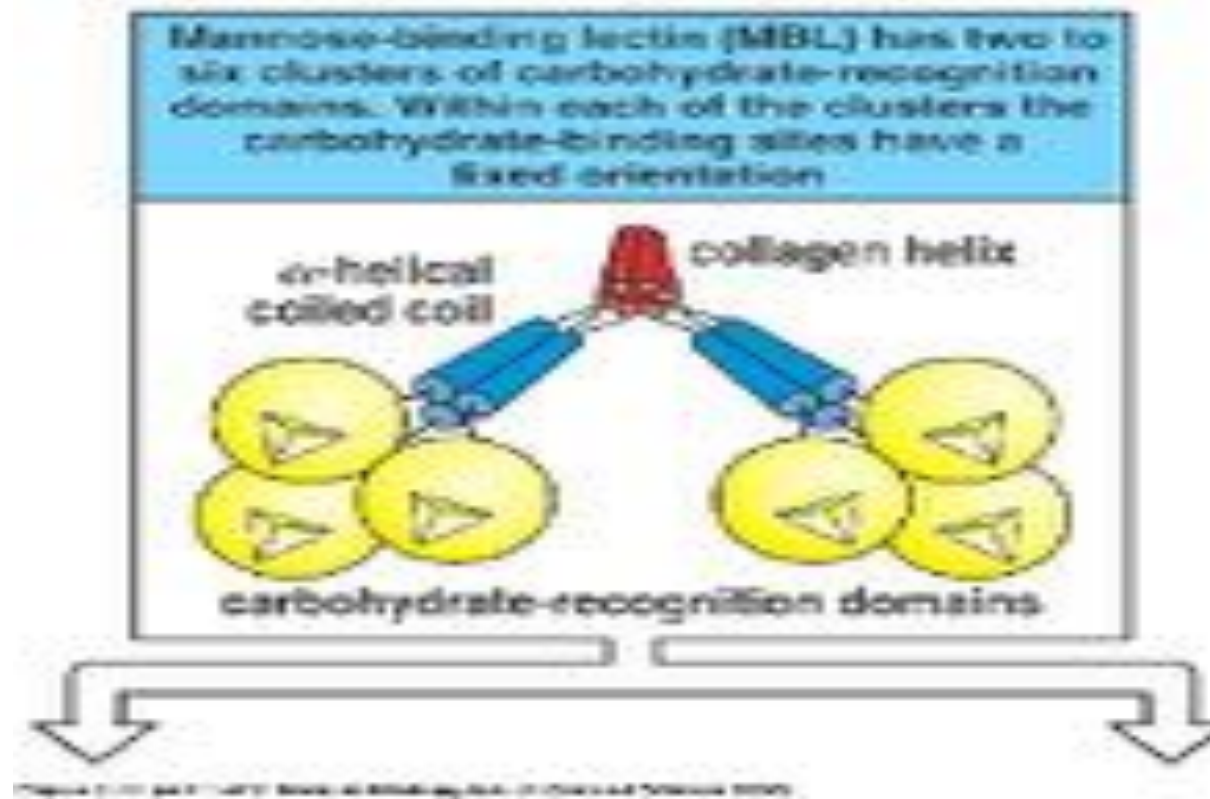
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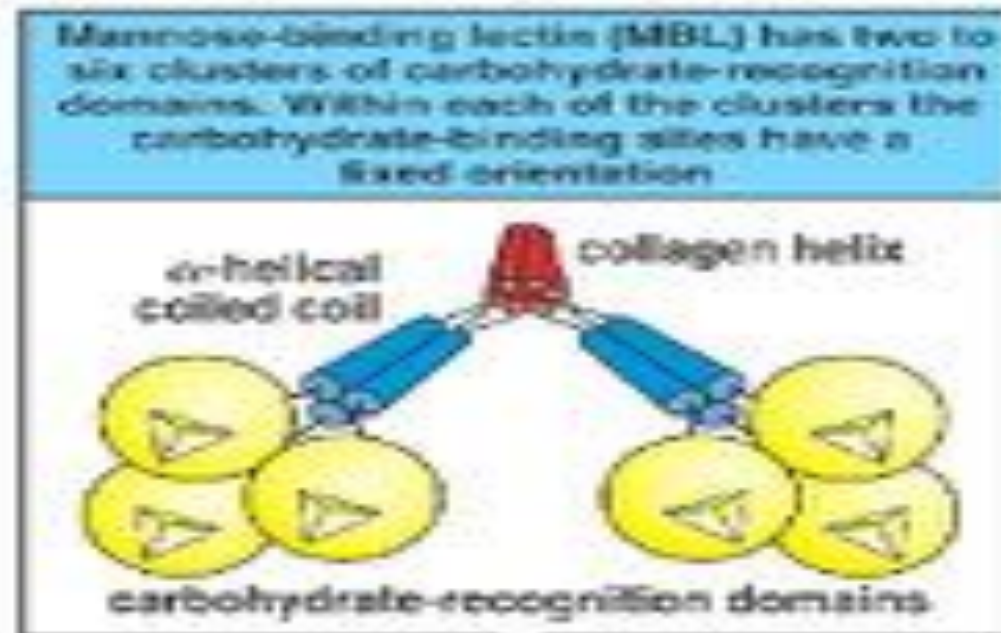
MBL is structurally related to the C1q and is part of the family of protein collectins, together with proteins A and D of the pulmonary surfactant.

It was recently discovered that a second group of proteins called ficolins, which includes the L-ficolin, the M-and H-ficolin, possesses lectin activity.

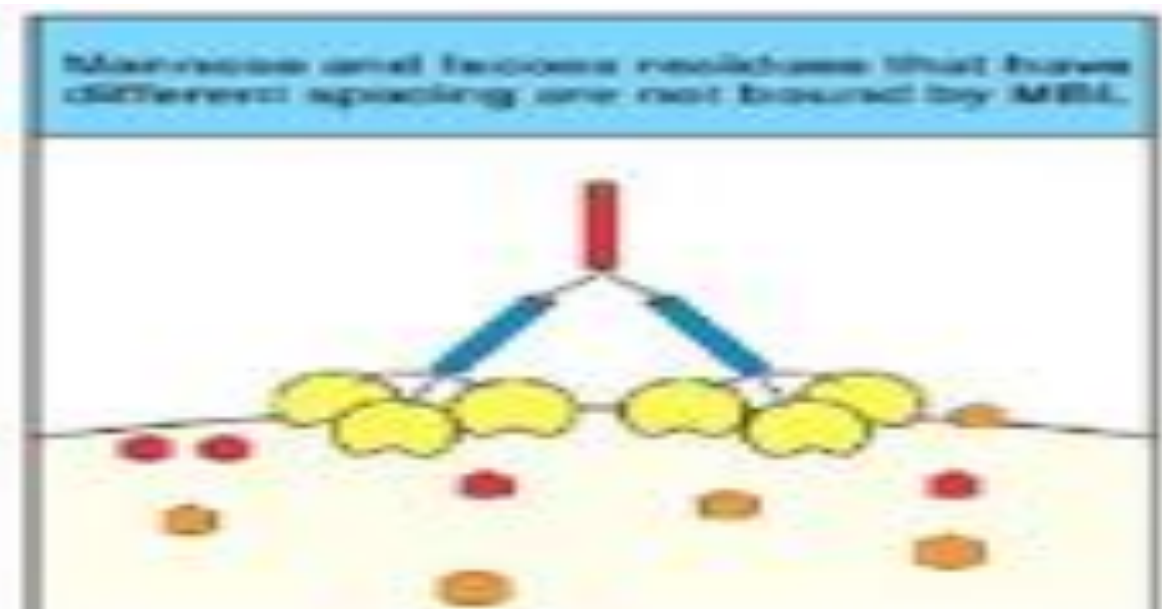
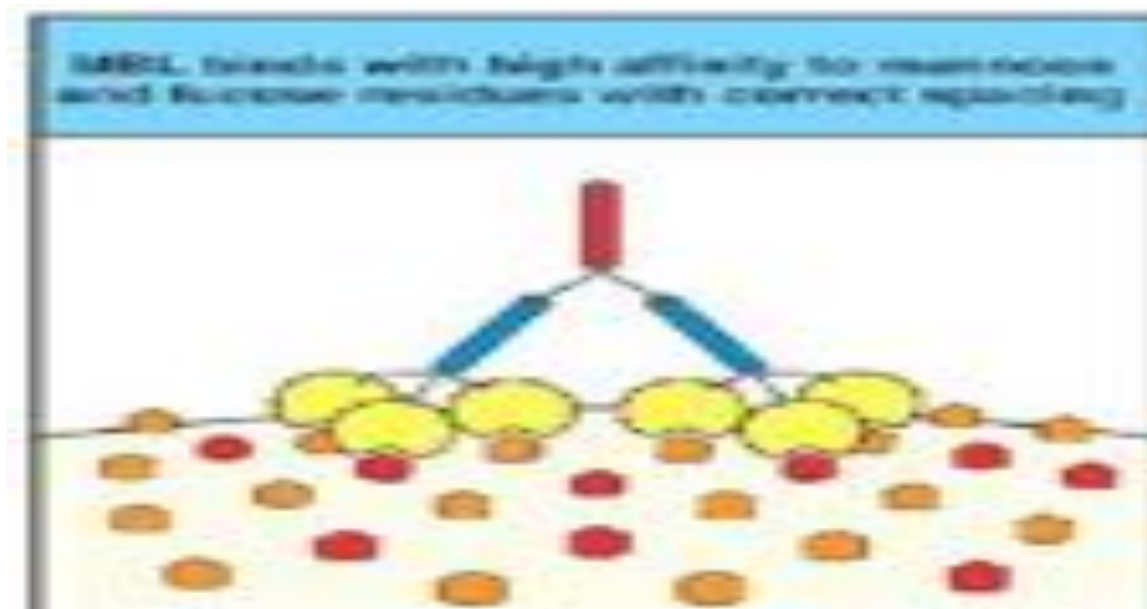
The MBL structure and function!



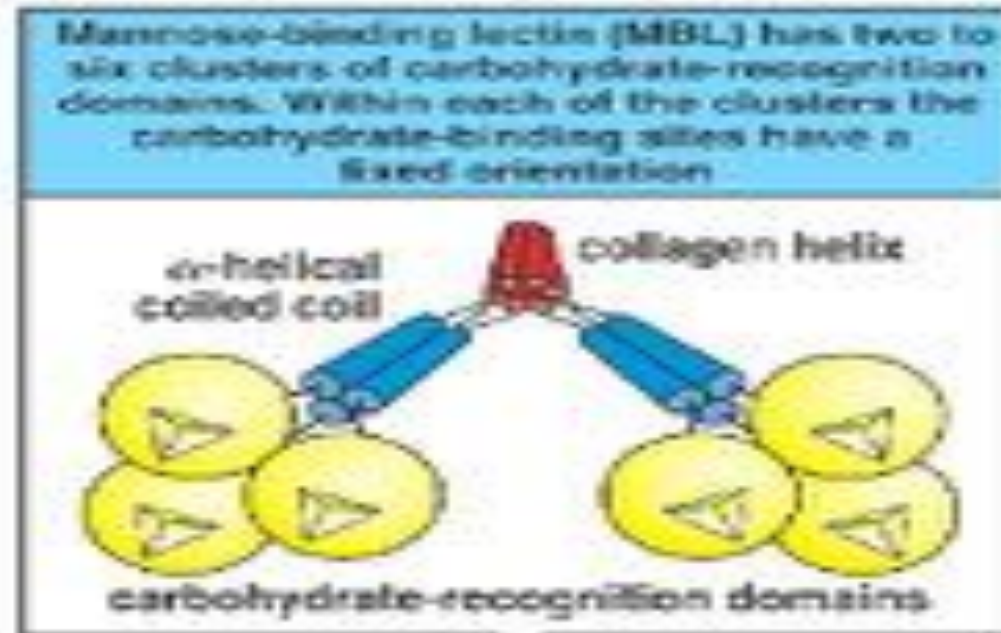
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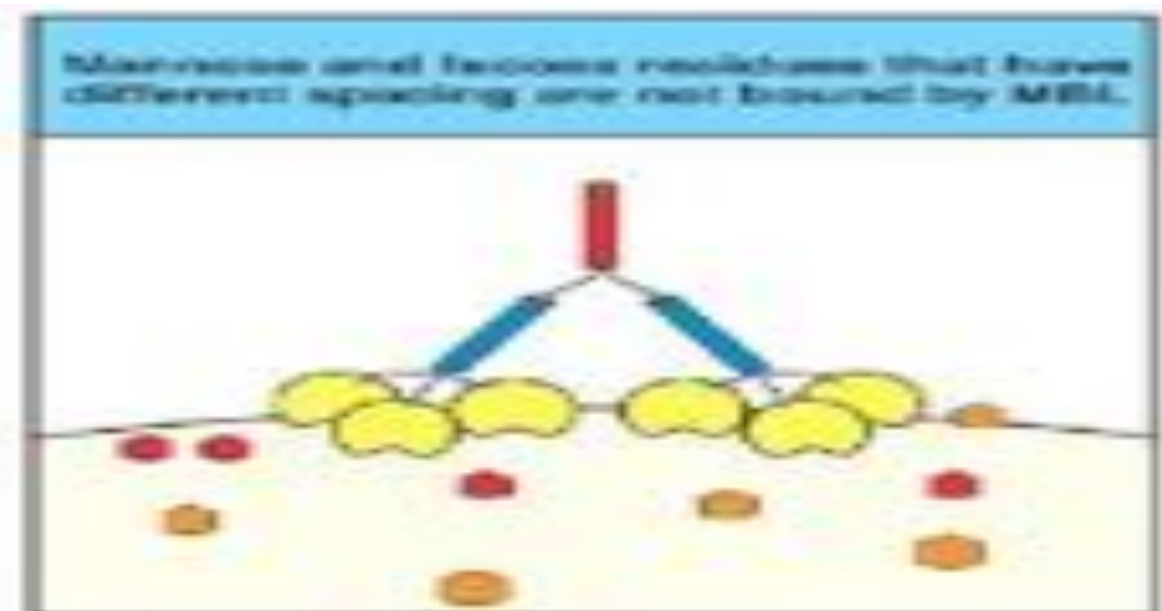
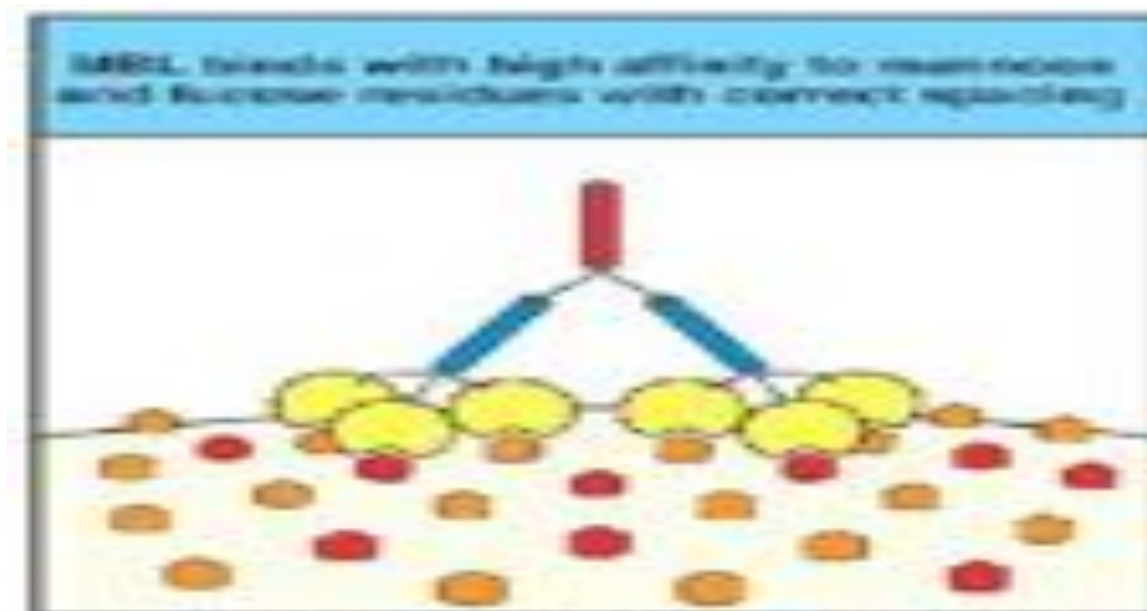
- The mannose-binding lectin (MBL), or mannose-binding protein, recognizes different carbohydrates present on the surface of many microorganisms, including bacteria, viruses, protozoa and fungi.



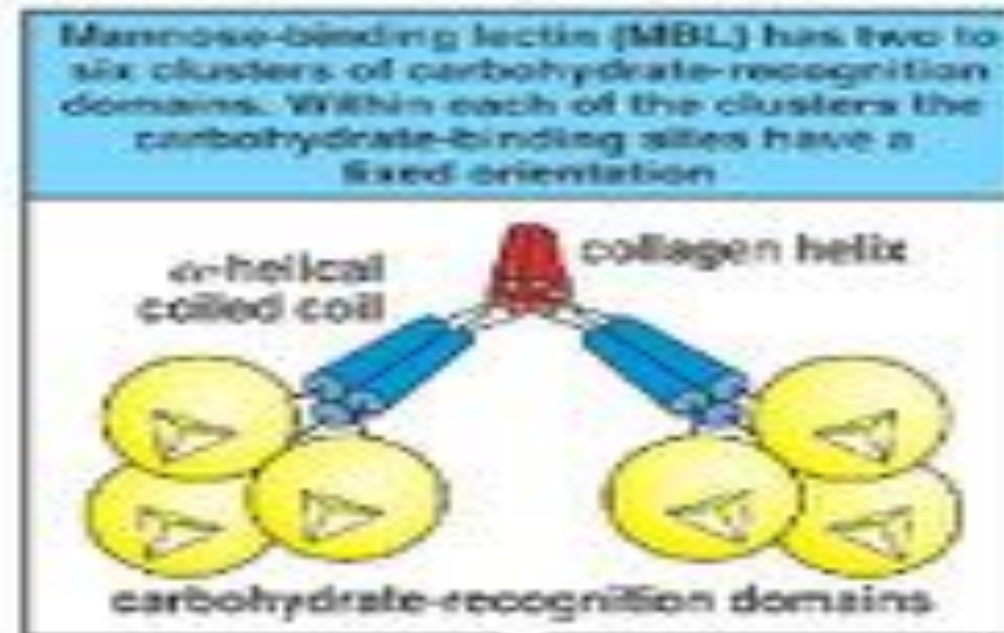
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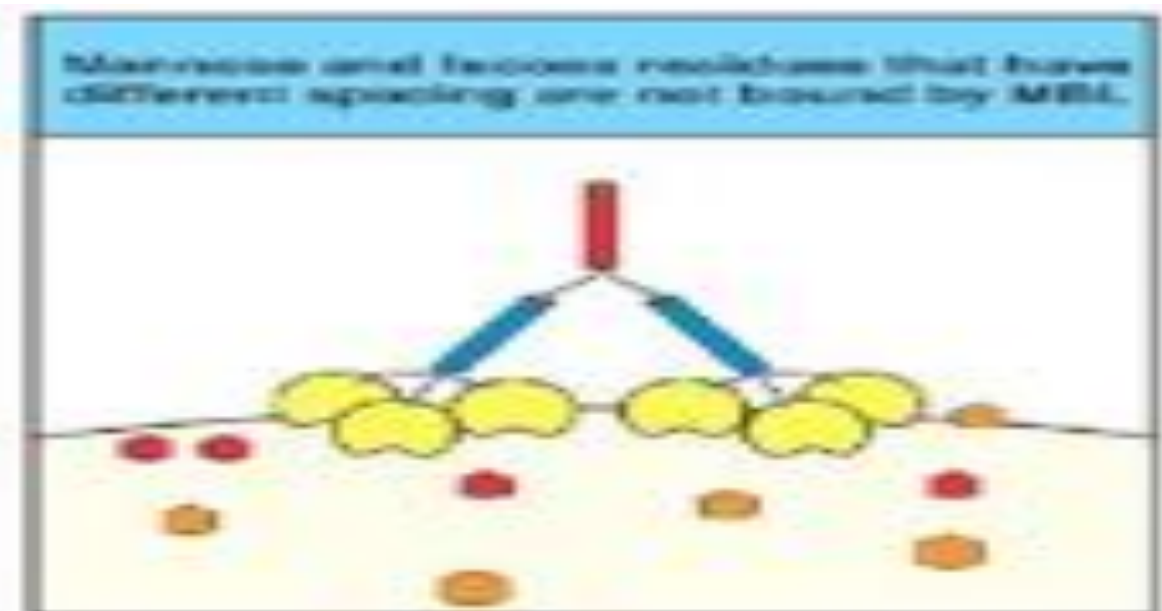
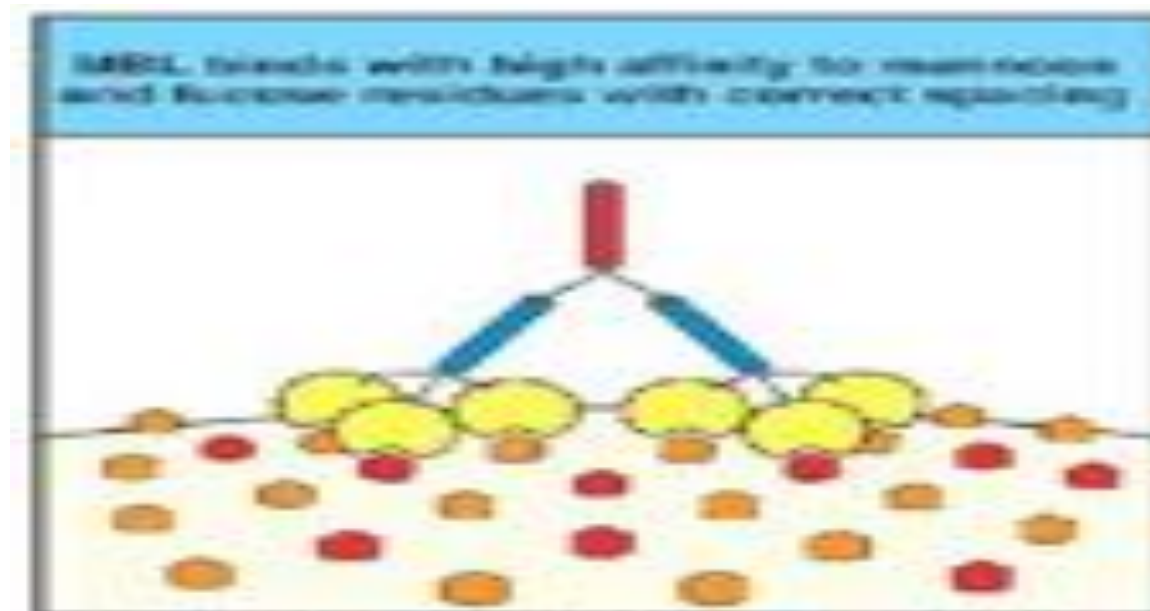
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- It has been shown that the mere presence of these sugar residues is not sufficient for the binding of the MBL, but their orientation is critical, as they are related only the residues that have a correct spatial arrangement. The bond has a low affinity (K_d 10⁻³) and, in order to be effective, it is essential that more "carbohydrate recognition sites" bind simultaneously.



The humoral collectins activate the COMPLEMENT SYSTEM!

The lectin-binding pathway of complement activation

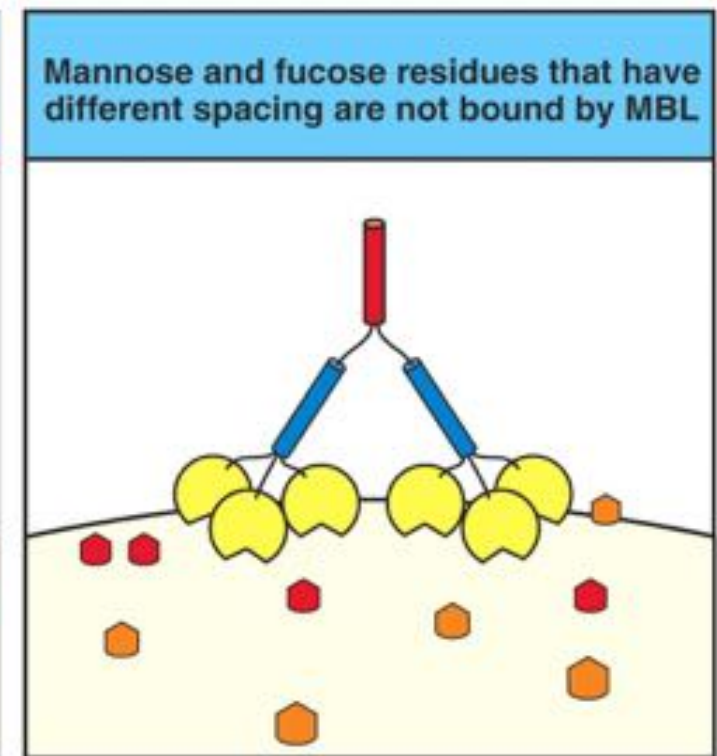
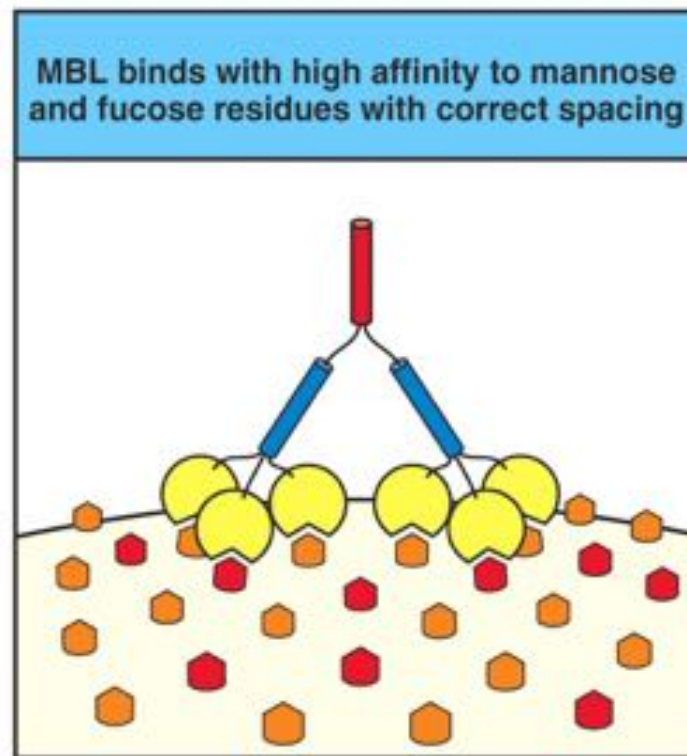
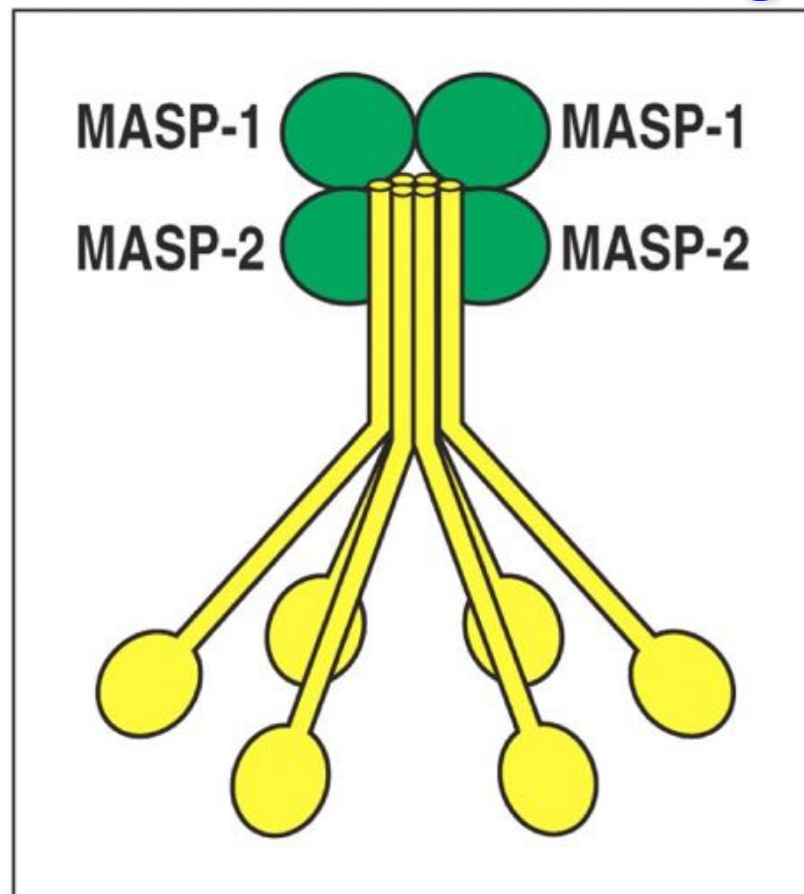
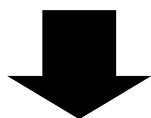


Figure 2-11 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

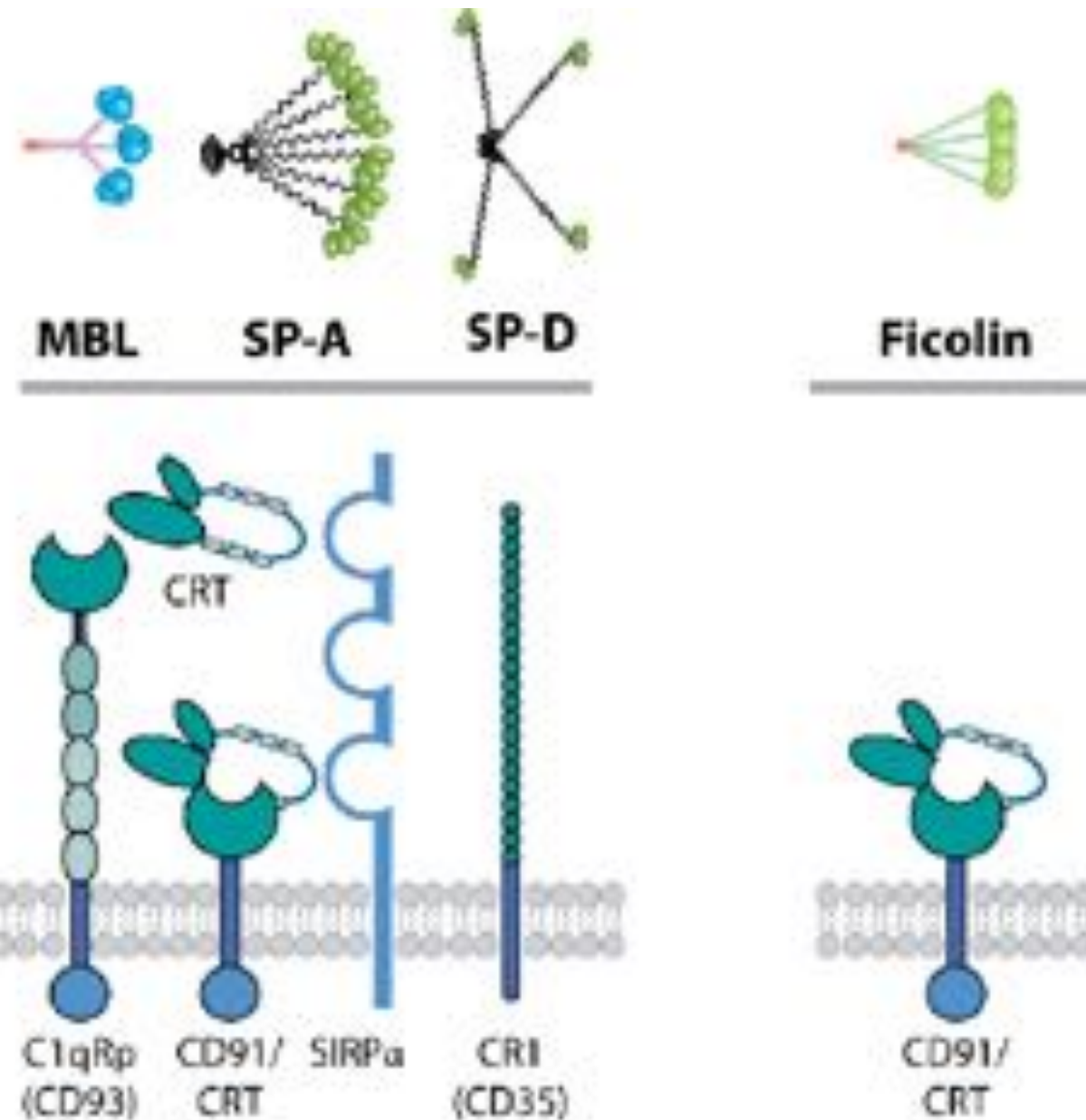
MBL
SPA
SPD
FICOLINs

➤ It interacts with **MASP1** and **MASP2** (Mannan Associated Serine Protease)

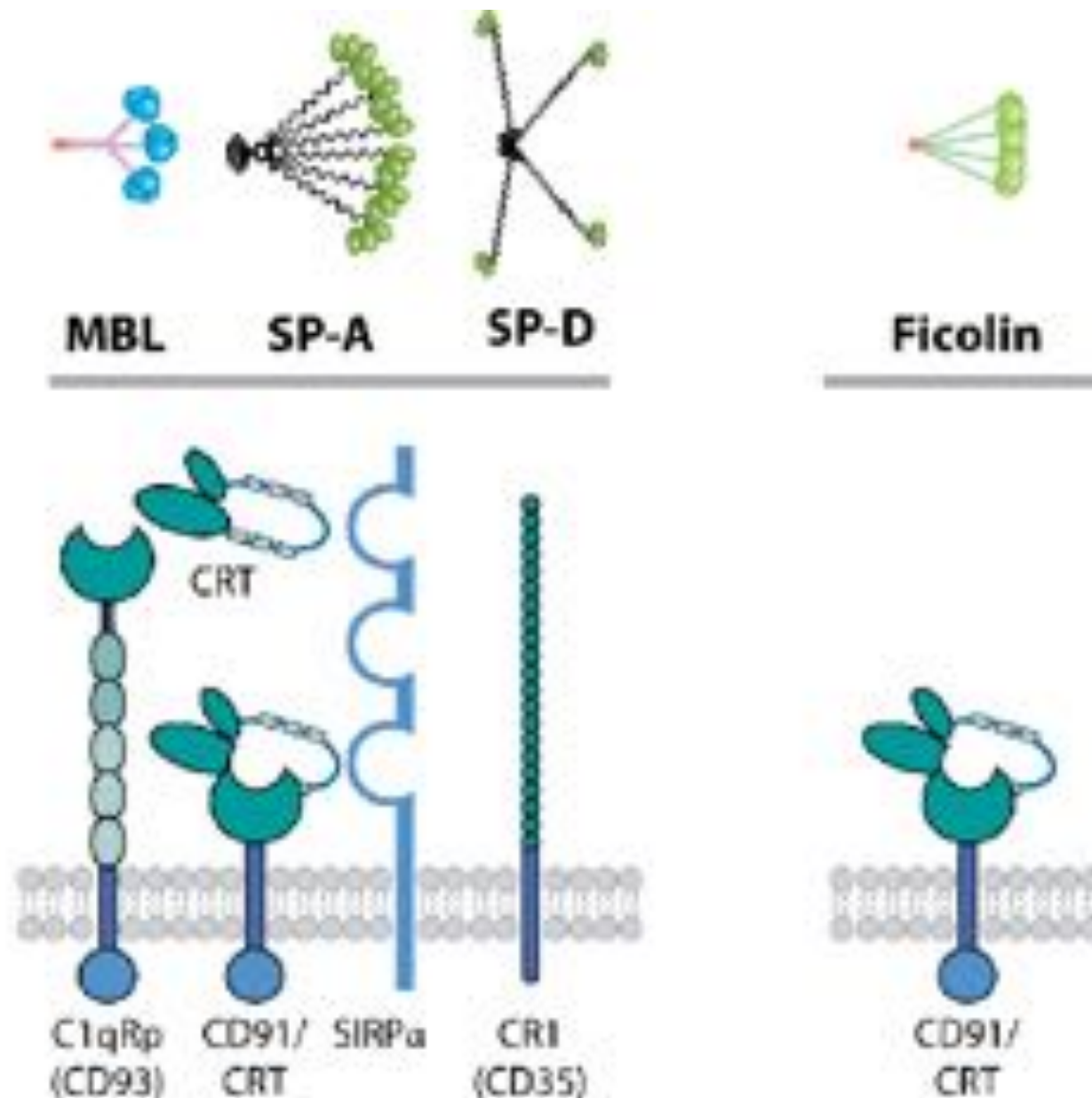


C4 and C2 activation ➡ **C3 convertase** of the classical pathway

The humoral collectins bind to specific receptors and are OPSONINS!



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The humoral collectins activate phagocytosis!!

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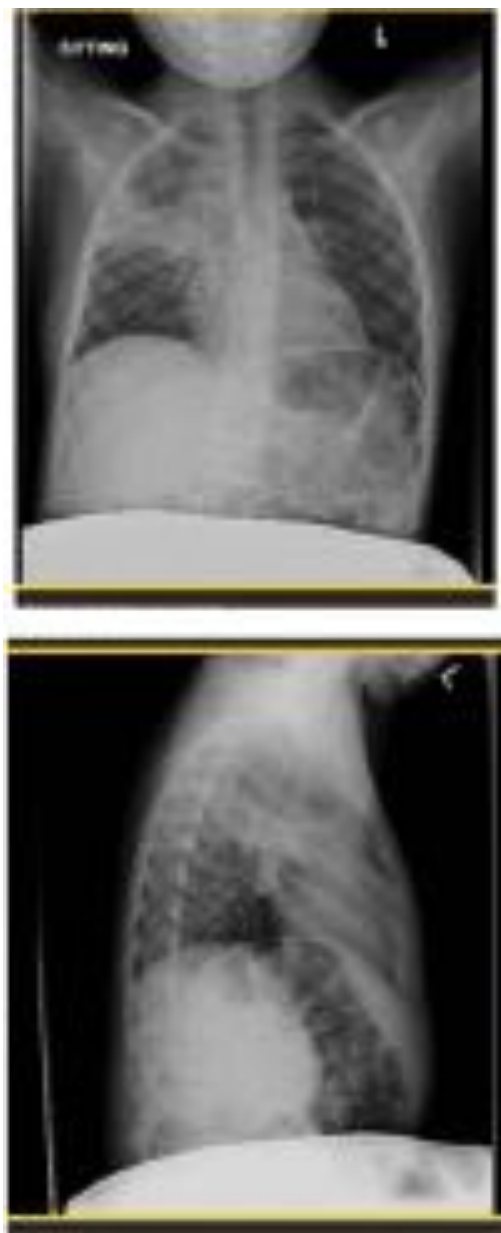
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- **The MBL deficiency is also associated with autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).**

Clin Med Insights Pediatr.
2012; 6: 89–94.
Mannose Binding Lectin
Deficiency: More than Meets
the Eye
Michelle Halbrich, Moshe Ben-
Shoshan, and Christine
McCusker

This case report describes a 5-year-old boy who presented to the emergency department with clinical symptoms and chest X-ray findings suggestive of pneumonia. Further history revealed multiple other infections, and workup for immunodeficiency revealed a deficiency of mannose-binding lectin (MBL), a pattern recognition receptor involved in activation of the complement system. Innate immunodeficiency may be more common than currently appreciated, with mutations of MBL affecting up to 50% of individuals in some populations. While pneumonia is a common presentation in the Pediatric Emergency Department, clinical presentations of children with defects of innate immunity can be unpredictable. Children may initially appear well with sudden deterioration. These cases pose particular challenges to physicians, and the level of suspicion for innate defects must remain high. It is crucial to identify patients with such impairments to better manage and prevent future complications.



X-ray from the emergency department demonstrating a right middle lobe pneumonia.



SAPIENZA
UNIVERSITÀ DI ROMA
AZIENDA POLICLINICO UMBERTO I
DIPARTIMENTO ASSISTENZIALE INTEGRATO
MEDICINA DIAGNOSTICA



U.O. IMMUNOLOGIA- IMMUNOPATOLOGIA DLC05
Responsabile F.F Prof. Fabrizio Mainiero
Tel: 06 49970966

Roma,

Sig..... PAZIENTE
(Cognome e Nome)

Prelievo del.....

Provenienza ...DAI Pediatria.....

DOSAGGIO LECTINA LEGANTE IL MANNOSIO (MBL) PER DEFICIT MBL

MBL 1470 (>100 ng/ml V.N.)

Il test è stato eseguito mediante MBL Oligomer ELISA kit (BioPorto Diagnostics).

Il Responsabile
Fabrizio Mainiero

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- **Only recently has been described a mutation of the gene FCN3, coding for the H - ficolin and cause defects of complement activation, and gene polymorphisms FCN1 which encodes the M-ficolin have been associated with susceptibility to develop arthritis rheumatoid arthritis.**

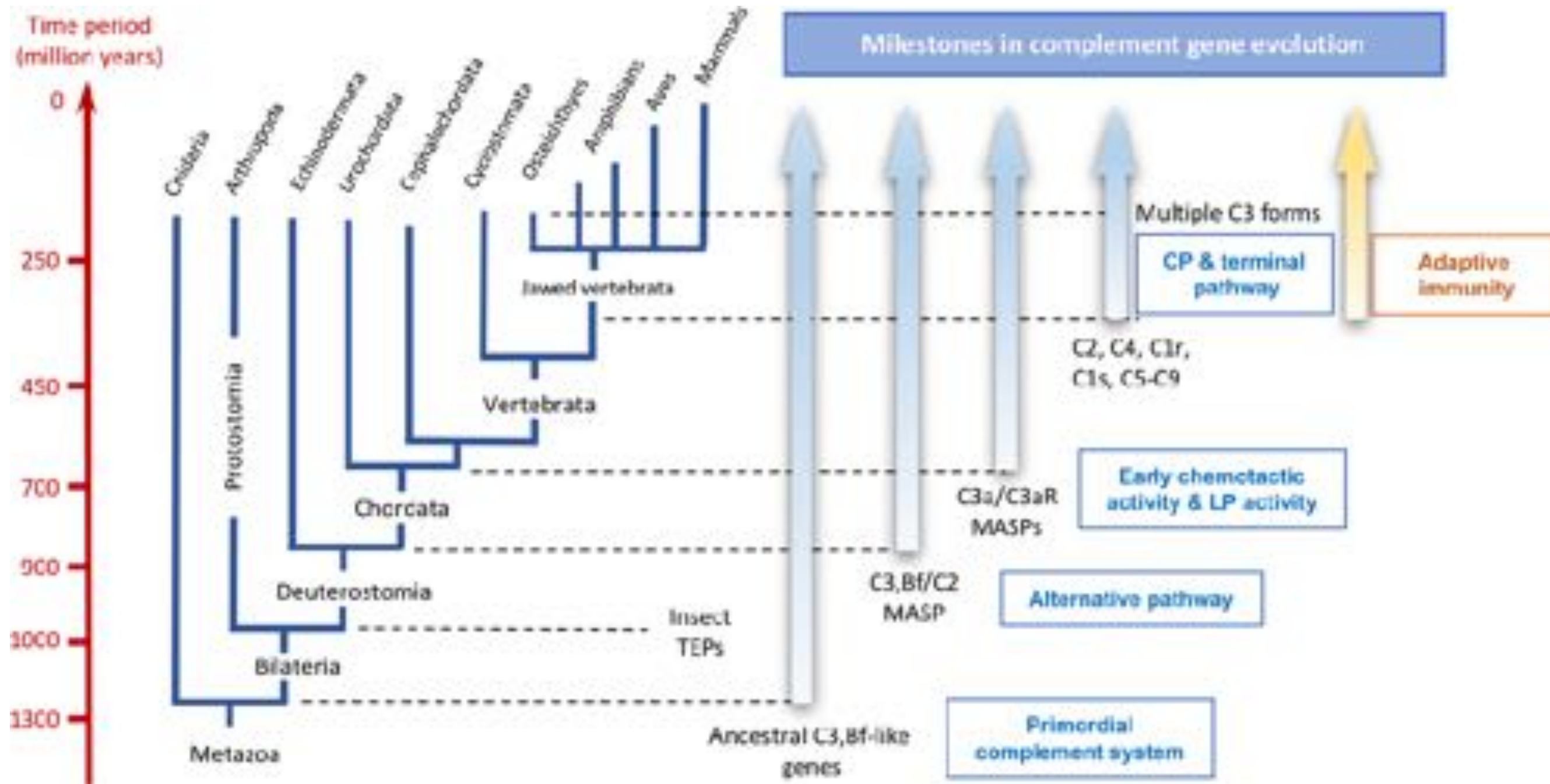
Proteins of the complement system!

| Functional protein classes in the complement system | |
|---|---|
| Binding to antigen-antibody complexes and pathogen surfaces | C1q |
| Binding to mannose on bacteria | MBL |
| Activating enzymes | C1r C1s C2 Bb D MASP-1 MASP-2 |
| Membrane-binding proteins and opsonins | C4b C3b |
| Peptide mediators of inflammation | C5a C3a C4a |

| Functional protein classes in the complement system | |
|---|---|
| Membrane-attack proteins | C5b C6 C7 C8 C9 |
| Complement receptors | CR1 CR2 CR3 CR4 C1qR |
| Complement-regulatory proteins | C1INH C4bp CR1 MCP DAF H I P CD59 |

Figure 2-29 Immunobiology, 6th ed. © Garland Science 2009

The COMPLEMENT is the oldest defense system!



Immunol Rev. 2016 Nov;274(1):33-58.

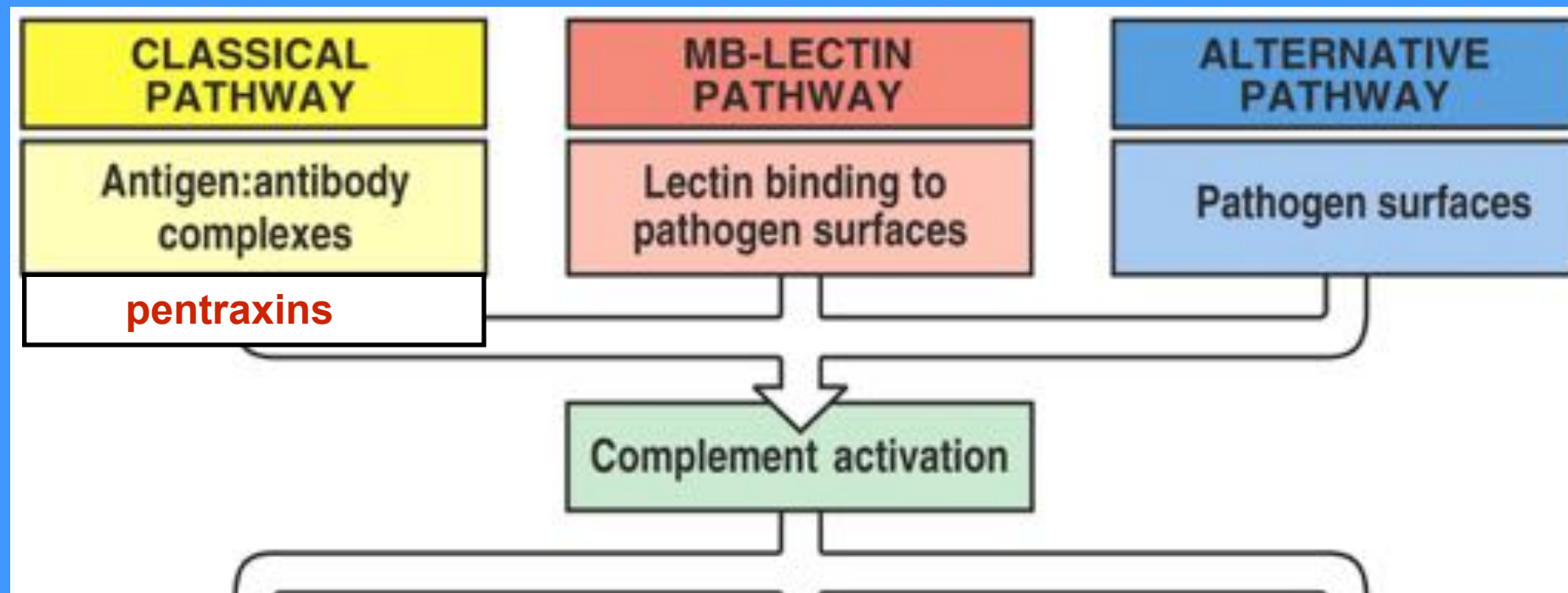
Complement component C3 - The "Swiss Army Knife" of innate immunity and host defense.

Ricklin D, Reis ES, Mastellos DC2, Gros P, Lambris JD.

PLASMA COMPLEMENT PROTEIN CONCENTRATIONS!

| Name | MW | mg/dl | fragments |
|--------------|-----|-----------|--|
| C1q | 410 | 0,7-3 | |
| C1r | 83 | 0,34-1 | |
| C1s | 85 | 0,3-0,8 | |
| C4 | 204 | 15-53 | C4a, C4b, C4c, C4d |
| C2 | 102 | 0,15-0,3 | C2a, C2b |
| C3 | 190 | 55-120 | C3a, C3b, C3c, C3d, C3f, C3g, C3dg, iC3b |
| C5 | 196 | 0,70-0,85 | C5a, C5b |
| C6 | 125 | 0,6-0,7 | |
| C7 | 120 | 0,55-0,7 | |
| C8 | 150 | 0,55-0,8 | |
| C9 | 66 | 0,5-1,6 | |
| Fattore B | 100 | 1,4-2,4 | Ba, Bb |
| P | 224 | 0,2-0,3 | |
| Fattore D | 24 | 0,01-0,02 | |
| MBL | 540 | 0,01 | |
| MASP-1 | 94 | 0,005 | |
| MASP-2 | 76 | 0,005 | |
| C1IH | 105 | 1,8-2,75 | |
| C4BP | 550 | 2,5 | |
| Fattore H | 150 | 3-5, 6 | |
| Fattore I | 100 | 0,34-0,55 | |
| CD59 | 20 | 0,005 | |
| DAF | 200 | 0,005 | |
| Clusterina | 80 | 0,03 | |
| Proteina S | 65 | 0,02 | |
| Vitronectina | | | |

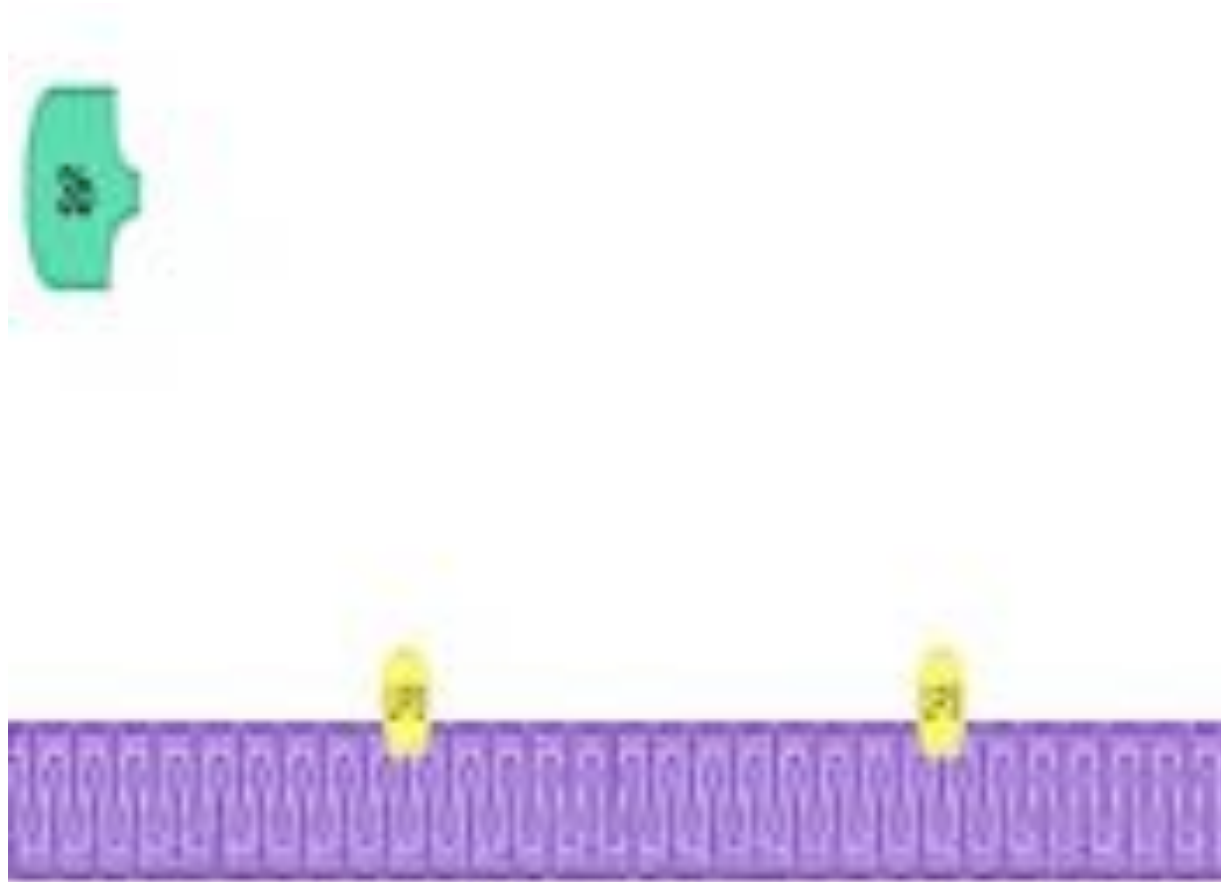
THE THREE MECHANISMS OF COMPLEMENT ACTIVATION!



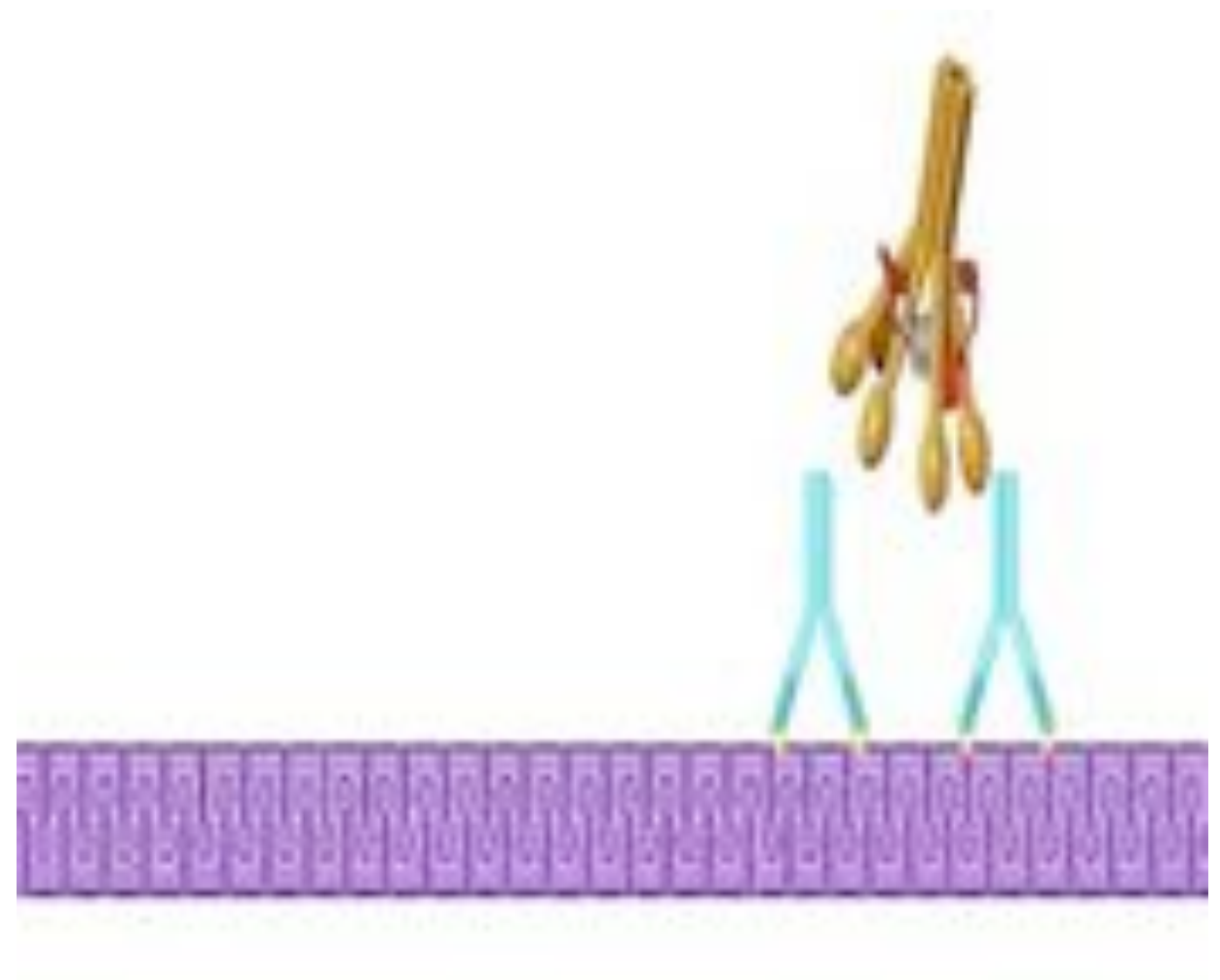
**ALL THREE MECHANISM HAVE C3
AS CENTRAL PROTEIN
AND CONVERGE
IN THE ACTIVATION OF C5!!!**

THE ALTERNATIVE AND CLASSICAL MECHANISMS OF COMPLEMENT ACTIVATION!

ALTERNATIVE PATHWAY

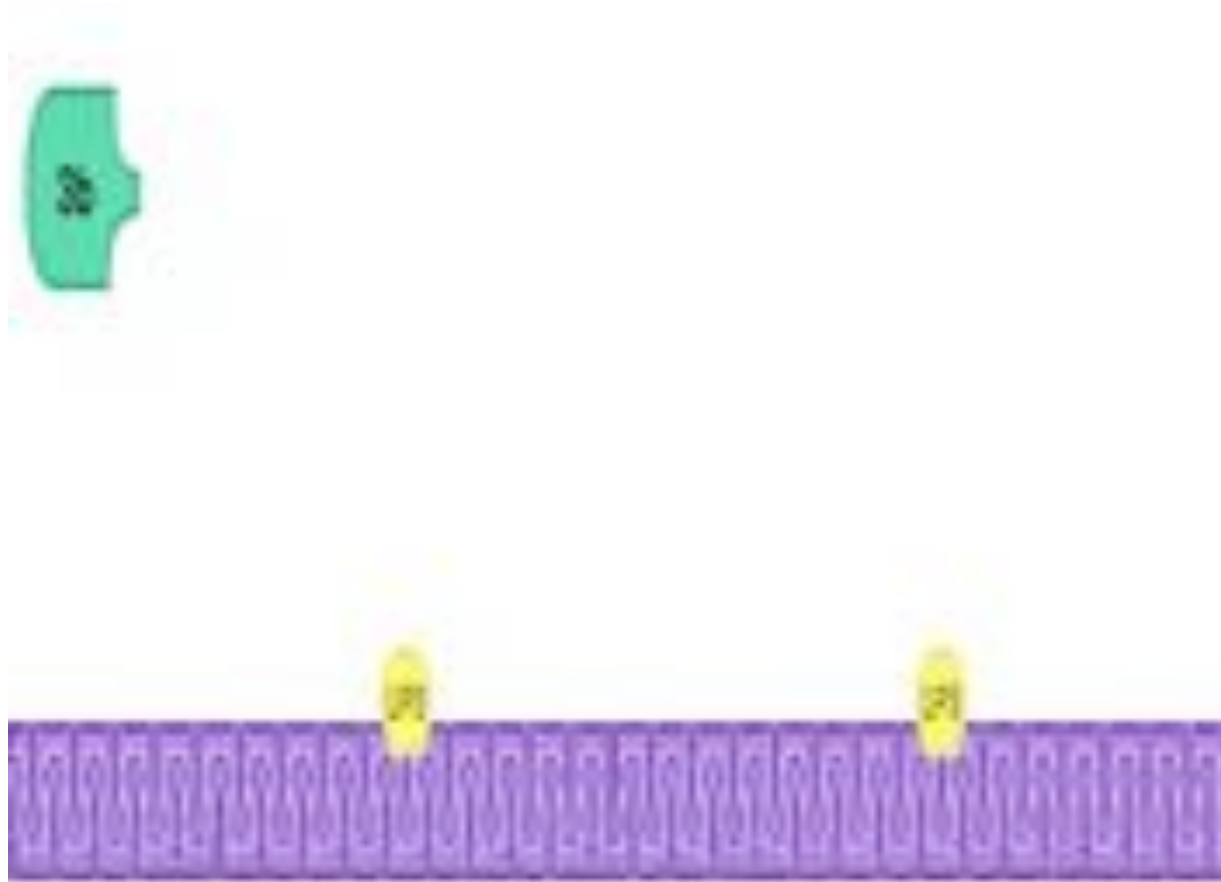


CLASSIC PATHWAY

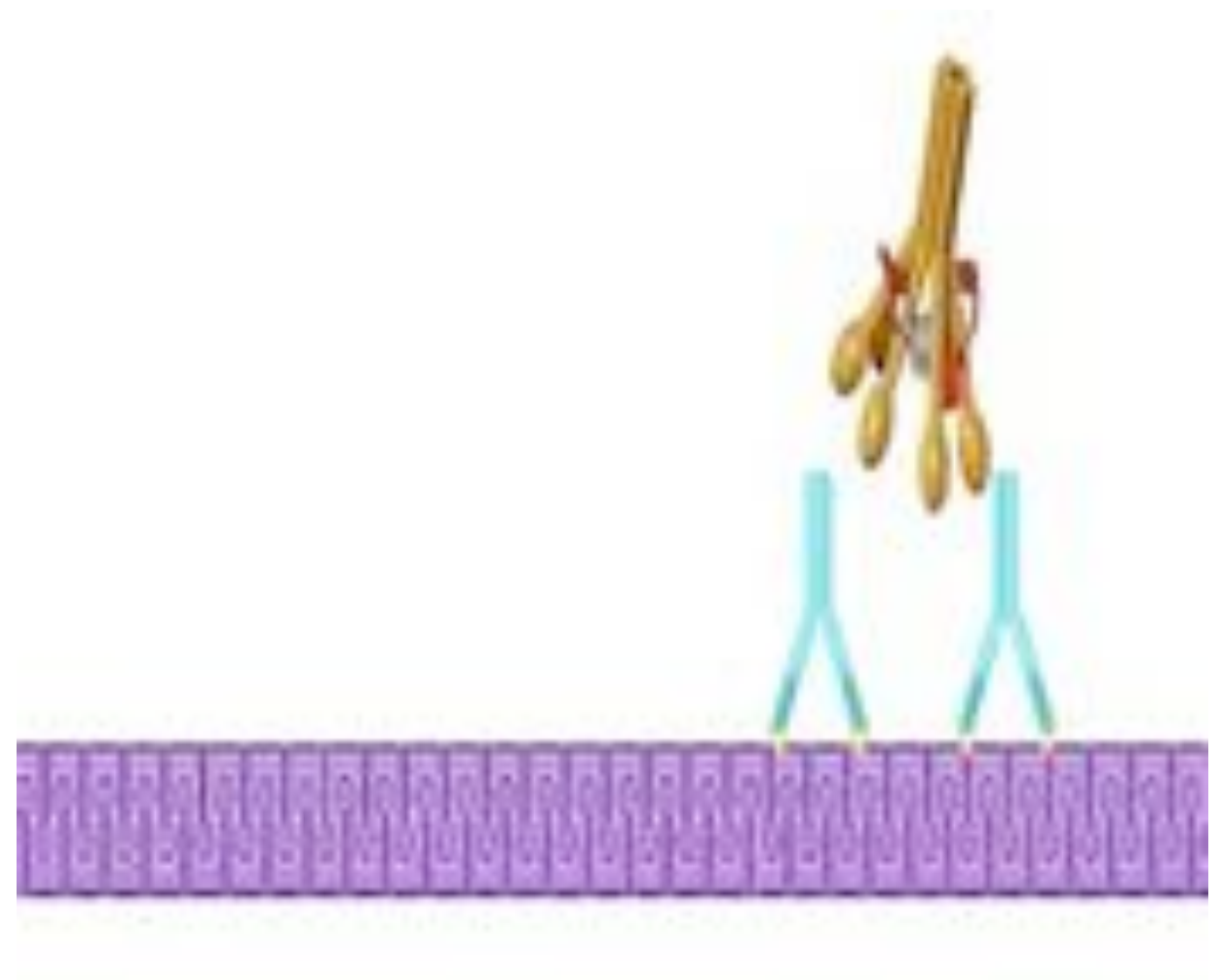


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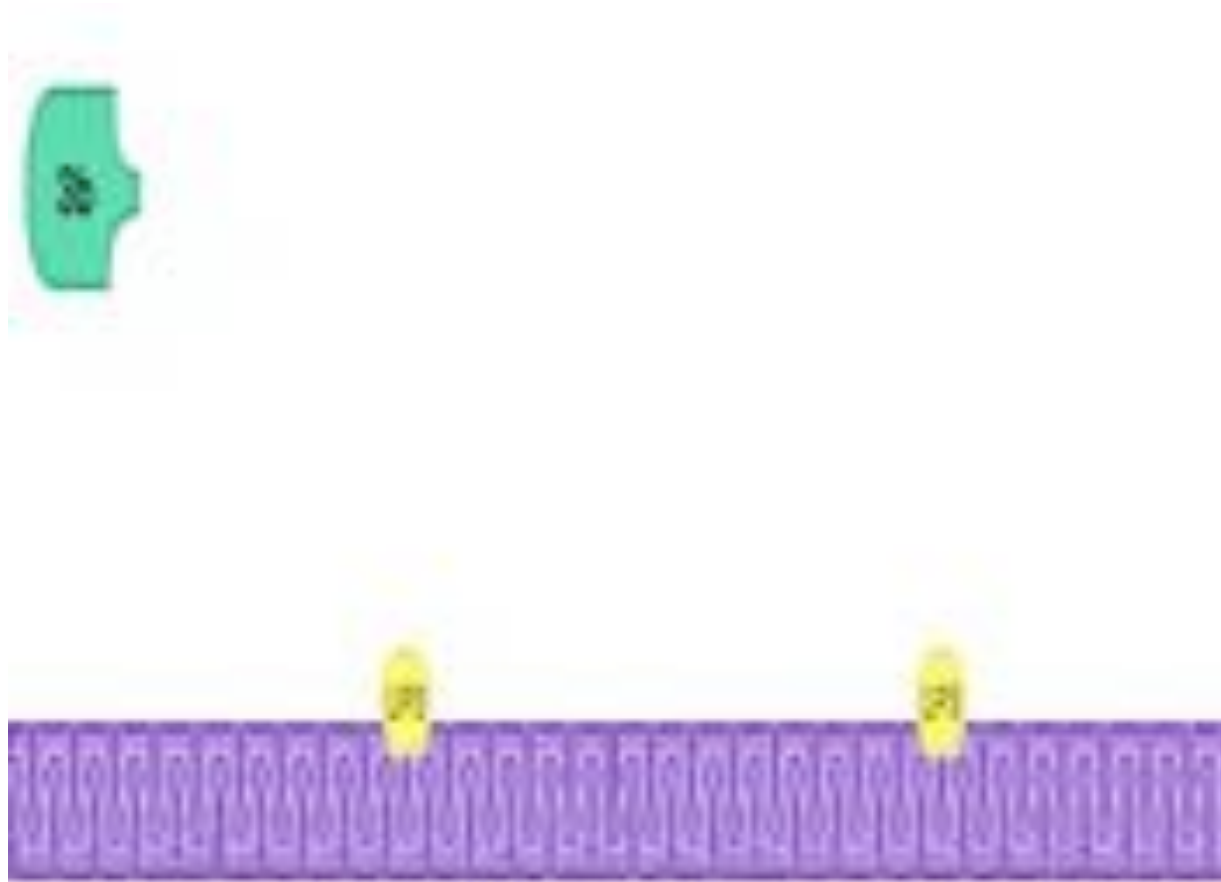


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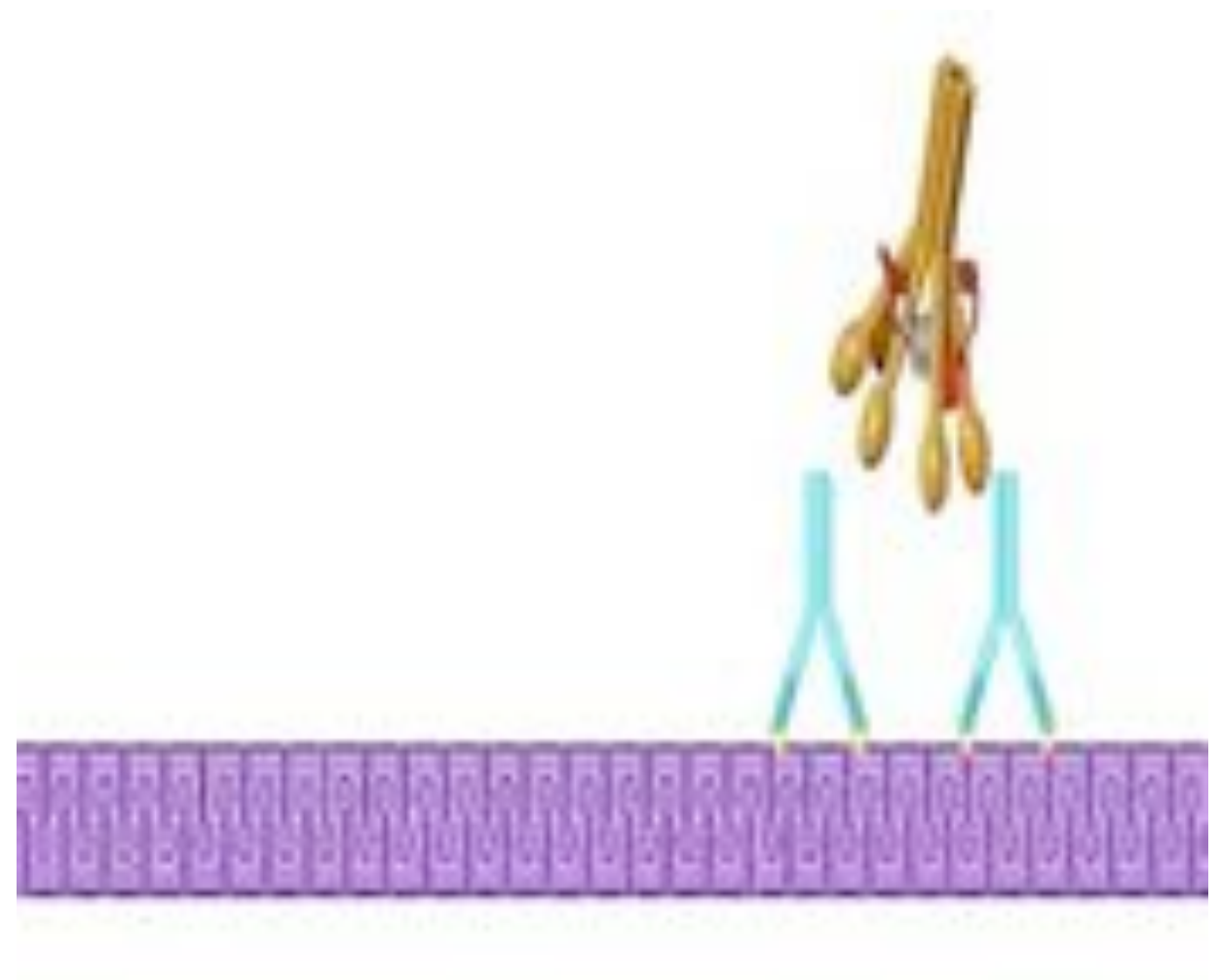


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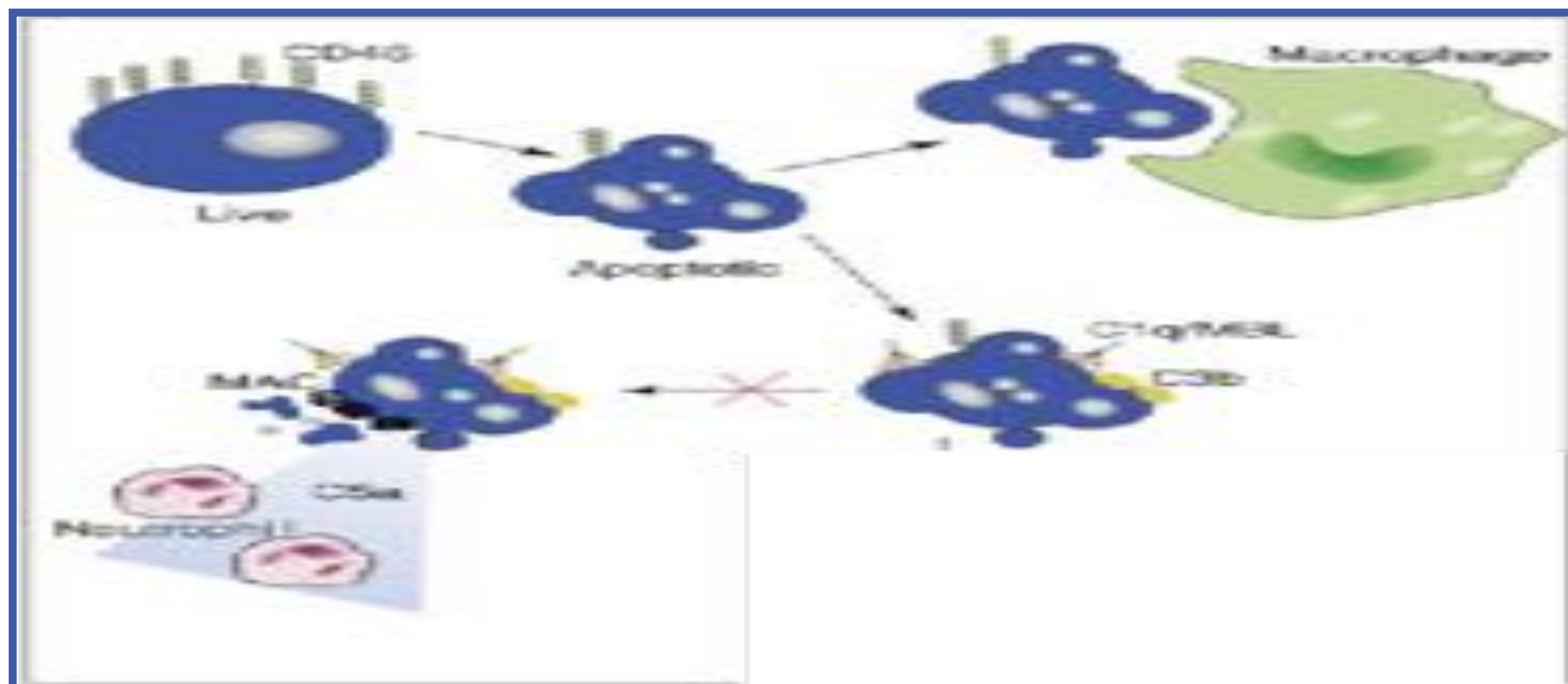


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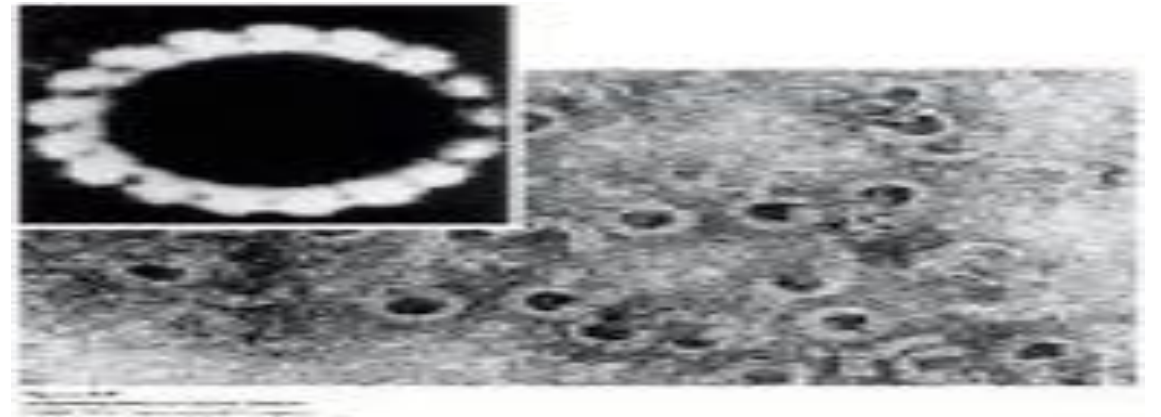


It has recently been shown that C1q, the 1st component of the classical pathway, as well as by antibodies, can be activated by pentraxins ! **CLASSICAL COMPLEMENT ACTIVATION IN THE NATURAL IMMUNITY AND INFLAMMATION!**

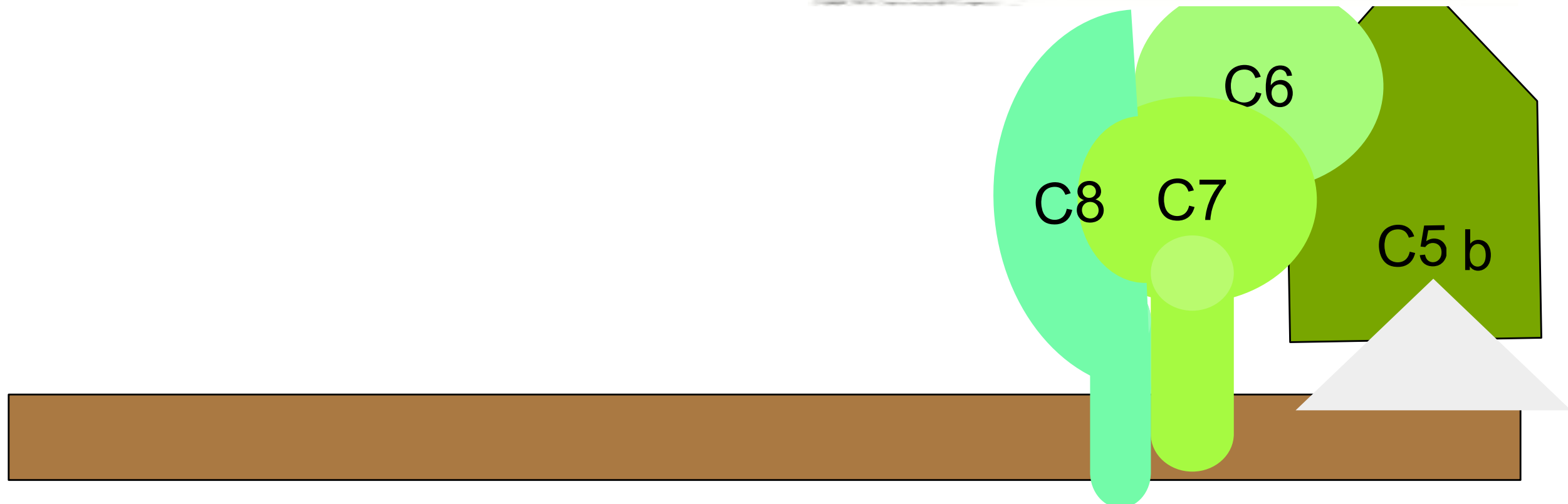
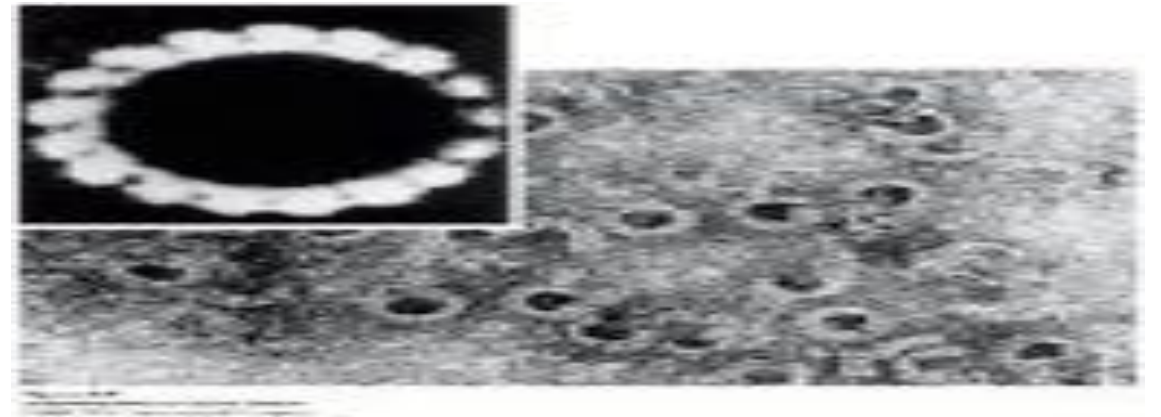
In addition to recognizing the Fc portion of antibodies, C1q binds to pentraxins (CRP, SAP, PTX3) through its gC1q domain. gC1q also binds directly to many gram-negative bacteria through Omp, LPS, or lipid A and to viruses (e.g., gp41 of HIV-1 or gp21 of HTLV-1). C1q also interacts with misfolded proteins, such as amyloid A β peptide and prion proteins found in neurodegenerative diseases and with several ECM proteins (such as fibromodulin, osteoadherin, fibronectin, and laminin). Finally, C1q binds via the globular head domain to surface blebs on apoptotic cells and to necrotic cells directly or through **pentraxins!!!!**



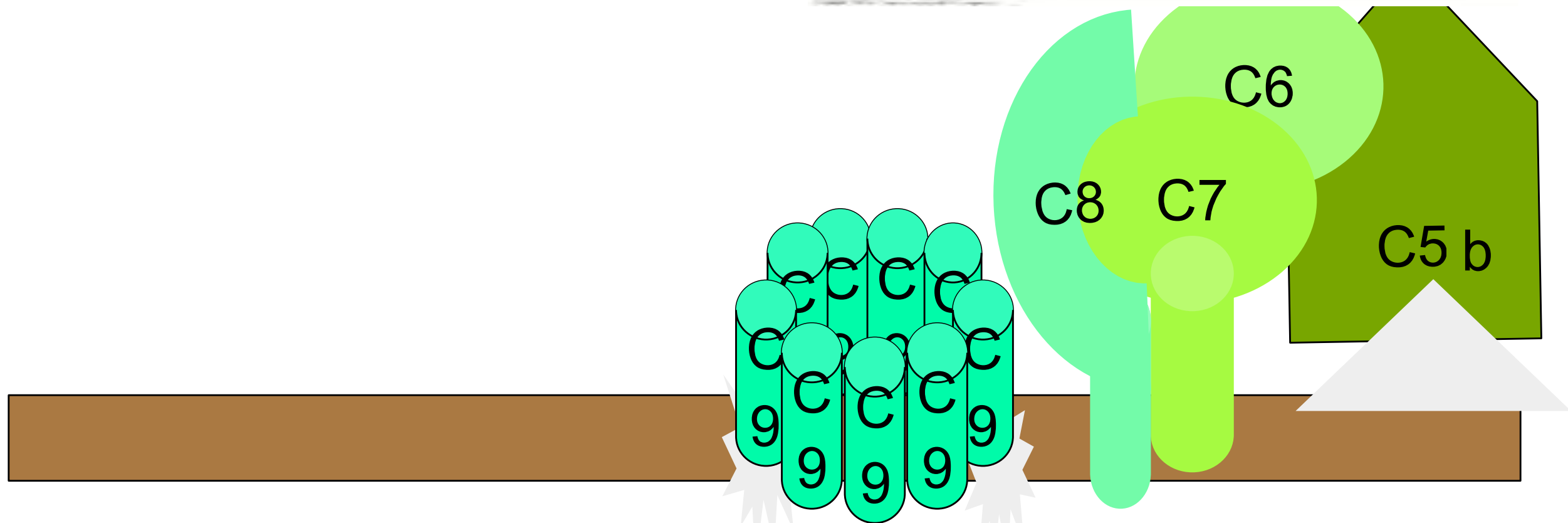
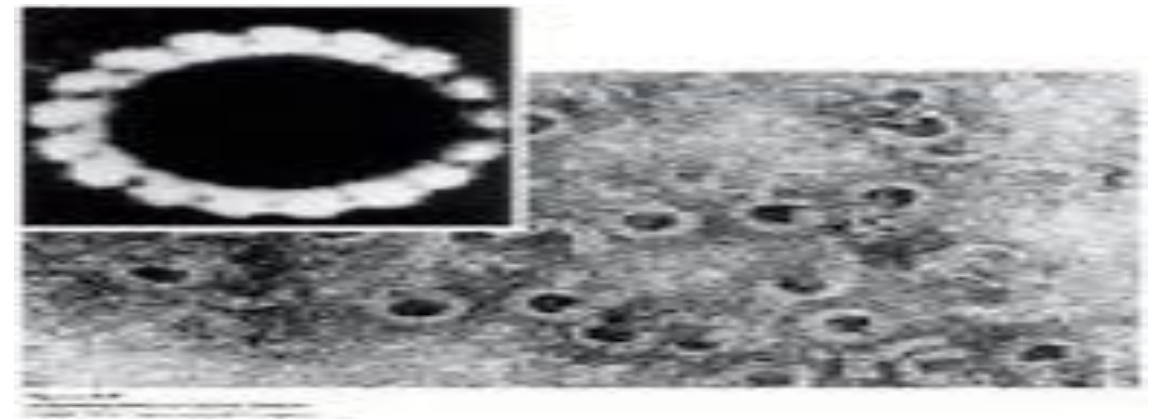
**ALL THREE MECHANISMS LEAD TO
LITHIC COMPLEX IN CELL MEMBRANE
or MAC, WHICH DESTROYS
PATHOGENS**



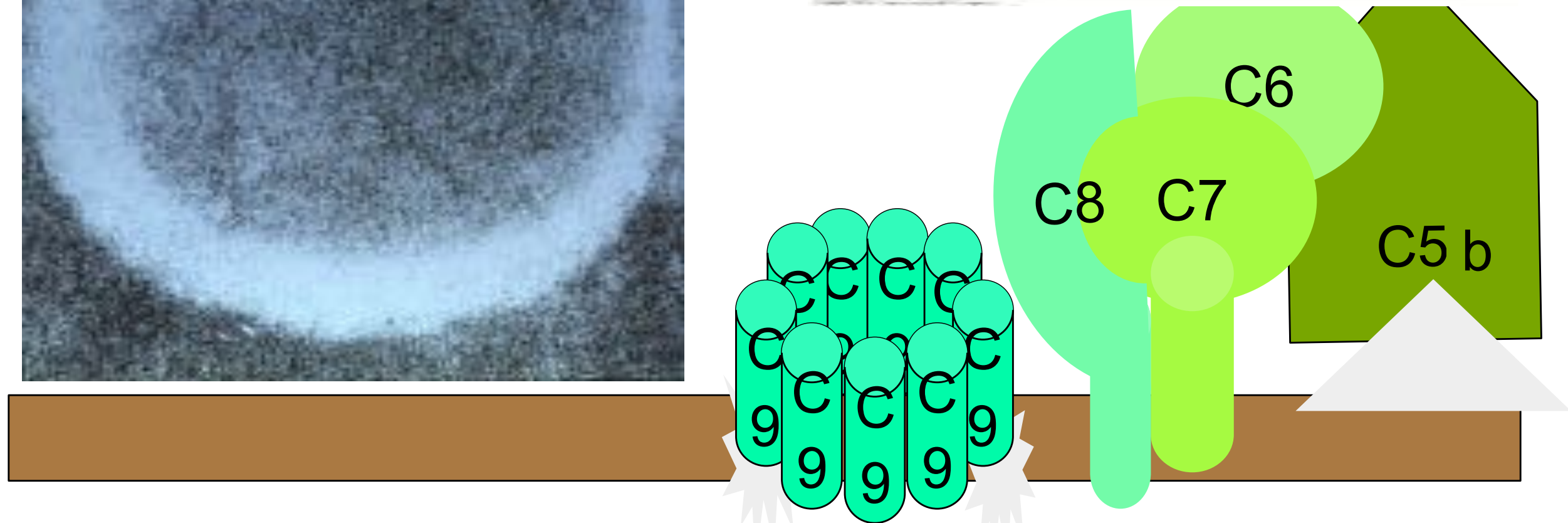
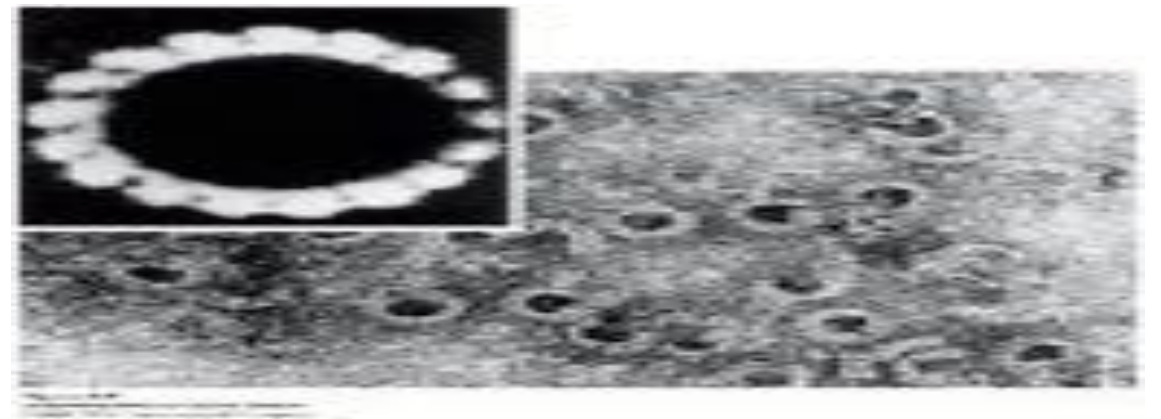
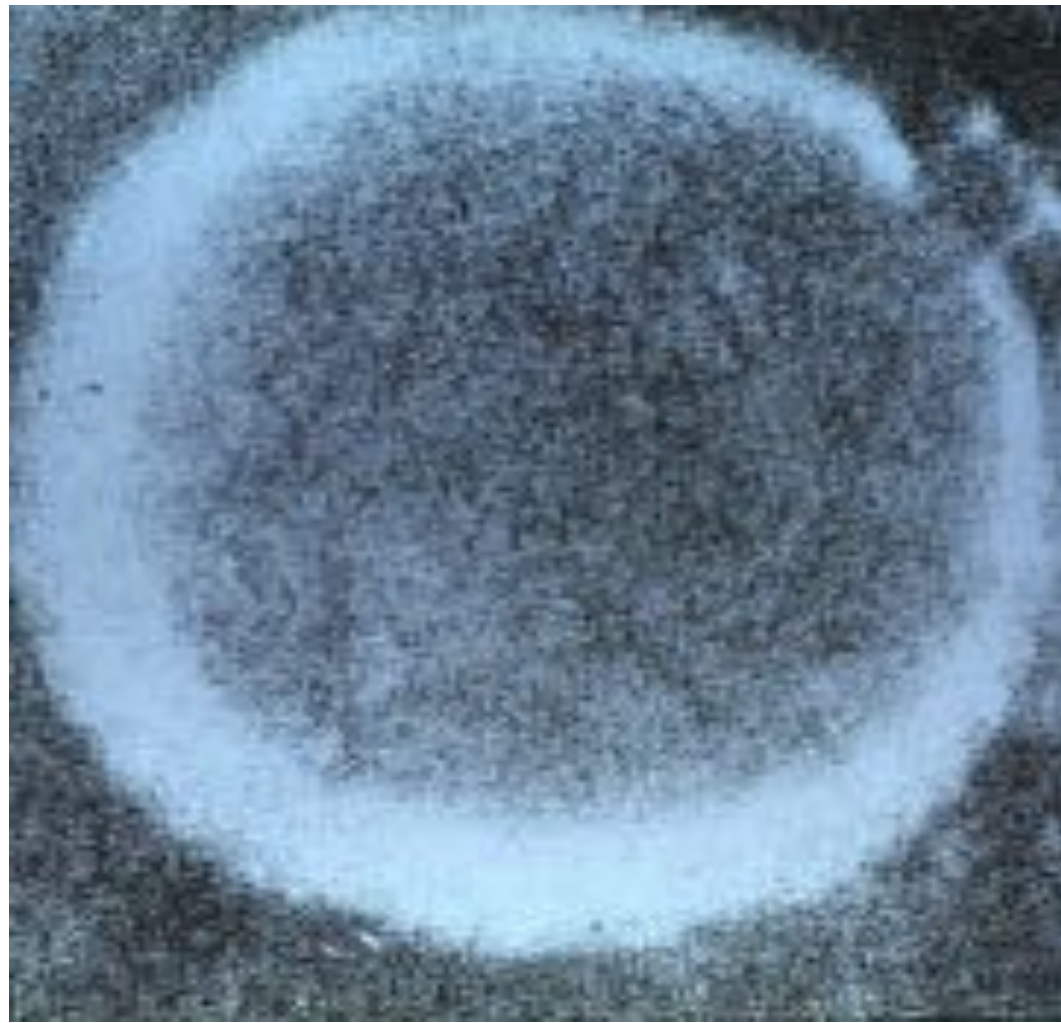
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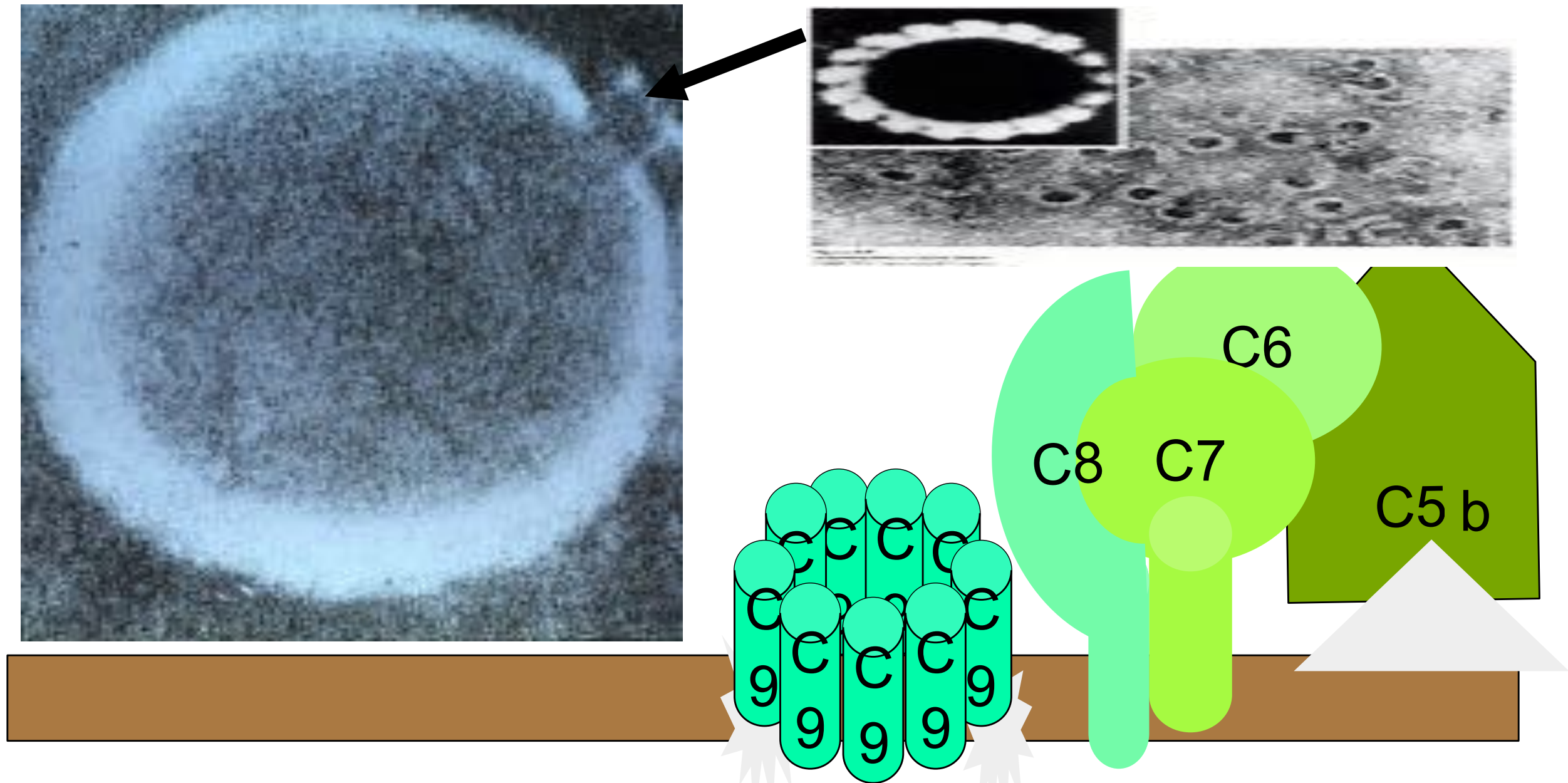
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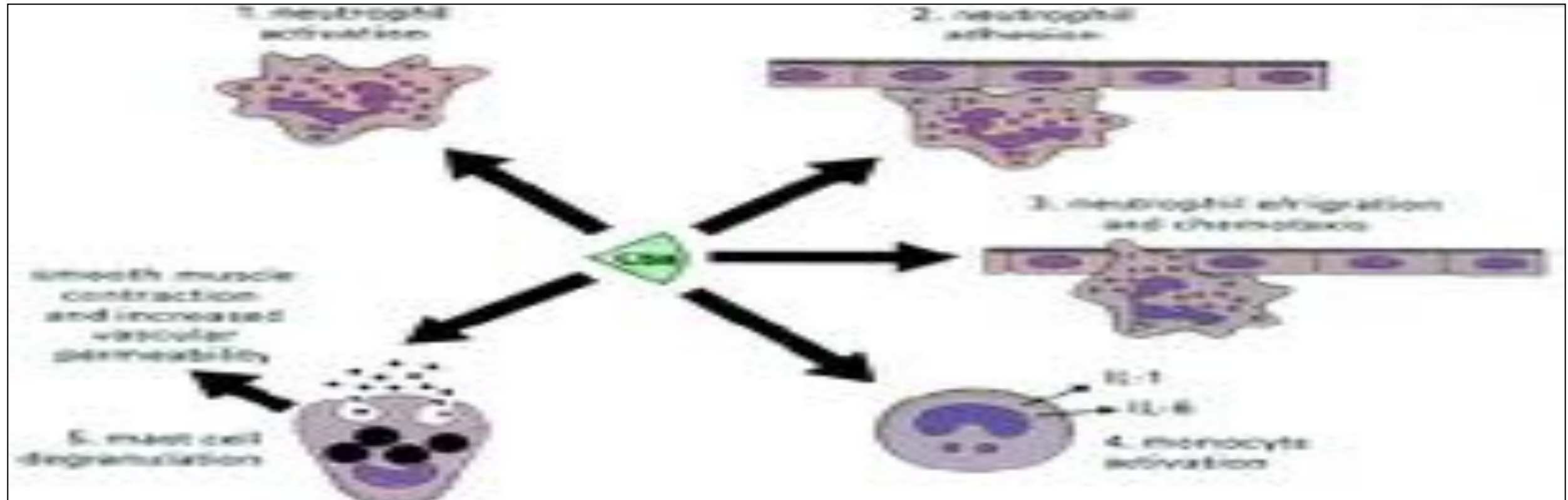


**ALL THREE MECHANISMS LEAD TO
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.....AND ALL THREE LEAD TO THE PRODUCTION OF ANAPHYLATOXINS (WHICH ACTIVATE INFLAMMATION) AND OF THE PHAGOCYTOSIS OSPONINS!

ANAPHYLATOXINS (C5a-C3a)



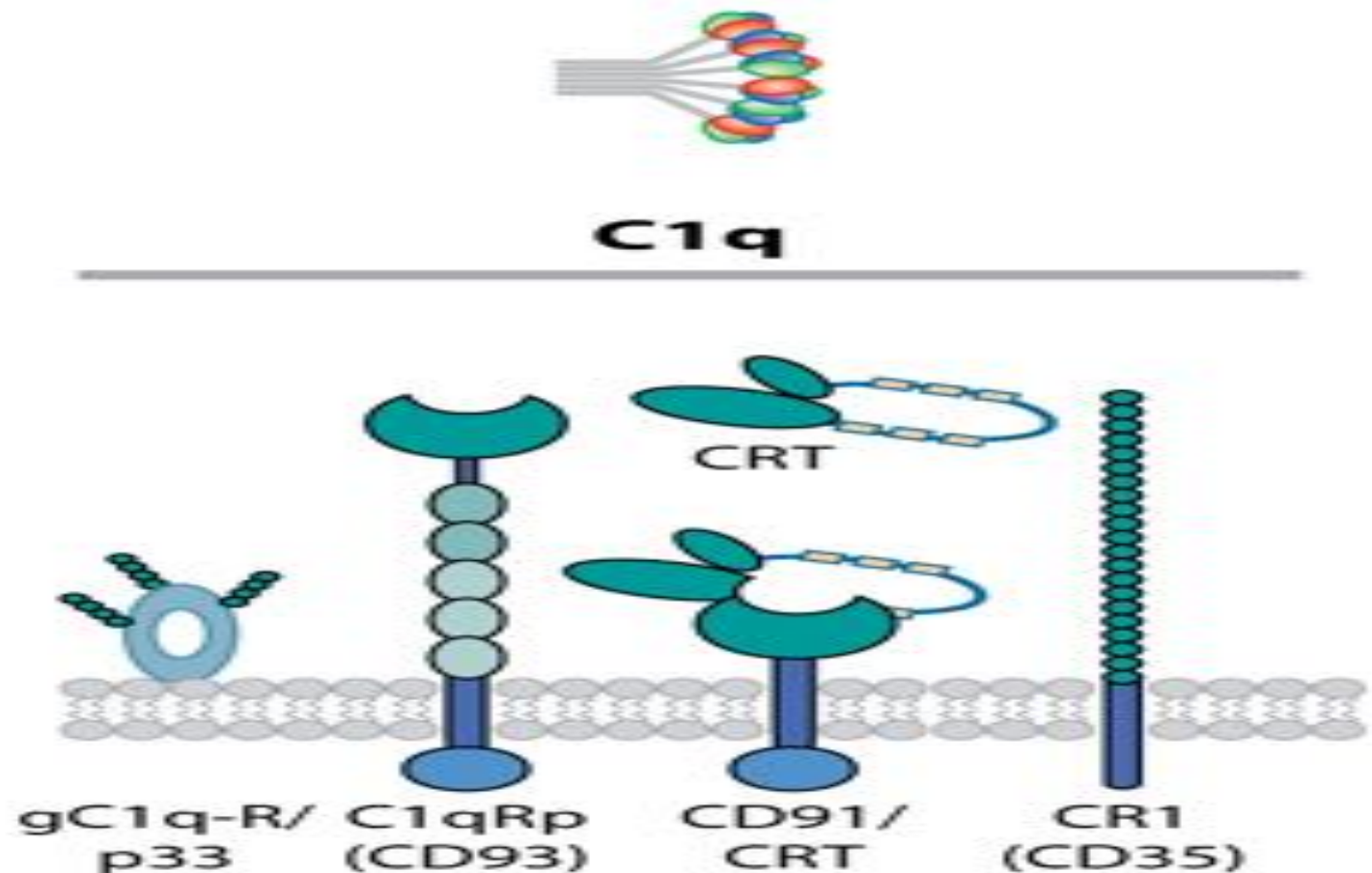
OSPONINS (C4b-C3b)-PHAGOCYTOSIS



C1q is also an opsonin and active phagocytosis!

C1q binds to a wide range of cell types (PMN, monocytes, lymphocytes, DCs, ECs, and platelets), resulting in the induction of cell-specific biological responses, which include phagocytosis, chemotaxis, the generation of procoagulant activity, activation of ECs, and enhancement of FcγR- and CR1-mediated phagocytosis and superoxide production.

Receptors for C1q humoral complement factor !

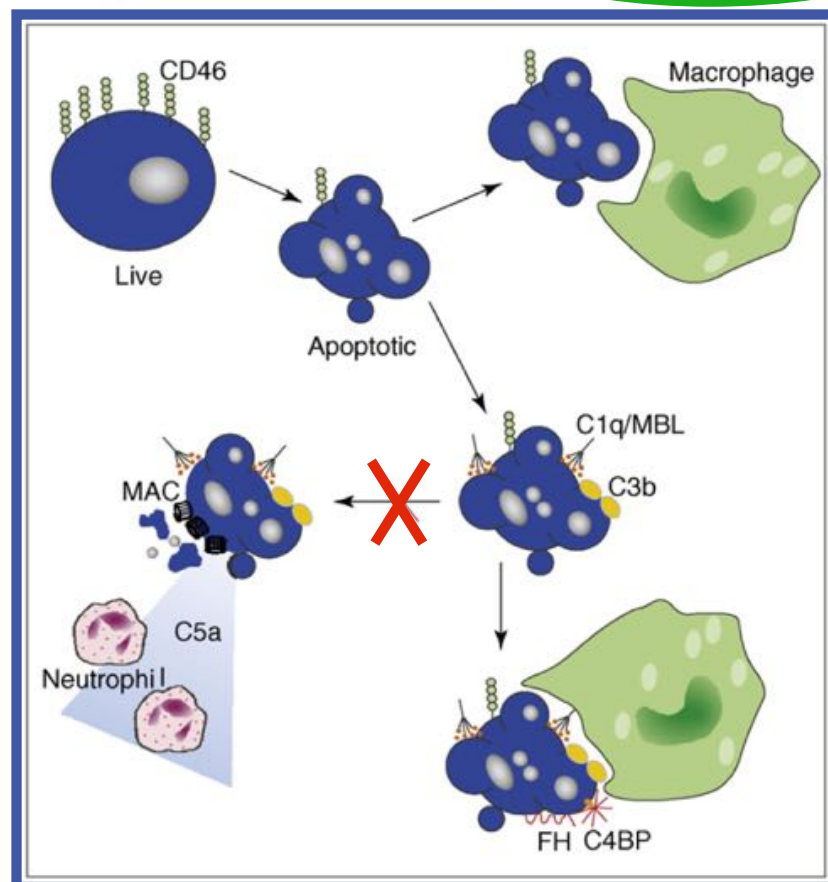


To date, investigators have described four types of C1q-binding proteins/receptors expressed on the cell surface. These include cC1q-R/calreticulin (CRT), a 60-kDa protein ; gC1q-R/p33, a 33-kDa homotrimeric protein; C1q-Rp (CD93), a 120-kDa O-sialoglycoprotein; and CR1 (CD35), the receptor for C3b. In addition to C1q, CRT reportedly serves as a receptor for collectins, such as the MBL, SP-A, SP-D, CL-43, and conglutinin, and, in association with CD91, initiates macropinocytosis and phagocytosis of apoptotic cells

Complement activation is tightly regulated!

Table 1 | Complement interactions with pathogens and self

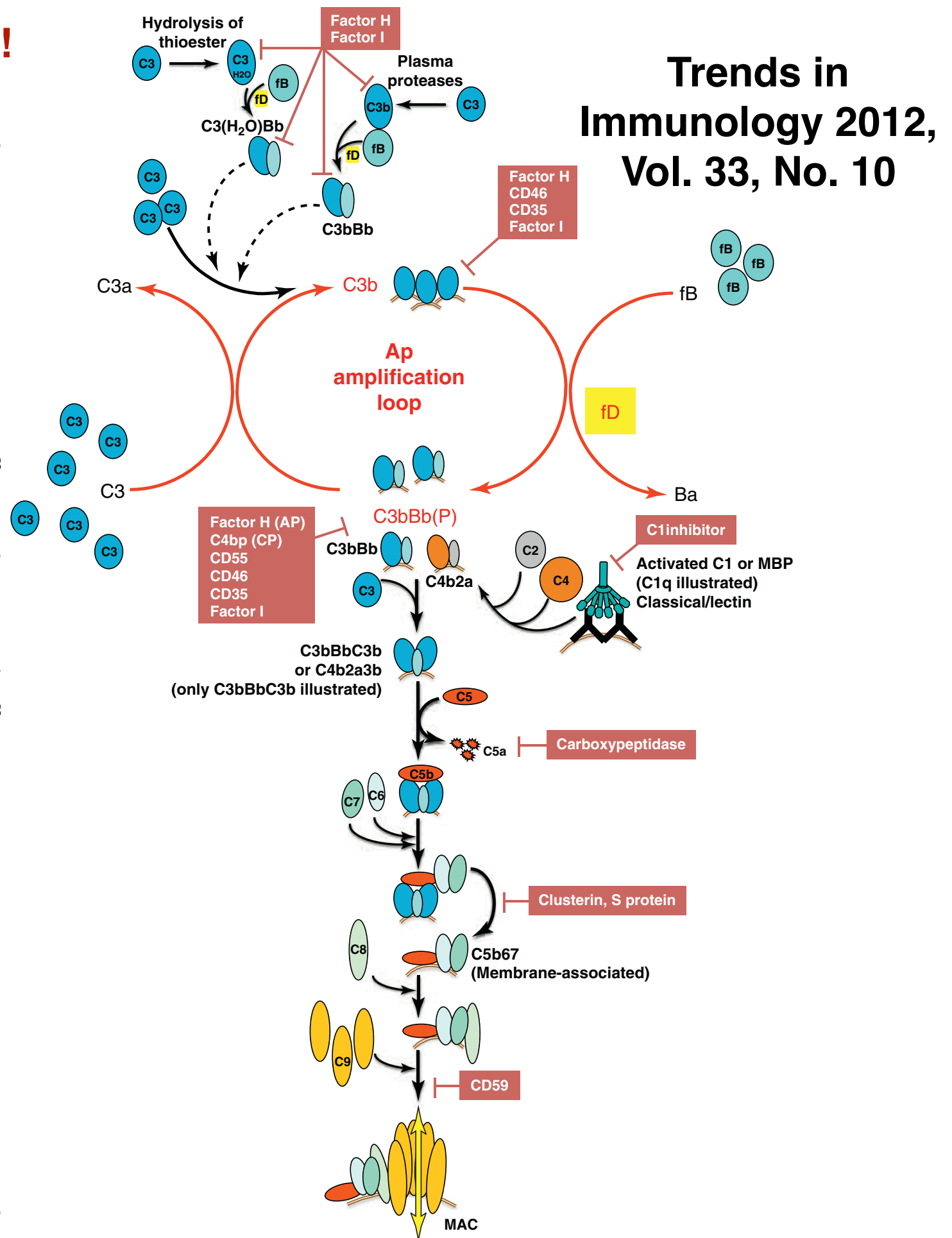
| | Activation profiles | Outcomes | Examples |
|--------------|-----------------------------|-----------------------------------|---|
| Pathogen | Robust and unrestricted | Inflammation and immunity | Bacteria and viruses |
| Altered self | Limited and targeted | Mild inflammation and no immunity | Apoptotic and injured cells and tissues; lipid and proteinaceous debris |
| Normal self | Baseline (through tickover) | No inflammation and no immunity | Healthy cells and tissues |



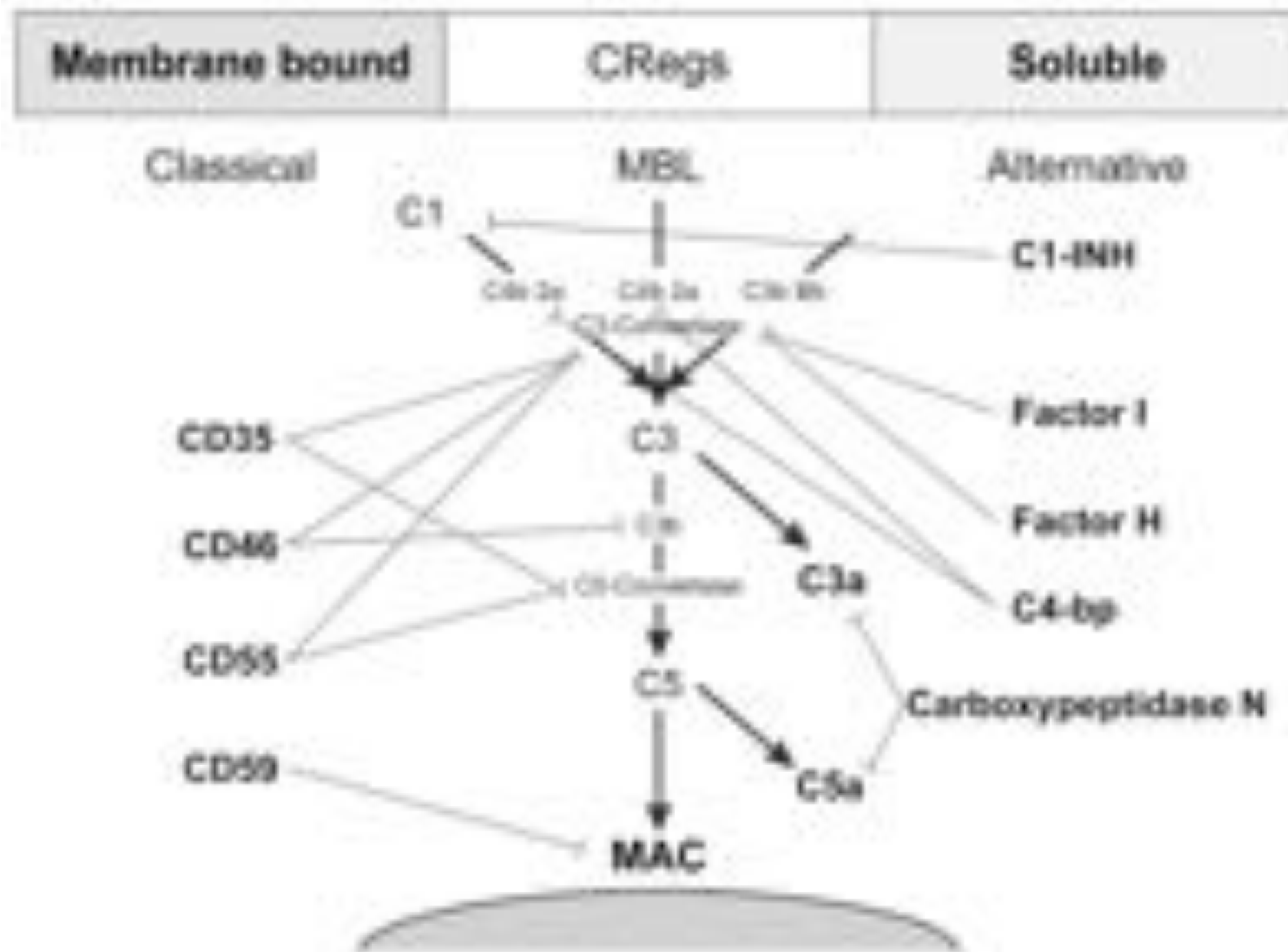
Complement activation on apoptotic cells depends on recognition by C1q and C3b/iC3b; the binding of Factor H and C4BP allows phagocytosis, without substantial activation of the terminal complement pathway and inflammation

Complement activation and regulation!

Complement tickover occurs through hydrolysis of the C3 thioester, or cleavage of C3 to C3b by plasma proteases. Fluid phase production of either molecule results in formation of the AP C3 convertase, C3bBb, and production of further C3b which either binds a surface or remains fluid phase. Each newly produced C3b can in turn form a convertase, which cleaves C3, resulting in exponential production of C3b. This self-propagation, referred to as the ‘amplification loop’ and indicated here in red, is responsible for amplifying a small trigger to yield large responses. C3b formed through any activation pathway feeds into the amplification loop. Binding of C3b to C3 convertase creates C5 convertase; cleavage of C5 and generation of C5b marks the start of the terminal pathway. C6 and C7 bind C5b to form C5b67, which is released from convertase, binds membrane and incorporates C8 and multiple C9 molecules to form the MAC. Self tissues are protected from accidental complement damage by regulatory proteins present in plasma and on membranes, indicated by maroon boxes. Note the abundance of regulators which control the amplification loop and C3 convertases.



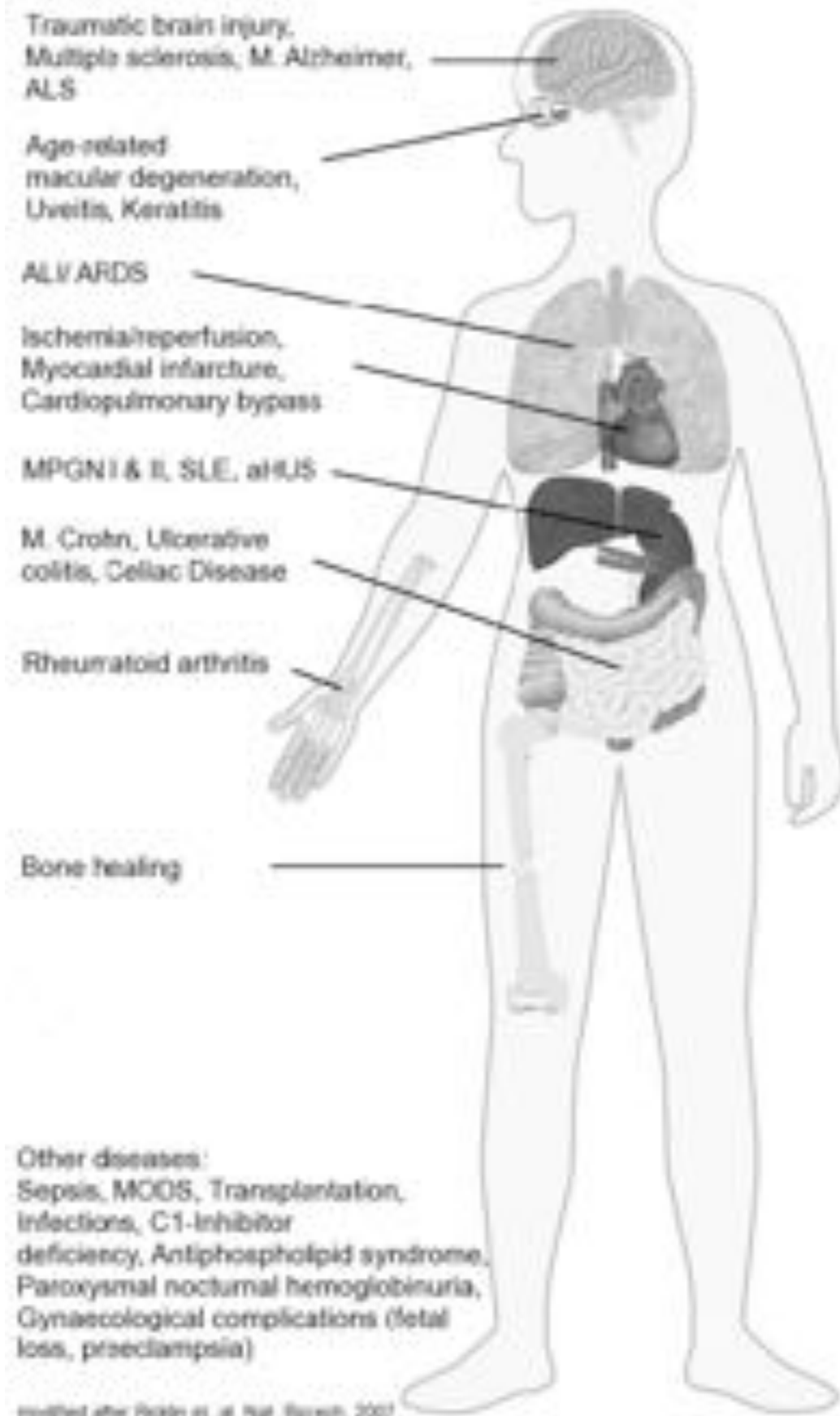
Scheme of the membrane bound and soluble complement regulators acting on different stages of the complement cascade!



Mol Med. 2011 Mar-Apr;17(3-4):317-29.

New insights of an old defense system: structure, function, and clinical relevance of the complement system.
Ehrnthaller C, Ignatius A, Gebhard F, Huber-Lang M.

Scheme of diseases in which complement has a major impact!



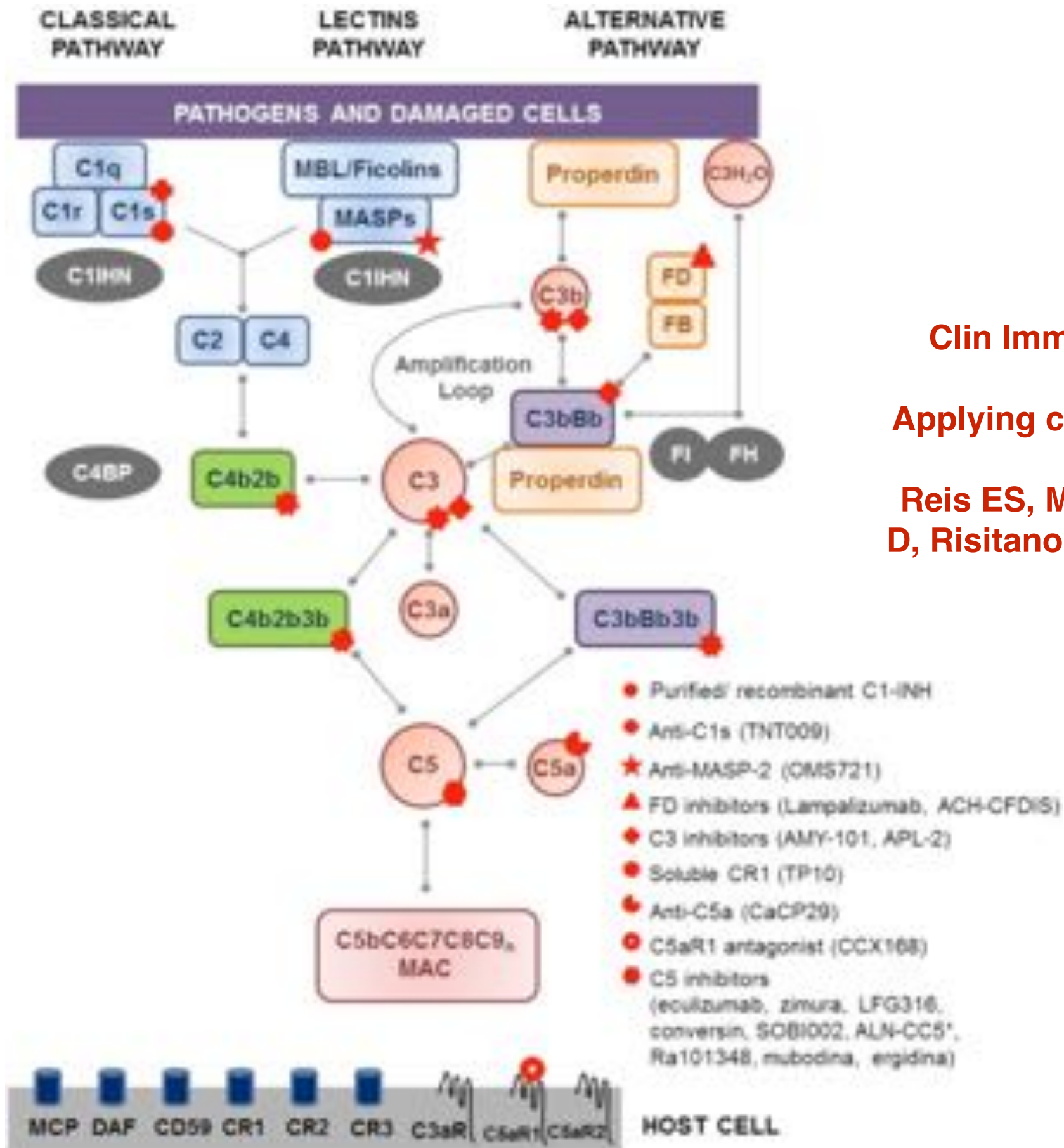
ALS, amyotrophic lateral sclerosis; ALI, acute lung injury; ARDS, adult respiratory distress syndrome; MPGN, membranoproliferative glomerulonephritis; SLE, systemic lupus erythematosus; aHUS, atypical hemolytic uremic syndrome; MODS, multiple organ dysfunction syndrome.

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New insights of an old defense system: structure, function, and clinical relevance of the complement system.

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Therapeutic regulators of C!

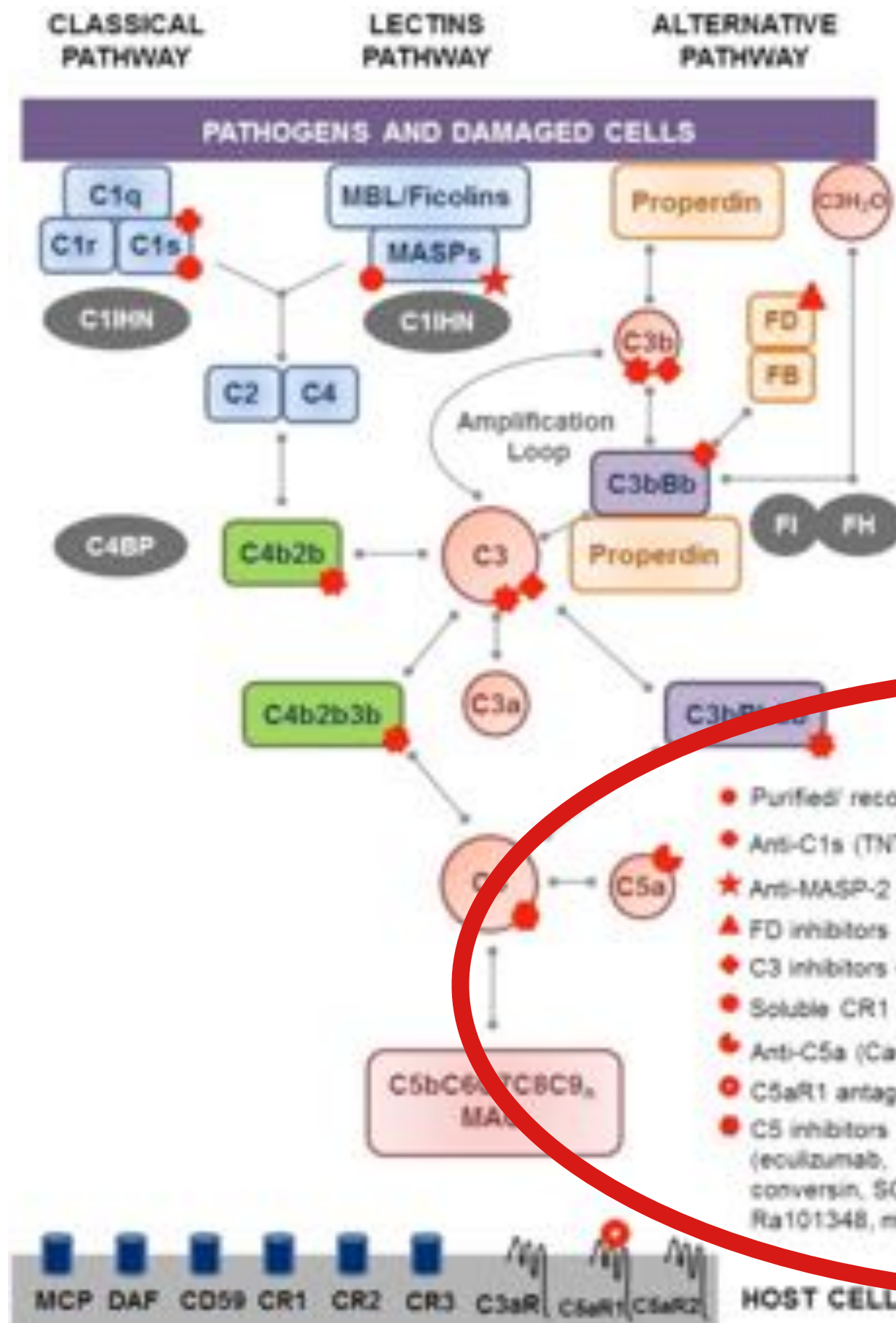


**Clin Immunol. 2015 Sep 1;161(2):
225-240.**

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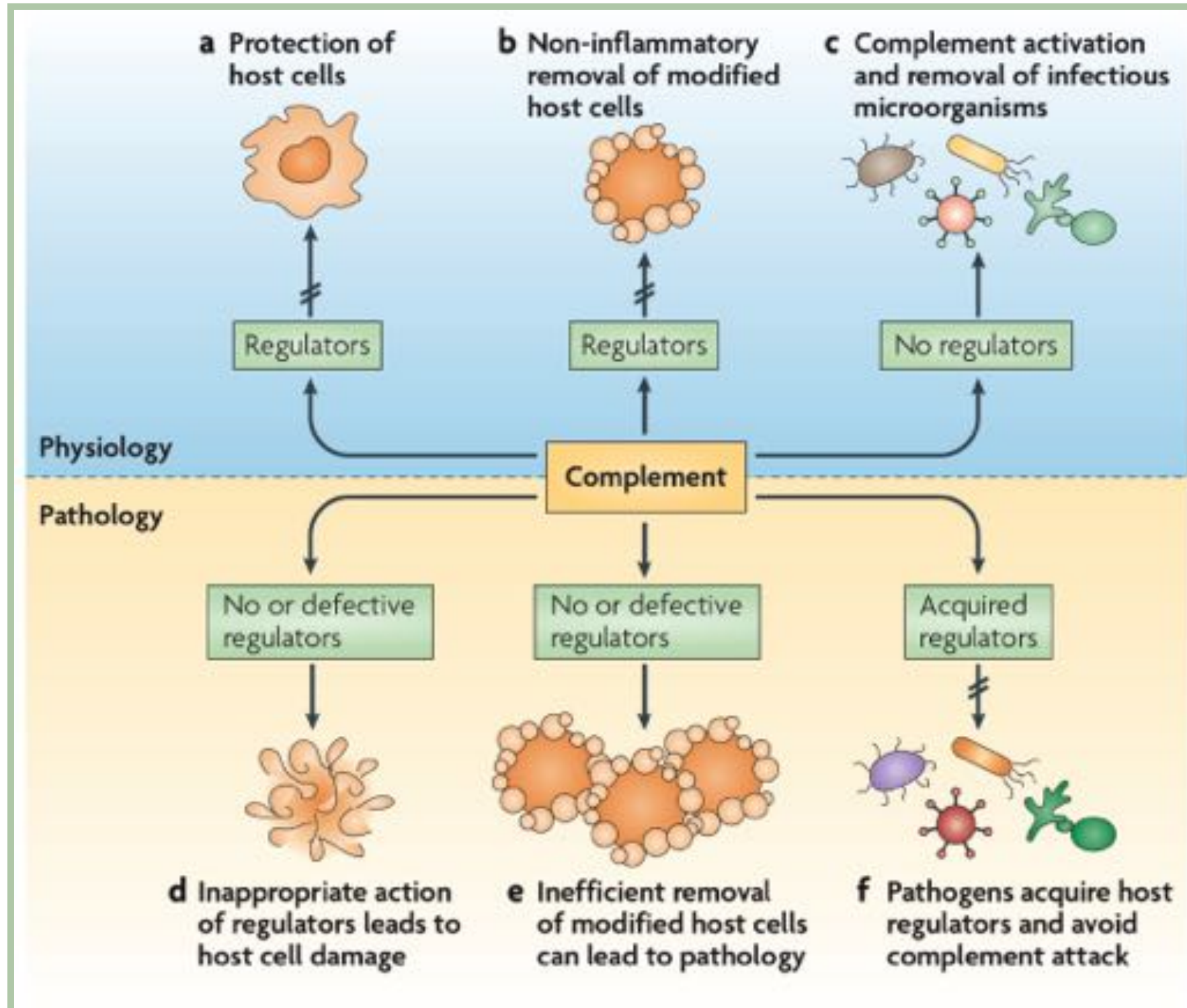
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The benefits and risks of complement

Complement activation has multiple effects, which can either benefit OR be detrimental to the host and possibly lead to pathology



Laboratory evaluation of Complement!

Hemolytic assay or CH50 (or AH50)!

CH50: defining the amount of complement required to induce 50% lysis of sensitized erythrocytes.

Is expressed as the reciprocal of the dilution serum that provides 50% lysis.

Serum sample

+

Sheep erythrocytes pre-sensitized
with specific antibodies.

Spectrophotometric measurement of the hemoglobin release.

Correlation between hemoglobin released,
percentage of hemolysis, and quantity of Complement.

The CH50 reduction correlated with the reduction of the levels of C3!

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It is observed reduction of complement by:

- 1. Consumption of C for the formation of immune-complexes;**
- 2. Decreased synthesis of C;**
- 3. Increased catabolism of C.**

The CH50 modulation correlated with the levels of C3!

| Pattern of Activation | CH50 | C4 | C3 | Factor B | Conditions with Activation Pattern |
|----------------------------------|-------------------------|-------------------------|-------------------------|------------------------|--|
| Classic | Decreased | Decreased | Decreased | No change | SLE, SS, RA, and cryoglobulinemia |
| Alternative | Decreased | No change | Decreased | Decreased | Endotoxemia; type II MPGN |
| Classical and alternative | Decreased | Decreased | Decreased | Decreased | SLE, shock, and immune complex diseases |
| Fluid phase activation—classical | Decreased | Decreased | No change | No change | Hereditary angioedema; malarial infection (P. vivax) |
| Acute phase pattern | Significantly increased | Significantly increased | Significantly increased | Significantly increase | Acute and chronic inflammation; pregnancy |

SLE, systemic lupus erythematosus; SS, Sjogren syndrome; RA, rheumatoid arthritis; MPGN, membranoproliferative glomerulonephritis.

The LPS binding protein or LBP

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The LPS binding protein or LBP

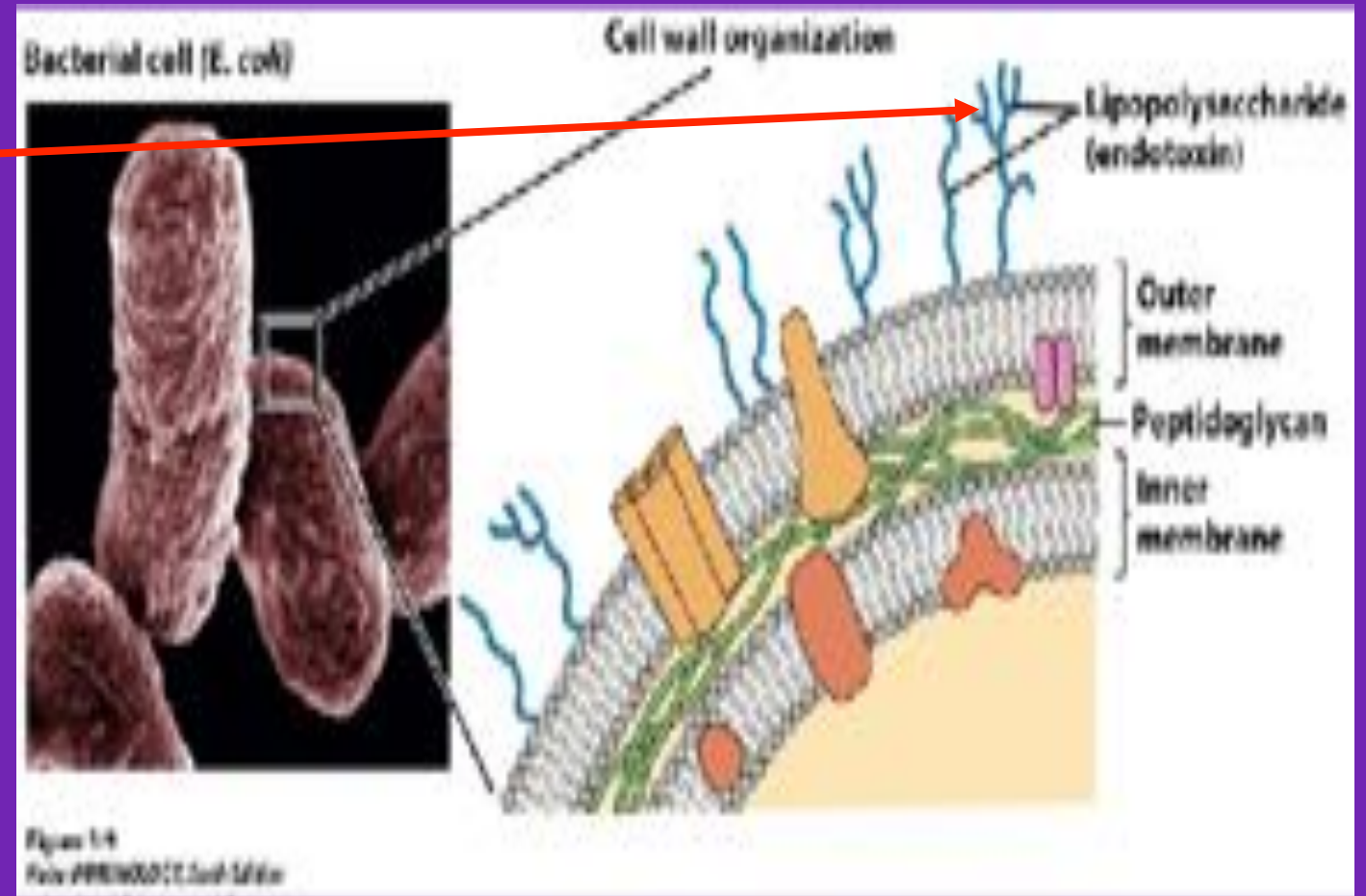
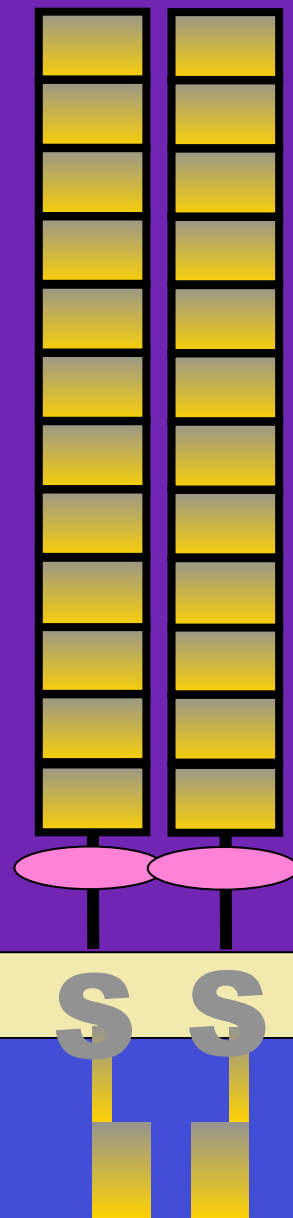
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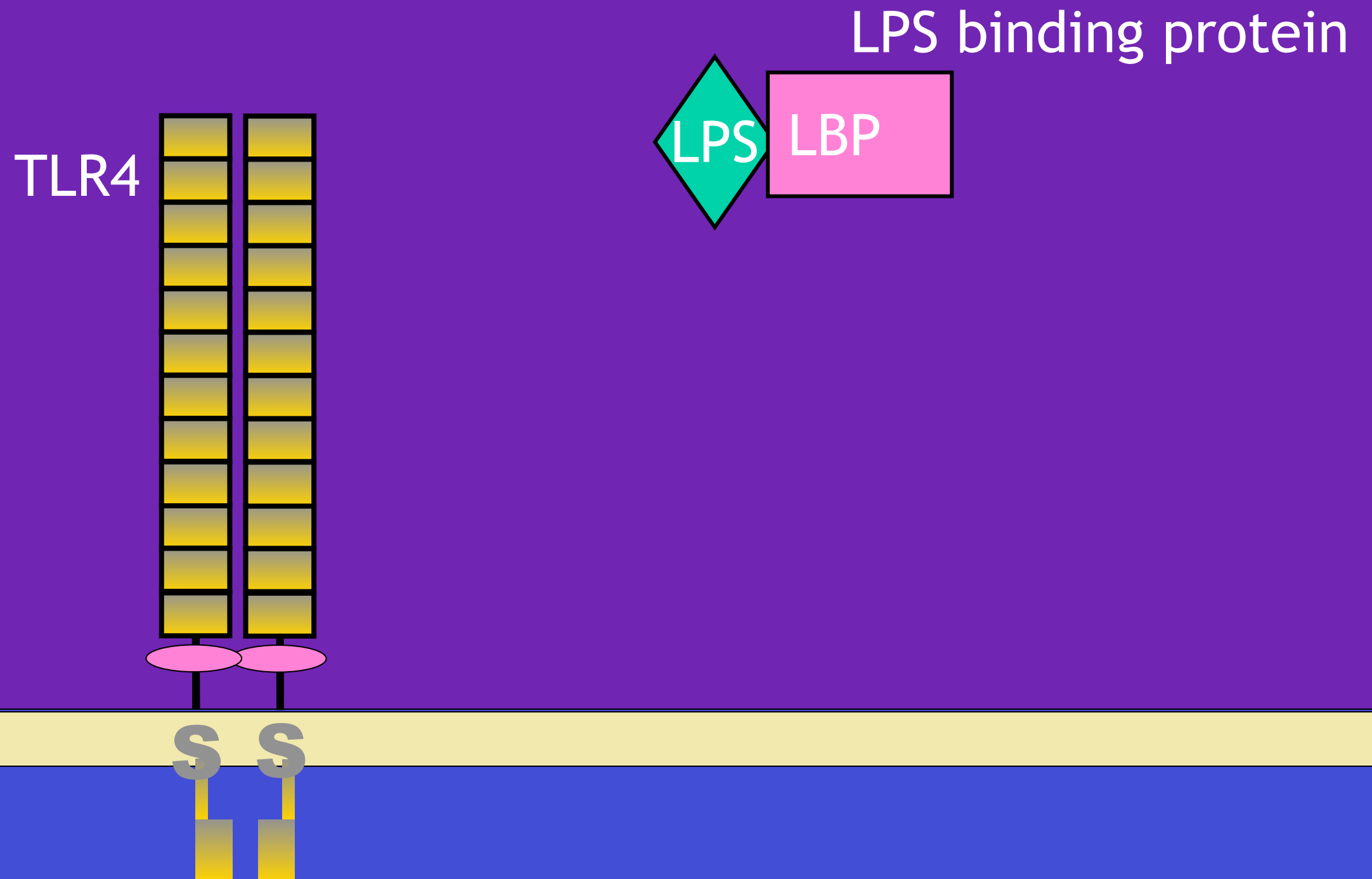
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- Although deficits have not been found in humans, the importance of LBP is underscored by the fact that **knockout mice (KO) to LPB are much more susceptible to Salmonella infections.**

THE LBP FUNCTION!

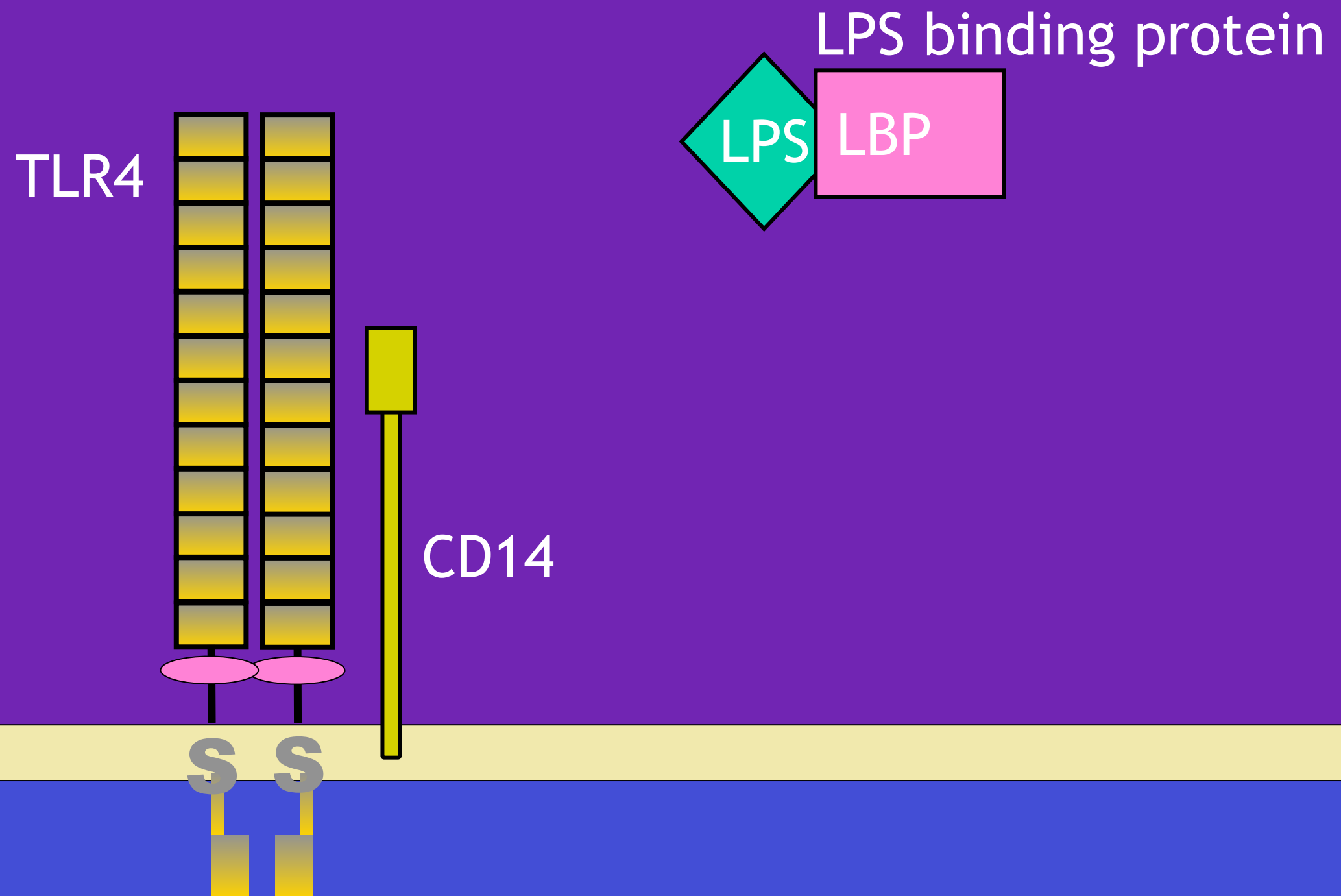
TLR4



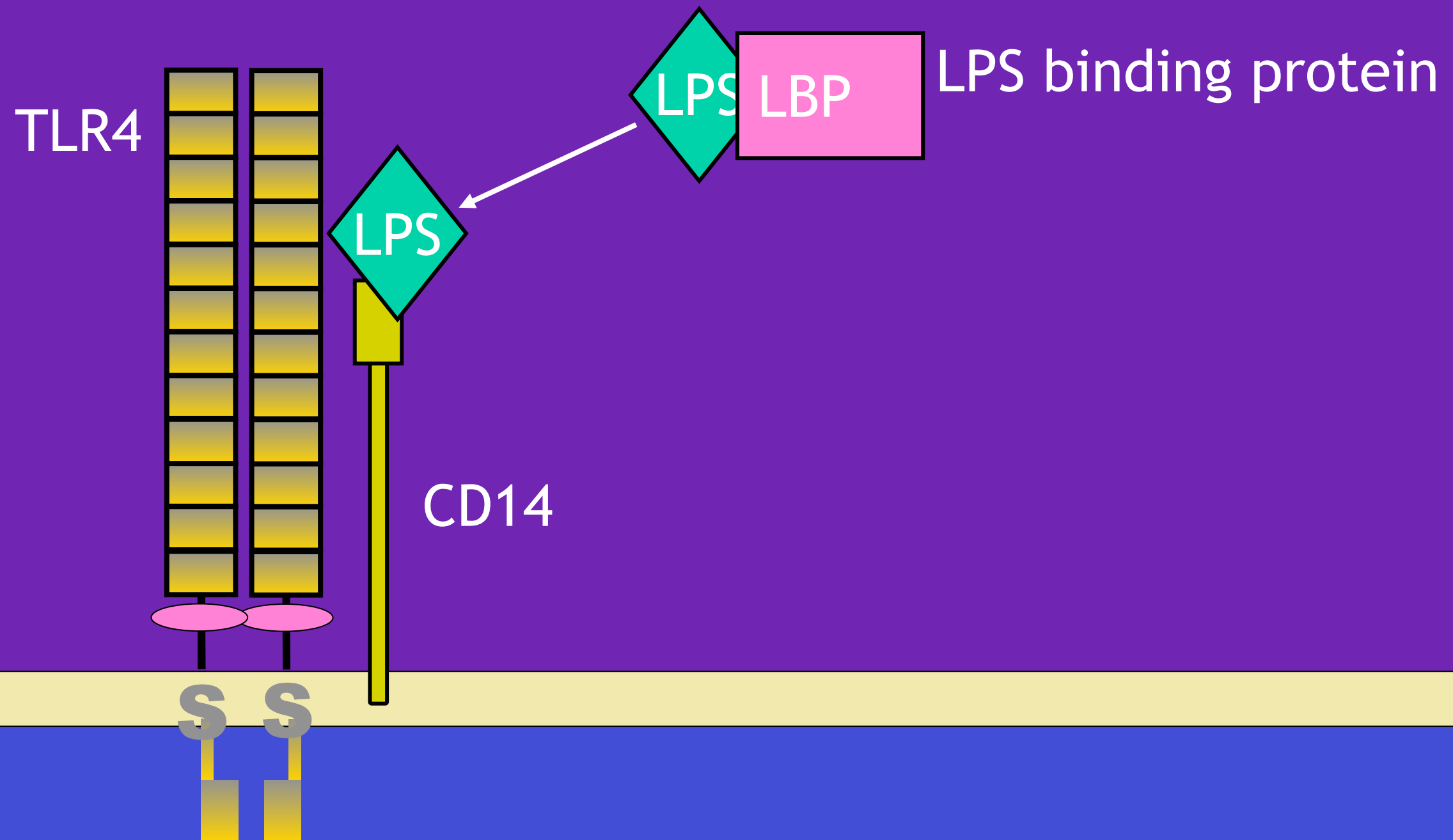
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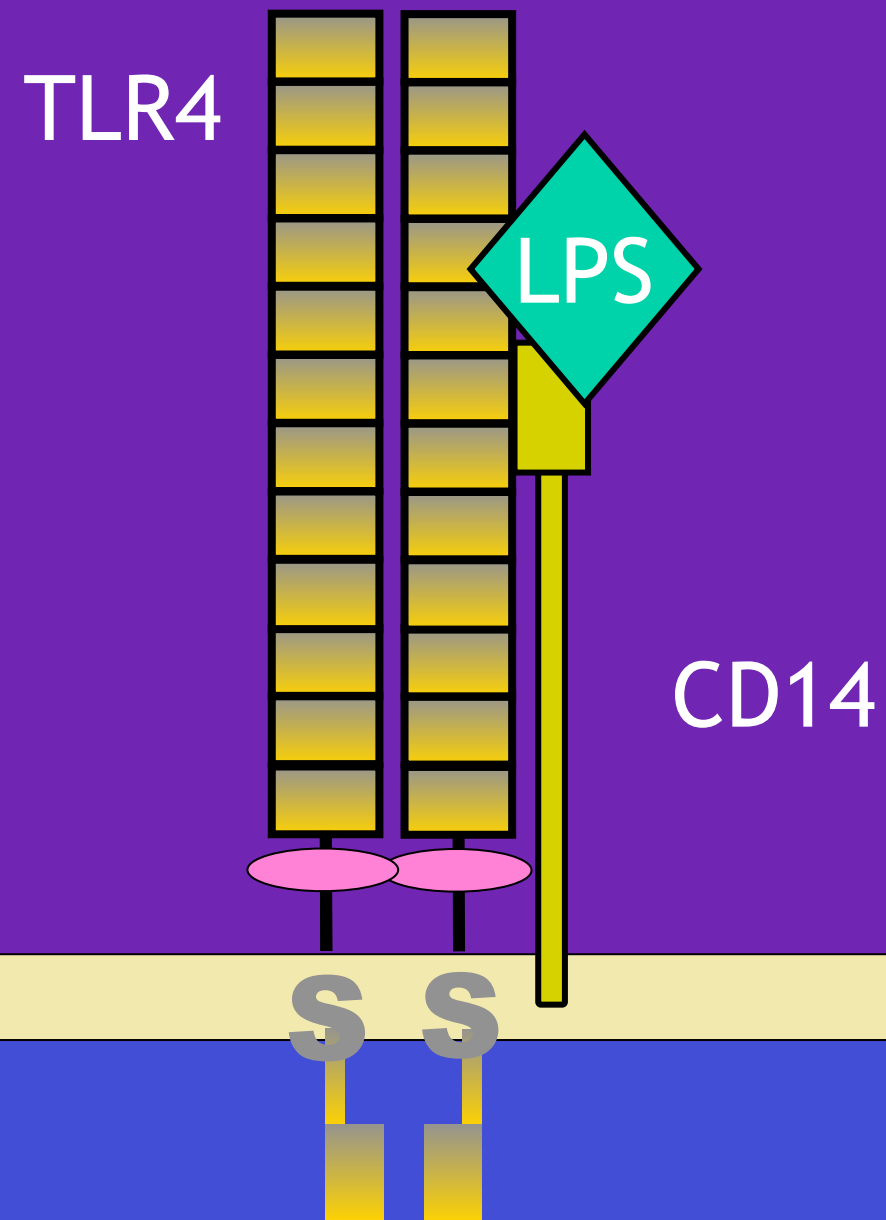
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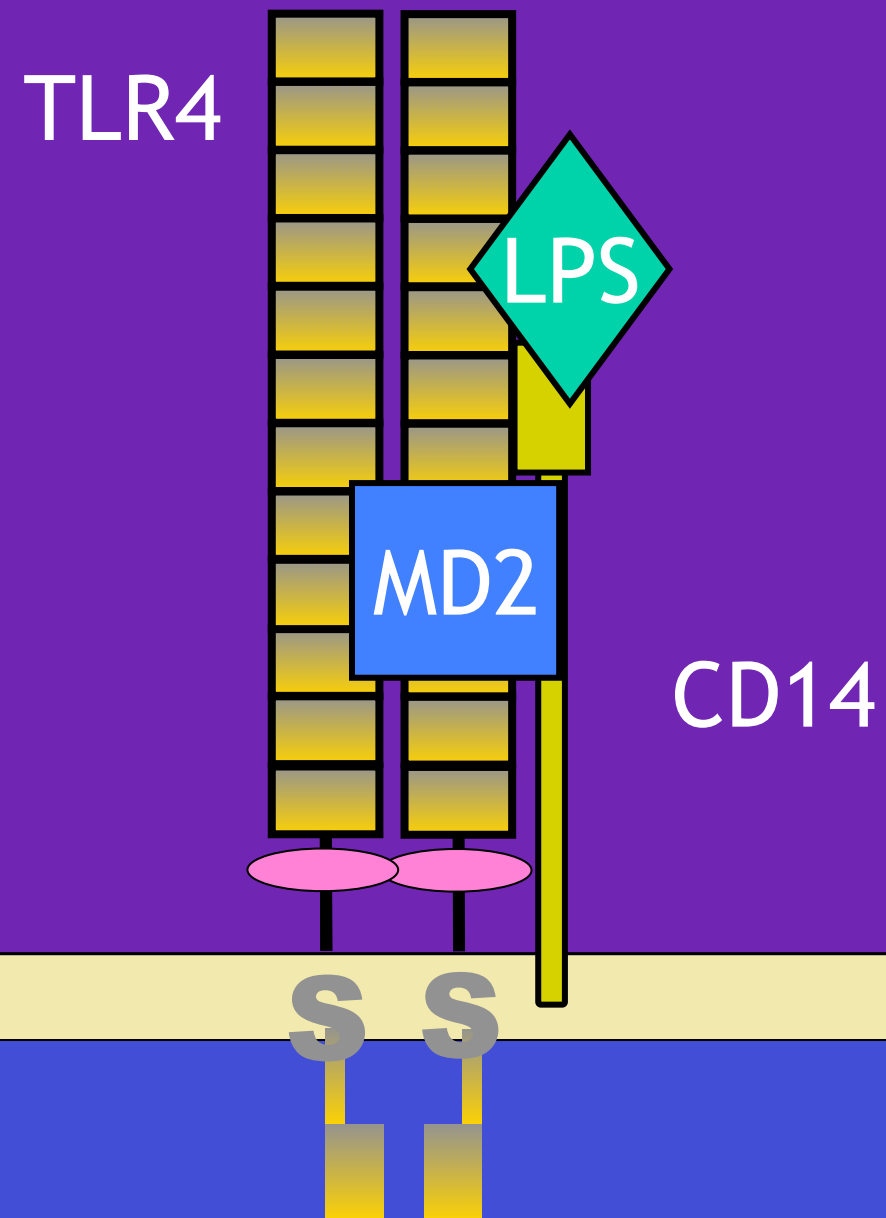
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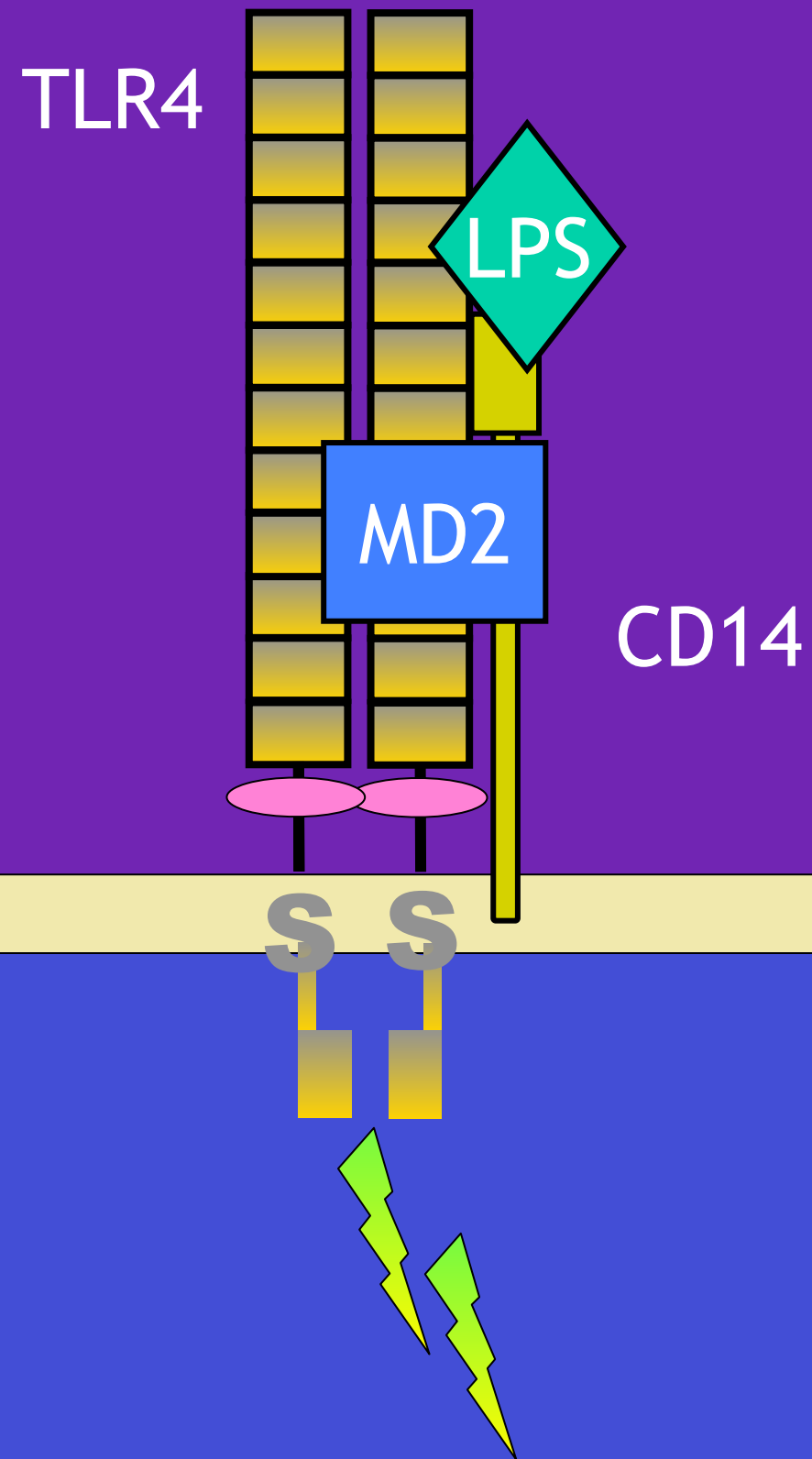
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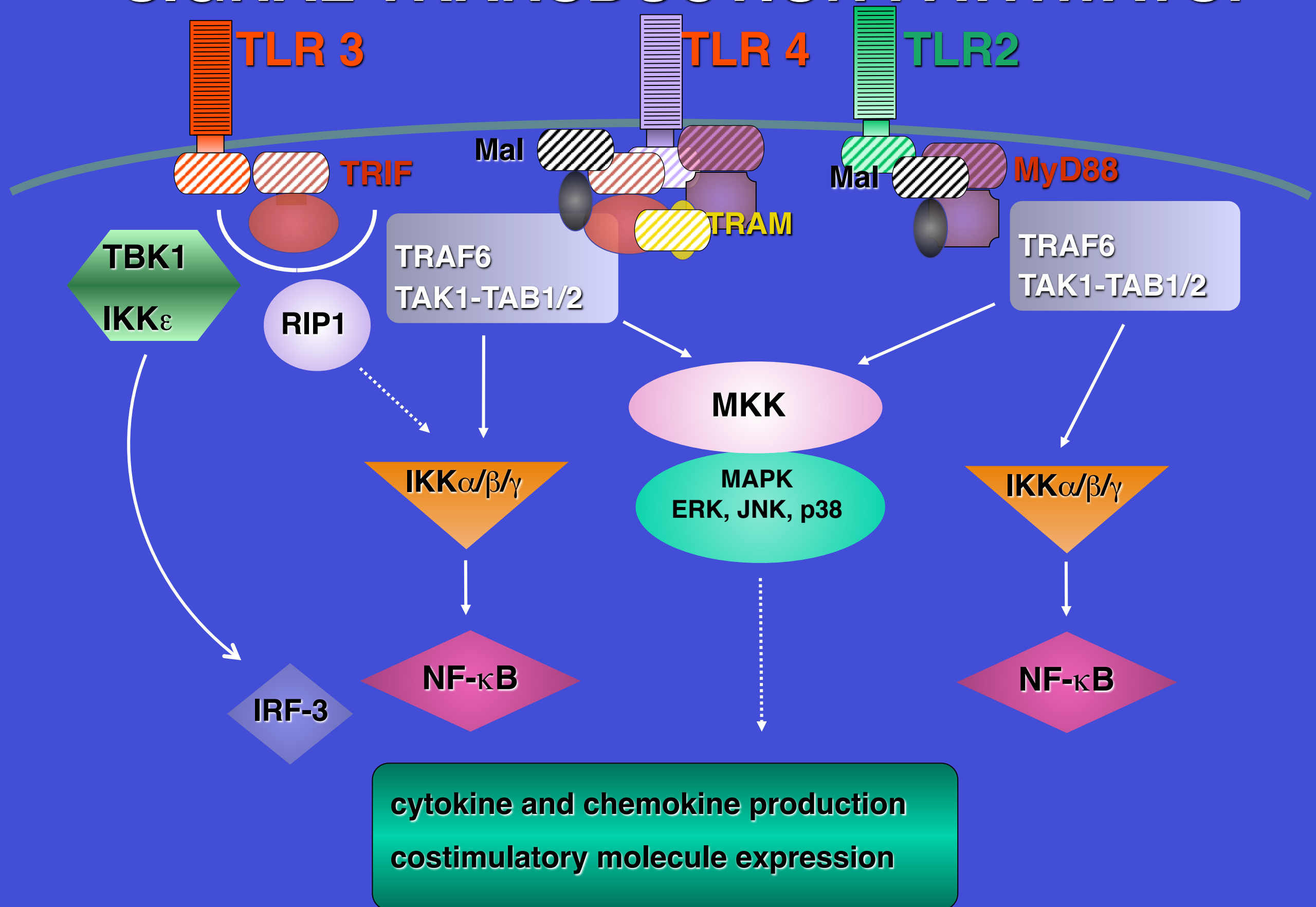
THE LBP FUNCTION!



THE LBP FUNCTION!



THE LBP IS INVOLVED IN TRL-INITIATED SIGNAL TRANSDUCTION PATHWAYS!



The major positive APP, their producers and their ligands!!!

Table 1. Ligands recognized by pattern-recognition molecules.

| Pattern-recognition molecules | Producers ¹ | Ligands |
|-------------------------------|--|--|
| Short pentraxins (CSP, SAP) | Liver (hepatocytes) | <ul style="list-style-type: none"> • Complement components (C1q, Factor H, L-ficolin, M-ficolin) • Microorganisms (bacteria, viruses, fungi, parasites) • Phosphorylcholine, carbohydrates • Modified LDLs • ECM protein (fibronectin, collagen IV, laminin, proteoglycans) • Amyloid fibrils • DNA |
| Long pentraxins (PTX3) | Monocytes, MΦ, PMN, EC, DC, fibroblasts, epithelial cells | <ul style="list-style-type: none"> • Complement components (C1q, Factor H, L-ficolin) • Microorganisms (bacteria, viruses, fungi) and microalleviations • OmpA • ECM protein (M, T32-5) • Apoptotic cells: PGE2 |
| CTLA | MΦ, DC, EC | <ul style="list-style-type: none"> • Is present at immunoglobulin • Pentraxins (CSP, SAP, PTX3) • Microorganisms and microbial moieties (LPS, LTA, OmpA) • 50 species of zoonosis • ECM protein (fibronectin, laminin, fibronectin, integrin) • Amyloid fibrils |
| Collectins (MCL, SP-A, SP-C) | Liver (hepatocytes), lung (type II alveolar cells), MΦ | <ul style="list-style-type: none"> • Carbohydrates • Microorganisms and microbial moieties (LPS, LTA, PGE2) |
| Ficolins | Liver (hepatocytes), lung (type II alveolar cells), PMN, monocytes | <ul style="list-style-type: none"> • Carbohydrates • Microorganisms and microbial moieties (LTA, PGE2, 1,3-β-D-glucan) |
| Properdin | Monocytes, MΦ, PMN, mast cells | <ul style="list-style-type: none"> • Complement components (C3b) • Microorganisms, zymosan |
| SAA | Liver (hepatocytes, monocytes, MΦ) | <ul style="list-style-type: none"> • Microorganisms and microbial moieties (OmpA) |
| Mincin | Spleen, lymph nodes | <ul style="list-style-type: none"> • Microorganisms and microbial moieties (LPS, LTA) |

¹This column reports the main cellular source of PRM, with particular emphasis on cells of the innate immune system.

Abbreviations: CSP, C-reactive protein; DC, dendritic cell; EC, endothelial cell; ECM, extracellular matrix; fcl, ficolin; Ig, immunoglobulin; LDL, low-density lipoprotein; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MΦ, macrophage; Omp, outer membrane protein; PGE2, prostaglandin E2; PMN, polymorphonuclear cell; SAA, serum amyloid A; SAP, serum amyloid P component; T32-5, TNF-induced protein 5.

Proteins of the coagulation system and fibrinolysis:

**fibrinogen, plasminogen, tissue plasminogen
activator, Protein S.**

Fibrinogen!

Fibrinogen levels become elevated in acute phase!

Fibrinogen!

Fibrinogen is the most abundant plasma contains from 100 to 400 mg/dl. With an overall molecular weight of 340 kDa, fibrinogen is a dimer composed of three pairs of peptide chains (A- α , β and gamma-B) linked by disulfide bridges, multiple proximate to the N-terminal.

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The chains extend outside in two other identical domains (D) at the C-terminal in which all three chains are intertwined. Thrombin detaches the fibrinopeptides of A and B from the N-terminal ends, forming a fibrin monomer, which polymerizes into fibrils, arranged longitudinally, which in turn form the clot macroscopic.

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- Fibrinogen levels increase during pregnancy and the use of contraceptives.**
- Low levels generally indicate an extensive activation of coagulation with consumption of fibrinogen.**
- There are several variants of hereditary fibrinogen pathologies, some with relative alteration of coagulation and bleeding diathesis, others with an increased tendency to thrombosis.**

Erythrocyte Sedimentation Rate

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- **The ESR measures the rate at which erythrocytes fall or settle in the plasma of a randomly drawn anticoagulated blood specimen over a specified period of time (usually 60 minutes) in millimeters (mm)/ hour; however, newer methods involving centrifugation can generate results in approximately 5 minutes. This phenomenon was first observed by Edmund Faustyn Biernacki, who found that the rate at which blood settled varied among individuals and that red blood cells (RBCs) settled more quickly in the presence of increased levels of fibrinogen.**

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- **In 1918, Dr Robert Fahraeus noted that ESR differed in pregnant versus nonpregnant women and saw the test as a possible indicator of pregnancy. In 1921, Dr Alf Vilhelm Albertsson Westergren used ESR as a laboratory indicator of the prognosis of patients with pulmonary tuberculosis. Dr Westergren defined the measurement standards for the ESR test that still are used widely today, including utilization of sodium citrate as an anticoagulant.**

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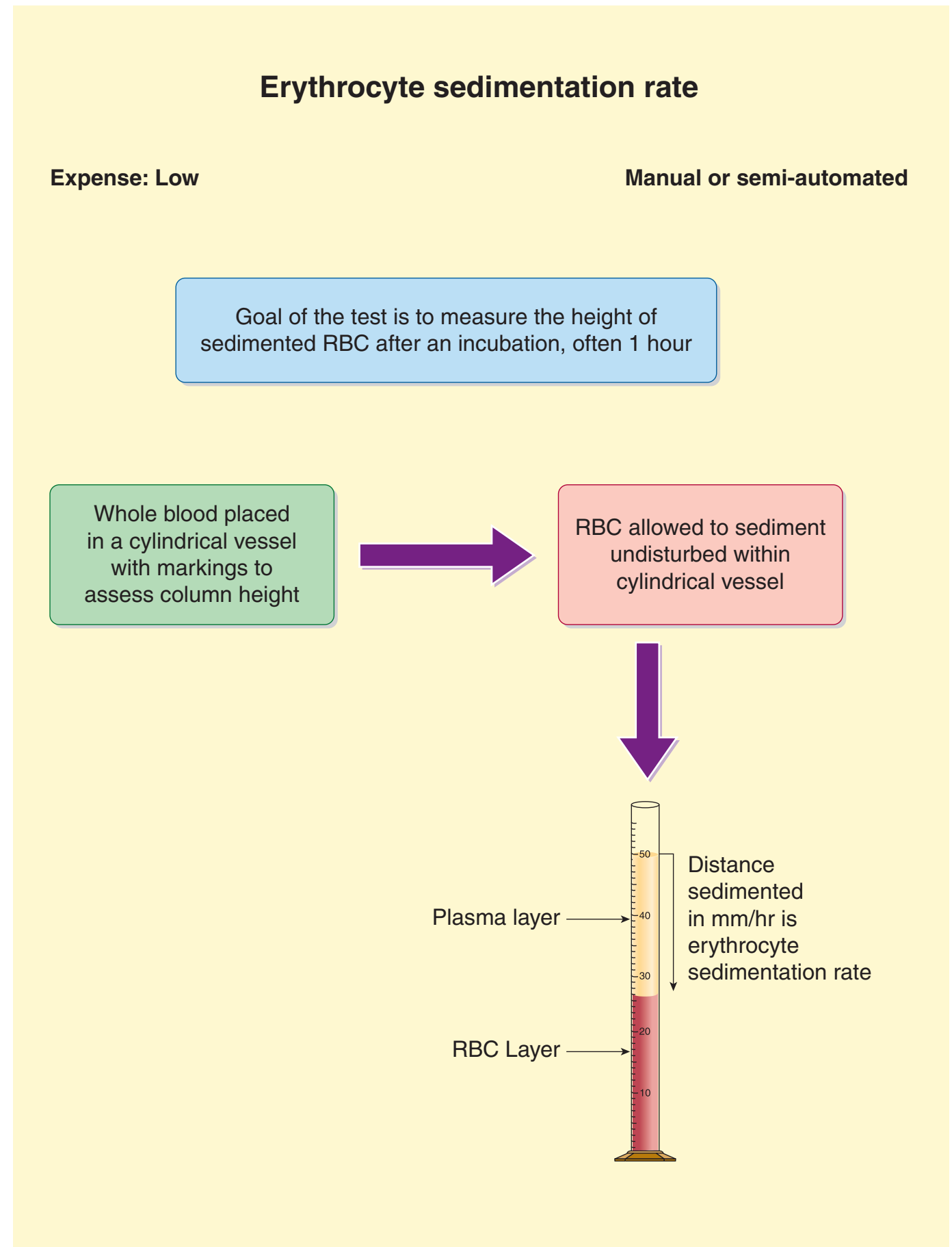
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- **The ESR can be confounded by many factors, leaving this widely used test vulnerable to misinterpretation in clinical practice. Aggregation of erythrocytes promotes falling and increases the ESR; however, RBCs are negatively charged and tend to repel one another. Thus, the presence of positively charged, large, asymmetric acute phase proteins such as fibrinogen and immunoglobulins increases the ESR. The rate of erythrocyte settlement can be influenced by a wide variety of immune and nonimmune factors, including alterations of the quality and quantity of the RBCs, as well as changes in the normal patterns and amounts of various plasma proteins.**

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Fibrinogen and erythrocyte sedimentation rate (ESR) or VES!

Fibrinogen levels become elevated in acute phase up to values of occasional over 1.0 g/dL. In this case also becomes markedly elevated the erythrocyte sedimentation rate (ESR): it is believed that 60-70 % of the increase of ESR is due to the fibrinogen neutralizing effect on the sialic acid residues of red blood cells that are known to inhibit the erythrocyte aggregation!



Antiproteases:

α 1 -antitrypsin (AAT) and the α 1 -anti-chymotrypsin.

α 1-antitrypsin (AAT)!

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Alpha I -antitrypsin is also referred to as alpha-I proteinase inhibitor (A1PI) because it inhibits a wide variety of proteases.

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Usually there are no appreciable amounts of trypsin in the circulating blood, it and other similar proteases, such as collagenases, are produced predominantly by leukocytes in response to inflammatory stimuli or irritative or damaged cells. The AAT is able to neutralize these proteases, which may cause tissue damage, and from this derives its physiological function of homeostatic control of endogenous proteolysis in the body.

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- **The serum protein electrophoresis can be used for screening for AAT deficiency, but it is necessary to perform confirmatory testing complex, such as **trypsin inhibitory capacity (TIC)**, so the phenotype seeking to cross electrophoresis or isoelectric focusing in order to exclude the presence of some other allele as PiS or PiF that migrates differently. The ZZ phenotype ICT has a very low which corresponds to very low concentrations of AAT. **It is essential that such persons should avoid cigarette smoke, as this activates alveolar macrophages to release proteases.****

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AAC, which has a molecular weight of 68 kDa with approximately 25% of the carbohydrate content and a normal serum concentration from 40 to 60 mg/dL, can rapidly increase up to five times during and for the duration of inflammation.

Transport proteins:

ceruloplasmin, haptoglobin and hemopexin.

Ceruloplasmin!

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- Ceruloplasmin consists of a single polypeptide chain, can bind six atoms of copper, which give a blue color to the protein and "in vitro" activity manifests oxidase. **Although at birth is lower, its serum level ranges from 20 to 40 mg/dL in young adults, increasing to twice in the treatment of contraception and pregnancy or as an acute-phase reactive.**

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- The aceruloplasminemia is a genetic disease with an autosomal recessive trait caused by a mutation of a gene located on chromosome 3. Unlike Wilson's disease, transmitted in an autosomal recessive and caused by mutations in the ATP7B gene coding for ATPase that controls the transport of copper into the bile and its incorporation in the enzyme ferroxidase, there are no apparent defects in the metabolism of copper but, due to the excessive accumulation of iron in the tissues, the damage is localized mainly at the level of the pancreas, liver and nervous tissue.

Hemopexin!

The hemopexin binds heme released after haemoglobin degradation. In this way the small molecule porphyrin, with its iron atom, is protected in respect of excretion, preserving the organic deposit of iron.

The normal serum concentration is from 50 to 120 mg/dl.

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- It binds hemoglobin released by lysis of erythrocytes in order to preserve the iron and protein reserves. The hemoglobin - haptoglobin complexes are removed by macrophages in the liver and spleen of the reticuloendothelial system to ensure the recovery of the heme-iron. Therefore, the physiological function of haptoglobin is mainly to allow the recovery of iron when red blood cells, at the end of their life in the circulation are destroyed (hemolysis saline).

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- **The concentration of haptoglobin is therefore inversely proportional to the extent of hemolysis.** The serum haptoglobin also increases in response to stress, infection, acute inflammation, tissue necrosis. In simultaneous presence of inflammation and hemolysis, the concentration of haptoglobin is more difficult to interpret.

**New APP that can be used as a
marker of systemic or localized
inflammation:**

**α 1-acid glycoprotein, soluble CD14 or
CD14S, phagocyte-specific S100 calcium-
binding proteins and procalcitonin!**

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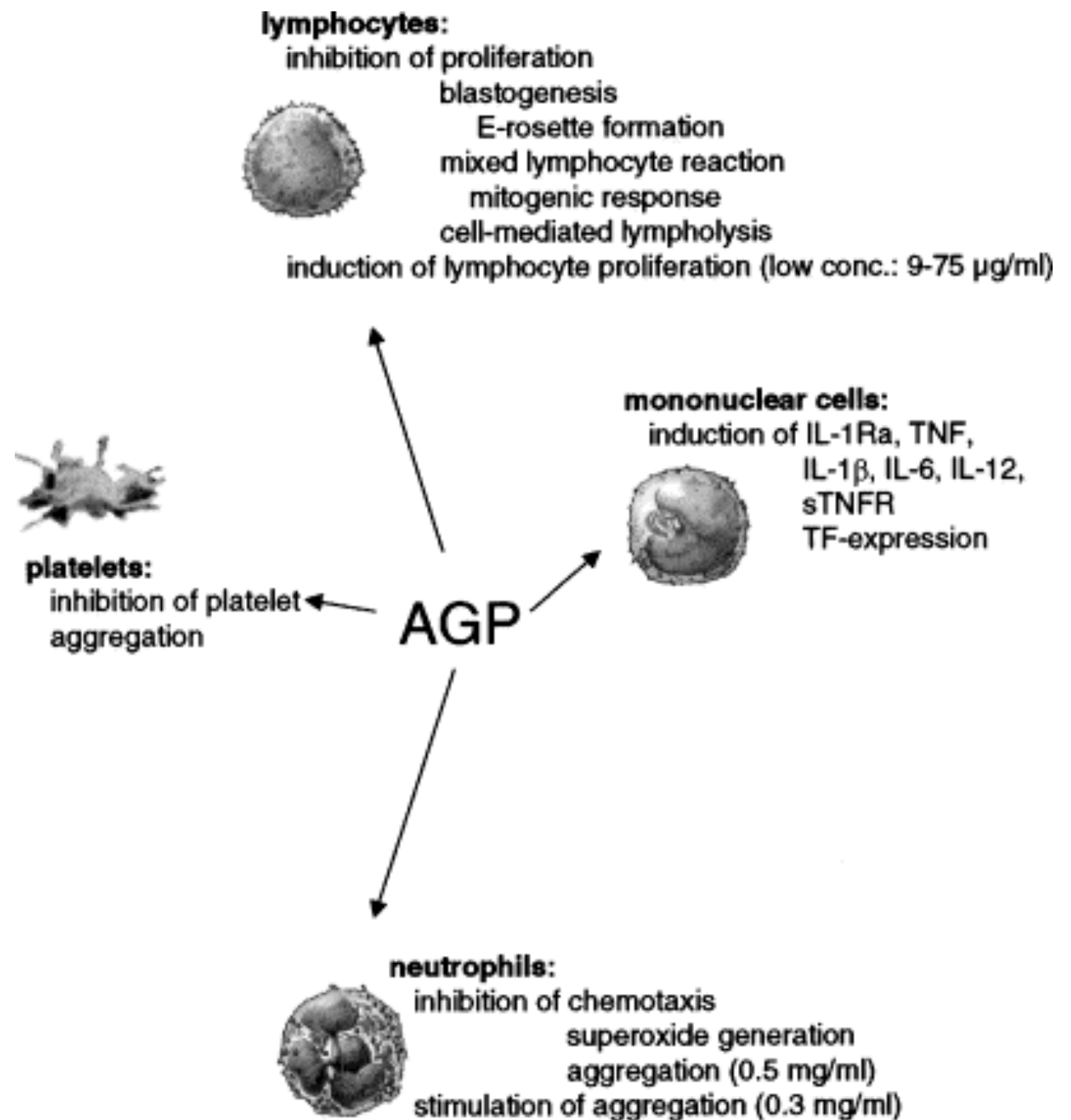
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Its biological role is not clear in vivo, but appears to have several effects in inflammation.

α 1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties!

Overview of effects of AGP on lymphocytes, platelets, mononuclear cells and neutrophils.



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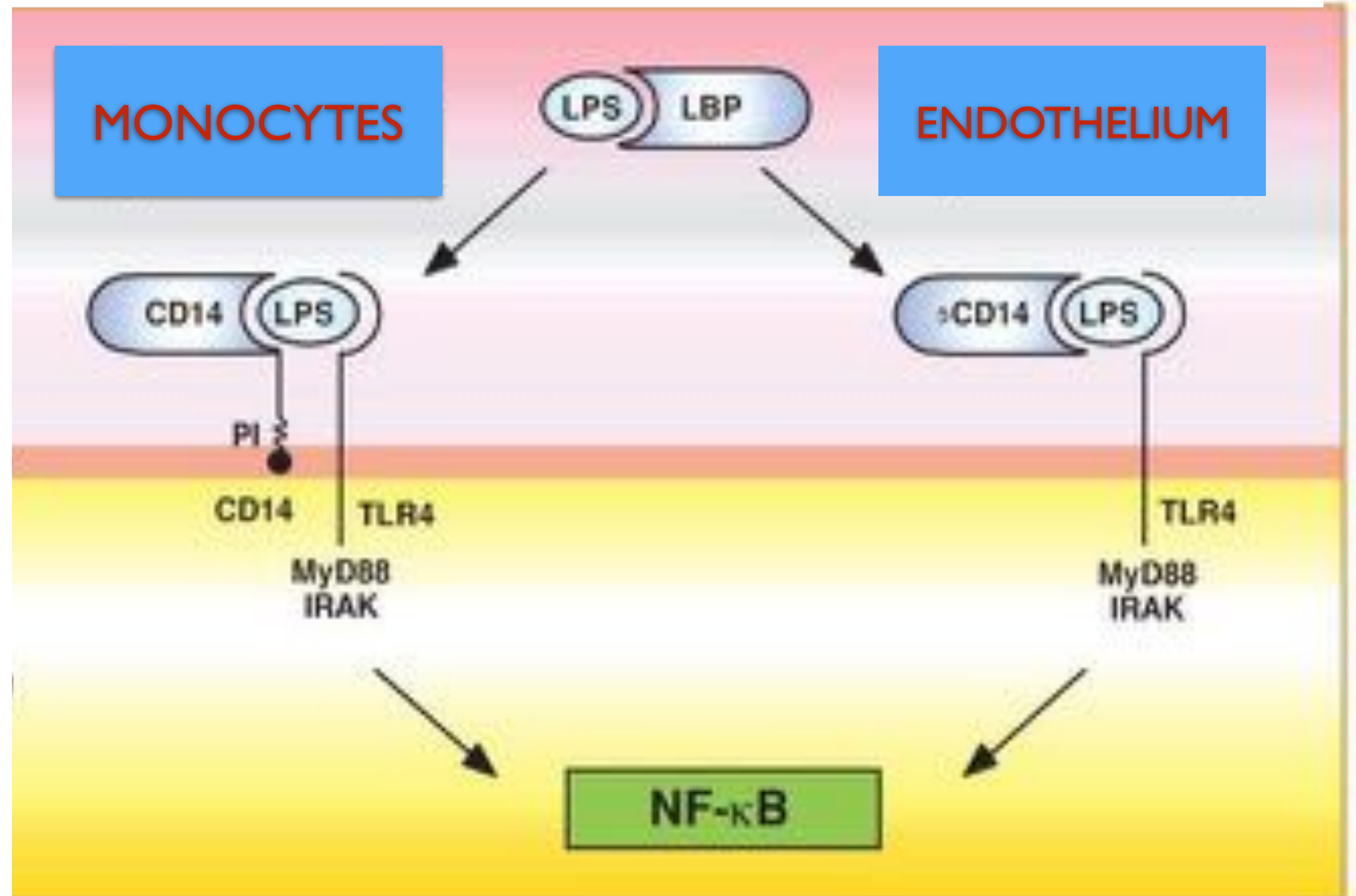
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- If it was initially postulated that the sCD14 was released to desensitize monocytes-macrophages and to limit their production of inflammatory cytokines. Recently, it has been detected its presence in breast milk, where it seems that enhances the differentiation of B lymphocytes. Plasma levels of sCD14 are predictive of mortality in patients with HIV infection.

Soluble CD14 or CD14S HELP ENDOTHELIUM TO PRODUCE INFLAMMATORY CYTOKINES AND CHEMOKINES!



Presepsin as a potential marker for bacterial infection relapse in critical care patients. A preliminary study.

Sargentini V, Ceccarelli G, D'Alessandro M, Colleparado D, Morelli A, D'Egidio A, Mariotti S, Nicoletti AM, Evangelista B, D'Ettorre G, Angeloni A, Venditti M, Bachetoni A.

Systemic bacterial infection carries a high risk of mortality in critical care patients. Improvements in diagnostic procedures are required for effective management of sepsis. Recently, **the soluble CD14 subtype, or presepsin, has been suggested as a reliable marker of sepsis**, and we set out to compare its diagnostic performance with that of procalcitonin (PCT). We focused on a cohort of septic patients who, during their hospitalization, relapsed after a period of clinical relief from symptoms.

Methods: In total 21 adult patients were studied during their hospitalization in the Critical Care Unit of Policlinico Umberto I hospital; 74 plasma samples were collected at multiple time points, and **presepsin levels were measured using a PATHFAST® analyzer.**

Results: Presepsin and PCT were significantly lower in healthy controls than in sepsis or severe sepsis ($p < 0.001$), both enabled a significant difference to be detected between systemic inflammatory response syndrome (SIRS) and severe sepsis ($p < 0.05$). The area under the curve (AUC) calculated from the receiver operating characteristic (ROC) curve analysis was 0.888 for presepsin and 0.910 for PCT. **In those patients in whom a clinical recurrence of sepsis was observed, while PCT levels normalized during the transient remission phase, presepsin levels (> 1000 pg/mL) remained high.**

Conclusions: This study confirms the importance of monitoring a combination of several biomarkers in order to obtain a reliable diagnosis. **Maximal presepsin levels could alert clinicians not to suspend antibiotic treatments and to carefully monitor septic patients' state of health, even after clinical symptoms have disappeared and PCT levels returned to normal.**

Intensive Care Med. 2014 Oct 16.

Circulating presepsin (soluble CD14 subtype) as a marker of host response in patients with severe sepsis or septic shock: data from the multicenter, randomized ALBIOS trial.

Masson SI, Caironi P, Fanizza C, Thomae R, Bernasconi R, Noto A, Oggioni R, Pasetti GS, Romero M, Tognoni G, Latini R, Gattinoni L.

PURPOSE:

Presepsin is a soluble fragment of the cluster-of-differentiation marker protein 14 (CD14) involved in pathogen recognition by innate immunity. We evaluated the relation between its circulating concentration, host response, appropriateness of antibiotic therapy, and mortality in patients with severe sepsis.

METHODS:

Plasma presepsin was measured 1, 2, and 7 days after enrollment of 997 patients with severe sepsis or septic shock in the multicenter Albumin Italian Outcome Sepsis (ALBIOS) trial. They were randomized to albumin or crystalloids. We tested with univariate and adjusted models the association of single measurements of presepsin or changes over time with clinical events, organ dysfunctions, appropriateness of antibiotic therapy, and ICU or 90-day mortality.

RESULTS:

Presepsin concentration at baseline (946 [492-1,887] ng/L) increased with the SOFA score, the number of prevalent organ dysfunctions or failures, and the incidence of new failures of the respiratory, coagulation, liver, and kidney systems. The concentration decreased in ICU over 7 days in patients with negative blood cultures, and in those with positive blood cultures and appropriate antibiotic therapy; it increased with inappropriate antibiotic therapy ($p = 0.0009$). Baseline presepsin was independently associated with, and correctly reclassified, the risk of ICU and 90-day mortality. Increasing concentrations of presepsin from day 1 to day 2 predicted higher ICU and 90-day mortality (adjusted $p < 0.0001$ and 0.01 , respectively). Albumin had no effect on presepsin concentration.

CONCLUSIONS:

Presepsin is an early predictor of host response and mortality in septic patients. Changes in concentrations over time seem to reflect the appropriateness of antibiotic therapy.

Review Article

Presepsin as a novel sepsis biomarker

Qi Zou, Wei Wen, Xin-chao Zhang

Emergency Medicine Department, Beijing Hospital, Beijing 100730, China

Corresponding Author: Xin-chao Zhang, Email: xinchaoz@163.com

BACKGROUND: In 2004, a new biomarker sCD14-subtypes (presepsin) was found and its value was shown in the diagnosis and evaluation of sepsis. This article is a brief overview of the new biomarker.

DATA SOURCES: A literature search using multiple databases was performed for articles, especially meta-analyses, systematic reviews, and randomized controlled trials.

RESULTS: Compared with other markers, presepsin seems to have a better sensitivity and specificity in the diagnosis of sepsis. Presepsin as a biomarker is not only suitable for the early diagnosis of sepsis, but also for the assessment of its severity and prognosis.

CONCLUSIONS: Presepsin has a higher sensitivity and specificity in the diagnosis of sepsis as a new biomarker, and is a predictor for the prognosis of sepsis. More importantly, presepsin seems to play a crucial role as a supplemental method in the early diagnosis of sepsis. Since there is no multicenter study on the relationship between presepsin and sepsis, further studies on the clinical values of presepsin are needed.

KEY WORDS: Presepsin; Sepsis; Diagnosis

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DOI: 10.5847/wjem.j.issn.1920–8642.2014.01.002

The phagocyte-specific S100 calcium-binding proteins

Clin Chim Acta. 2004 Jun;344(1-2):37-51.

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- They are index of activation of phagocytes more than any other parameter of inflammation. These proteins are able to detect minimal residual levels of inflammation and can be predictive for the prognosis of the patient.

Calprotectin recent reviews!

[The use of fecal calprotectin and lactoferrin in patients with IBD. Review.](#)

Stragier E, Van Assche G.

Acta Gastroenterol Belg. 2013 Sep;76(3):322-8.

[Diagnostics and prognostics of inflammatory bowel disease with fecal neutrophil-derived biomarkers calprotectin and lactoferrin.](#)

Sipponen T. Dig Dis. 2013;31(3-4):336-44.

[Role of fecal calprotectin in gastrointestinal disorders.](#)

Montalto M, Gallo A, Santoro L, D'Onofrio F, Landolfi R, Gasbarrini A.

Eur Rev Med Pharmacol Sci. 2013 Jun;17(12):1569-82.

[The Diagnostic Accuracy of Fecal Calprotectin During the Investigation of Suspected Pediatric Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis.](#)

Henderson P, Anderson NH, Wilson DC.

Am J Gastroenterol. 2013 May 14.

[Crohn's disease: small bowel motility impairment correlates with inflammatory-related markers C-reactive protein and calprotectin.](#)

Bickelhaupt S, Pazahr S, Chuck N, Blume I, Froehlich JM, Cattin R, Raible S, Bouquet H, Bill U, Rogler G, Frei P, Boss A, Patak MA.

Neurogastroenterol Motil. 2013 Jun;25(6):467-73.

[Clinical utility of calprotectin and lactoferrin in patients with inflammatory bowel disease: is there something new from the literature?](#)

Caccaro R, D'Inca R, Pathak S, Sturniolo GC.

Expert Rev Clin Immunol. 2012 Aug;8(6):579-85.

[Fecal calprotectin in pediatric inflammatory bowel disease: a systematic review.](#)

Kostakis ID, Cholidou KG, Vaiopoulos AG, Vlachos IS, Perrea D, Vaos G.

Dig Dis Sci. 2013 Feb;58(2):309-19.

Fecal calprotectin (FC) has been proposed as a useful and non-invasive marker of acute intestinal inflammation.

AIM:

We summarize recent evidences on FC, providing practical perspectives on its diagnostic and prognostic role in different gastrointestinal conditions.

RESULTS:

Most of relevant data derived from studies on inflammatory bowel disease (IBD). FC concentrations (FCCs) showed a good diagnostic precision for separating organic and functional intestinal diseases and well correlated with IBD activity. FCCs were higher in subjects with NSAID enteropathy, but the actual correlation between FC and endoscopy is under investigation.

CONCLUSIONS:

FC has been widely proposed as a filter to avoid unnecessary endoscopies. Nevertheless, it should not be considered as a marker of organic intestinal disease at all; rather it represents a marker of "neutrophilic intestinal inflammation". In IBD, more and larger studies are needed to confirm FC's capacity to correlate with IBD extent, to predict response to therapy and relapse, and the presence of a subclinical intestinal inflammation in asymptomatic first-degree relatives of patients.



Indice di infiammazione intestinale

Calprotectina, il test per individuare pazienti con possibile infiammazione dell'intestino: scopri i test disponibili e il loro funzionamento

CALPROTECTINA: DIAGNOSI E TEST

- Calprest
- CalFast
- Device per prelievo feci
- Calprotectina nelle feci
- Utilizzo in età adulta
- Utilizzo in età pediatrica

MALATTIE INFIAMMATORIE CRONICHE INTESTINALI (IBD)

- COLITE ULCEROSA
- MORBO DI CROHN
- SINDROME DELL'INTESTINO IRRITABILE (IBS)
- FAQ
- BIBLIOGRAFIA

CONTATTI per ulteriori informazioni ►

Calprest

CHE COS'E' CALPREST
Calprest è il test immunoenzimatico di Eurospital che consente di verificare, in modo accurato e non invasivo, la presenza di uno stato infiammatorio a carico del tratto intestinale. Calprest permette di effettuare una diagnosi differenziale fra patologie di tipo organico (**Malattie Infiammatorie Croniche Intestinali** - MICI, note anche come Inflammatory Bowel Disease - IBD) e di tipo funzionale (**Sindrome dell'Intestino Irritabile** - SII, Irritable Bowel Syndrome - IBS). Se Calprest fornisce un risultato negativo, si può, con quasi assoluta certezza, escludere un'infiammazione a carico della mucosa intestinale.



UN TEST SEMPLICE E ACCURATO
Fino ad oggi, per valutare lo stato infiammatorio della mucosa intestinale era necessario ricorrere ad esami invasivi (colonscopia e conseguente esame istologico). Di recente, però, ha trovato sempre più credito l'uso di marcatori non invasivi: tra questi, uno dei più attendibili e sicuri è rappresentato dalla determinazione della concentrazione fecale della **calprotectina**, una proteina antimicrobica presente nei neutrofili che, in presenza di processi infiammatori a carico dell'intestino, viene rilasciata nel lume intestinale e pertanto può essere rilevata nelle feci. Il principio diagnostico di Calprest si basa sulla determinazione quantitativa nelle feci della **calprotectina**: nei pazienti affetti da **Malattie Infiammatorie Croniche Intestinali** il livello di **calprotectina** è infatti generalmente molto elevato. Nei soggetti con **Sindrome dell'Intestino Irritabile** (IBS) il livello di **calprotectina** è invece decisamente inferiore a quello riscontrato nei pazienti con malattia attiva, talvolta superiore al limite di riferimento ma in ogni caso sempre superiore rispetto a quello rilevabile nei soggetti sani. Calprest permette di utilizzare questo marcatore per selezionare i pazienti con infiammazione da avviare a ulteriori esami e risulta in tal senso maggiormente accurato rispetto ai normali test biochimici (VES, PCR).

SENSIBILITA' E SPECIFICITA'
La determinazione della calprotectina fecale viene impiegata per la diagnosi differenziale tra IBD ed IBS grazie al suo elevato valore predittivo negativo che permette di escludere un'eventuale patologia organica.

| SENSIBILITA' DIAGNOSTICA | SPECIFICITA' DIAGNOSTICA | VALORE PREDITTIVO NEGATIVO |
|--------------------------|--------------------------|----------------------------|
| 95% | 93% | 98% |

INTERPRETAZIONE DEI RISULTATI
I campioni con una concentrazione di **calprotectina** superiore a 50 mg calprotectina/kg devono essere considerati positivi al test. Nei soggetti adulti sani il valore medio della calprotectina è di 25 mg calprotectina/kg. Un risultato positivo di Calprest è indice di infiammazione intestinale e permette di selezionare con sicurezza i pazienti da avviare a ulteriori indagini diagnostiche.

| VALORE | INTERPRETAZIONE |
|------------------------|---------------------------------------|
| < 50 mg/kg di feci | Negativo |
| 50 - 100 mg/kg di feci | Zona Grigia, si consiglia di ripetere |
| > 100 mg/kg di feci | Positivo |



SAPIENZA
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AZIENDA POLICLINICO UMBERTO I
DIPARTIMENTO ASSISTENZIALE INTEGRATO
MEDICINA DIAGNOSTICA

U.O. IMMUNOLOGIA- IMMUNOPATOLOGIA DLC05
Responsabile F:F Prof. Fabrizio Mainiero
Tel: 06 49970966



Roma,

Sig..... PAZIENTE
(Cognome e Nome)

Prelievo del

Provenienza ...DAI Pediatria.....

DOSAGGIO CAPROTECTINA FECALE

- < 50 mg/kg di feci Negativo
- 50 - 100 mg/kg di feci Zona Grigia, si consiglia di ripetere
- > 100 mg/kg di feci Positivo

Il kit Calprest (Eurospital, Trieste, Italia) è utilizzato x l'analisi della calprotectina fecale. Calprest è un test immunoenzimatico che sfrutta l'uso di anticorpi policlonali (riconoscimento del massimo numero di epitopi) diretti contro la calprotectina e permette un dosaggio quantitativo di essa.

Il Responsabile
Fabrizio Mainiero

Among S100 proteins, the S100P is a novel marker and therapeutic target for cancer!

S100P expression is described in many different cancers, and its expression is associated with drug resistance, metastasis, and poor clinical outcome. S100P is member of the S100 family of small calcium-binding proteins that have been reported to have either intracellular or extracellular functions, or both. Extracellular S100P can bind with the receptor for advanced glycation end products (RAGE) and activate cellular signaling. Through RAGE, S100P has been shown to mediate tumor growth, drug resistance, and metastasis. S100P is specifically expressed in cancer cells in the adult. Therefore, **S100P is a useful marker for differentiating cancer cells from normal cells, and can aid in the diagnosis of cancer by cytological examination.** The expression of S100P in cancer cells has been related to hypomethylation of the gene. Multiple studies have confirmed the beneficial effects of blocking S100P/RAGE in cancer cells, and different blockers are being developed including small molecules and antagonist peptides.

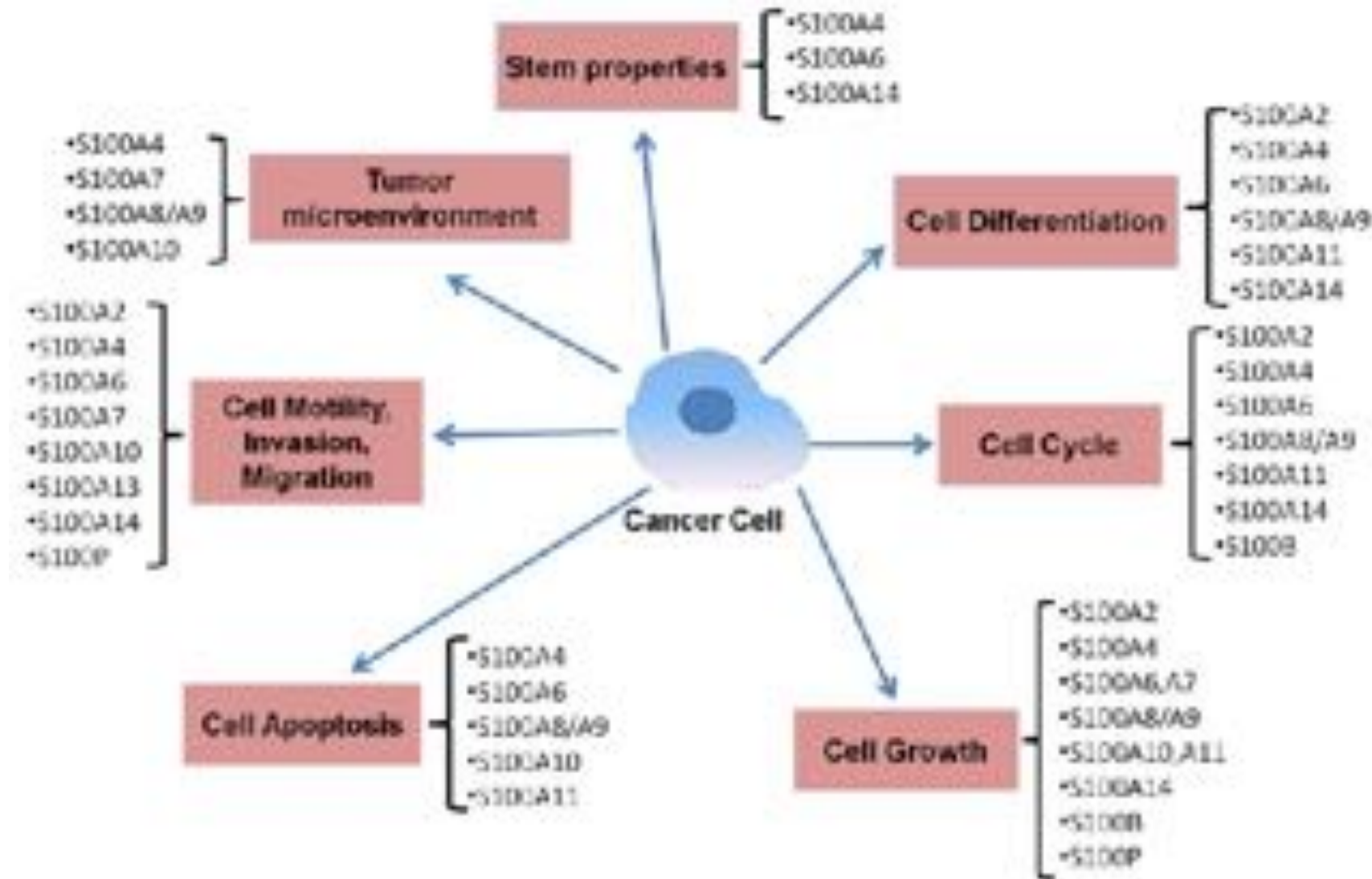
[Amino Acids](#)

October 2011, Volume 41, [Issue 4](#), pp 893–899

S100 protein family in human cancer!

Chen H, Xu C, Jin Q, Liu Z.

S100 protein family has been implicated in multiple stages of tumorigenesis and progression. Among the S100 genes, 22 are clustered at chromosome locus 1q21, a region frequently rearranged in cancers. S100 protein possesses a wide range of intracellular and extracellular functions such as regulation of calcium homeostasis, cell proliferation, apoptosis, cell invasion and motility, cytoskeleton interactions, protein phosphorylation, regulation of transcriptional factors, autoimmunity, chemotaxis, inflammation and pluripotency. Many lines of evidence suggest that altered expression of S100 proteins was associated with tumor progression and prognosis. Therefore, S100 proteins might also represent potential tumor biomarkers and therapeutic targets.



Procalcitonin!

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- **In sick infants, PCT appears to be a good indicator of early onset and late onset sepsis.**
- **PCT can also be used to distinguish between septic ARDS and ARDS not septic. Because of these characteristics, the PCT is currently used in intensive care in the early diagnosis and monitoring of sepsis.**

Laboratory evaluation of APP!

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- **Although APP are considered to be stable at -20 °C, the long term storage at -70 °C is recommended.**

PEP and NEPHELOMETRY in the Laboratory evaluation of APP!

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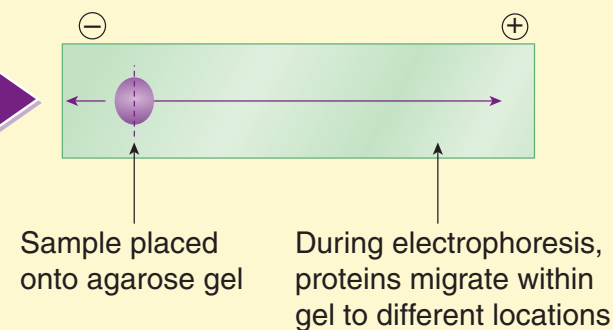
Protein electrophoresis (PEP)

Expense: Moderate

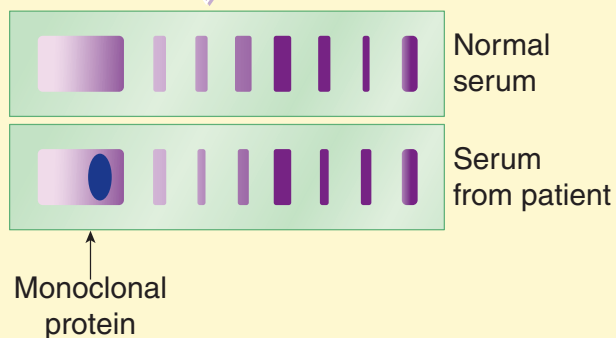
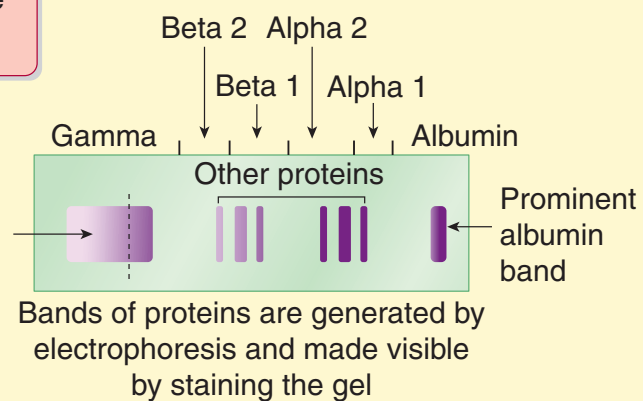
Semi-automated

Sample can be:
Serum for SPEP analysis
Urine for UPEP analysis
Cerebrospinal fluid (CSF)

Urine and CSF are usually concentrated prior to testing to increase the concentration of proteins in sample



Area of gel:



PEP and NEPHELOMETRY in the Laboratory evaluation of APP!

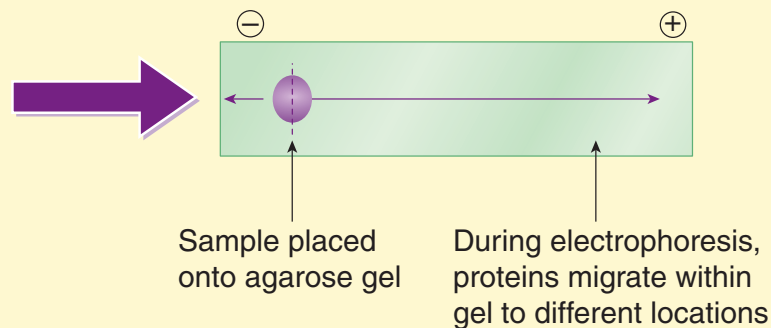
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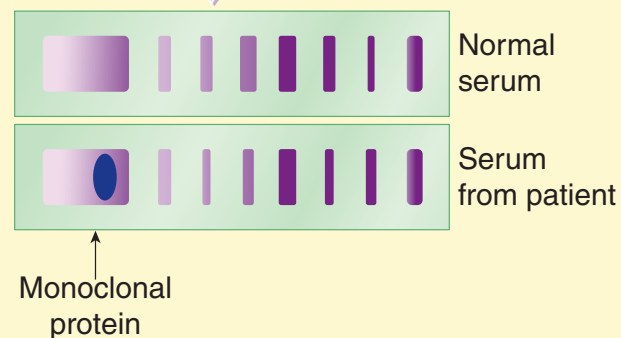
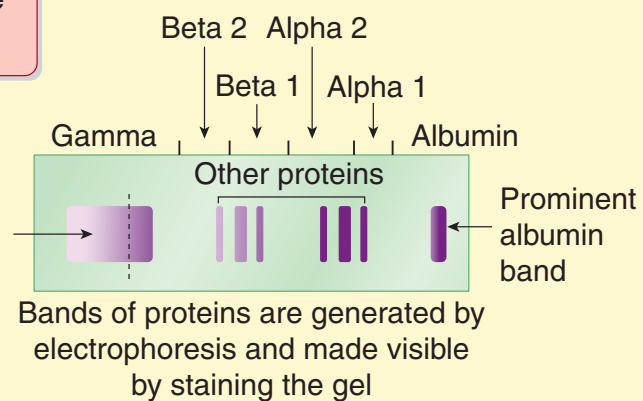
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A sample with an additional monoclonal protein, which can appear in multiple myeloma, for example, shows a dense band of protein not present in a sample from a healthy individual

Area of gel:



Nephelometry for quantitation of selected proteins and other compounds

Expense: Moderate

Semi-automated

Sample of any body fluid is incubated with an antibody to the compound being measured

When the compound is present, antigen-antibody complexes form

Antibody to the compound is the reagent added to the sample

Antigen is compound being measured

The amount of scattered light is proportional to the amount of compound being measured

Antigen-antibody complexes scatter light from a beam of light shown through the sample

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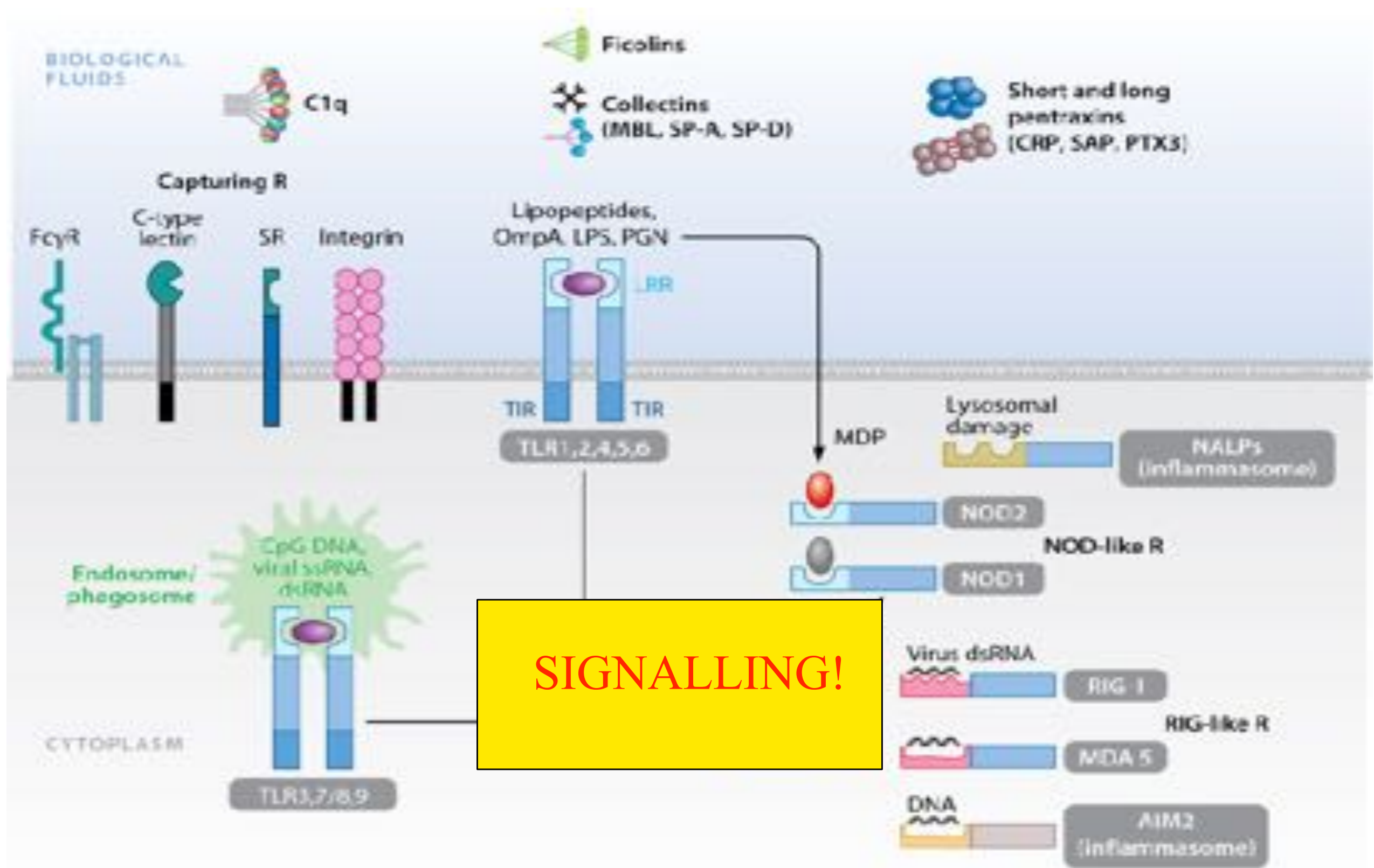
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THEY CAN BE NAMED:

**THE SOLUBLE DAMAGE RECEPTORS OF
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THE SOLUBLE, CYTOPLASMIC AND MEMBRANE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION!



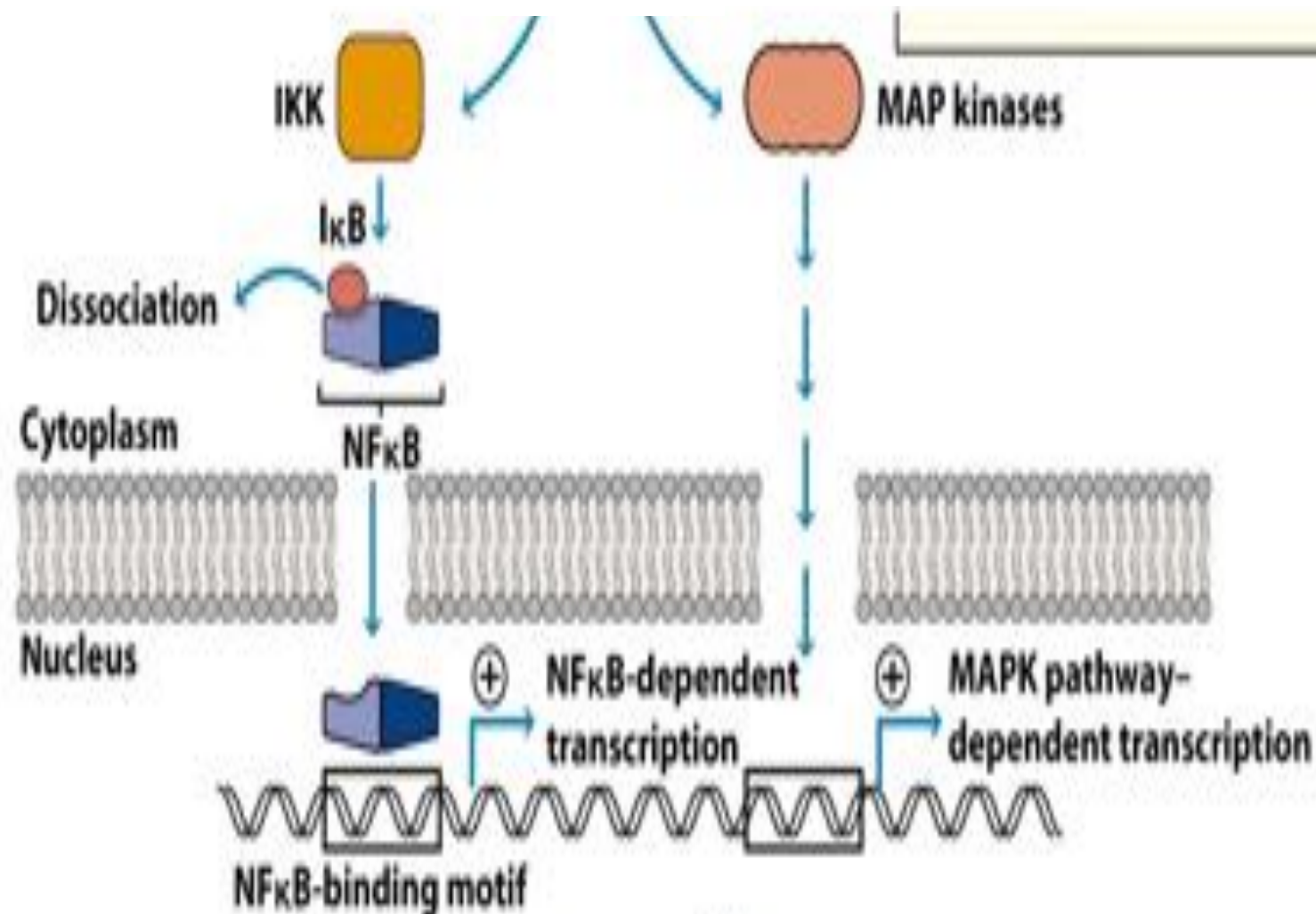
**THE APP POSITIVE AS THE SOLUBLE
DAMAGE RECEPTORS**

**CAN COOPERATE WITH THE
CYTOPLASMIC AND MEMBRANE
RECEPTORS**

TO ACTIVATE

**THE TRANSCRIPTIONAL
PROGRAM OF NATURAL IMMUNITY
AND INFLAMMATION!**

THE TRANSCRIPTIONAL PROGRAM OF NATURAL IMMUNITY AND INFLAMMATION INVOLVE **NFKB AND MAPK ACTIVATION!**



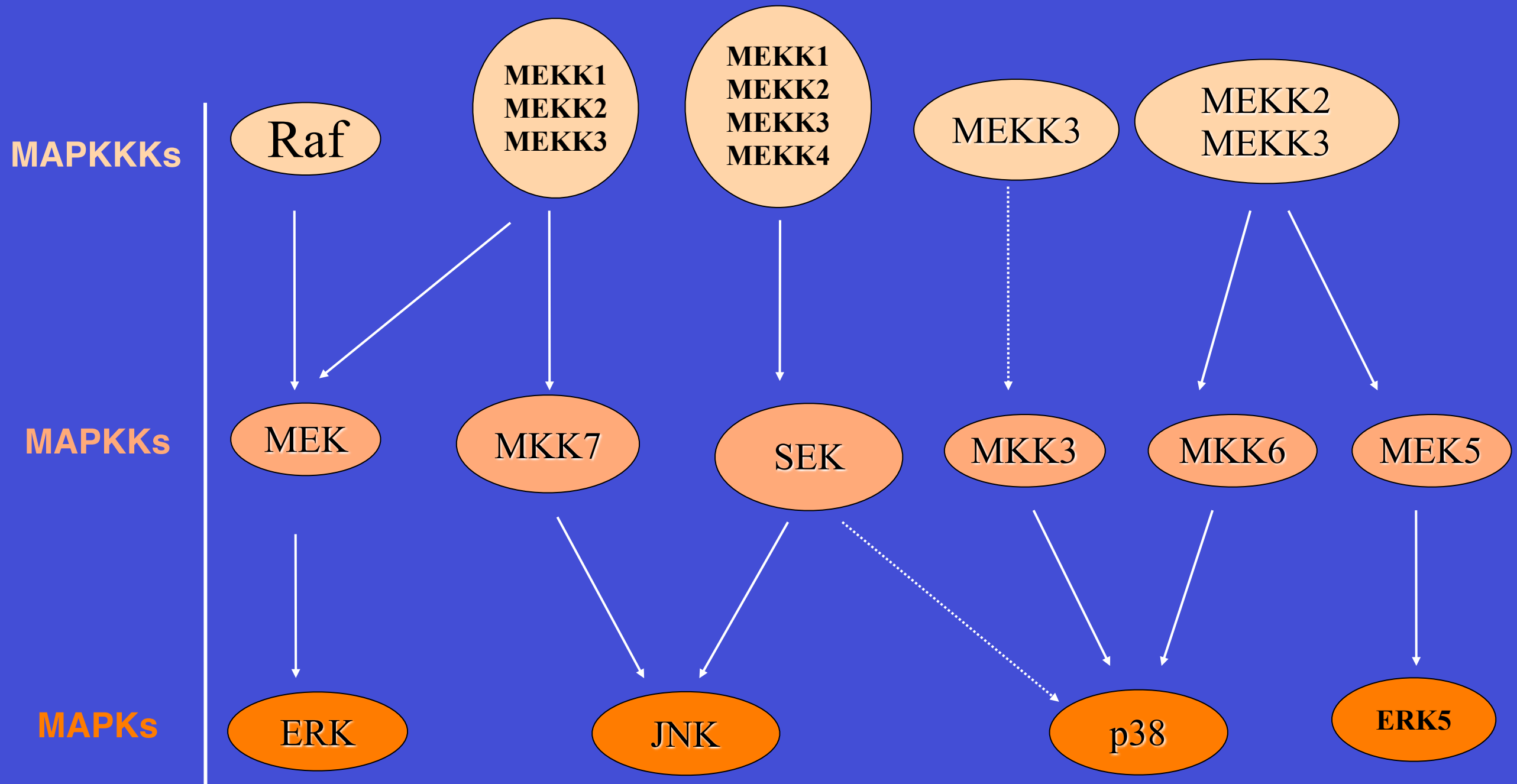
6a

The freed NFκB translocates from the cytoplasm into the nucleus, where it serves as a transcriptional activator for NFκB-dependent genes

6b

The MAPK cascade results in translocation of a transcriptional activator from the cytoplasm into the nucleus, where it activates transcription of MAPK-dependent genes

THE MAPK SIGNALING CASCADE!



TRANSCRIPTION FACTORS

THE MAPK SIGNALING CASCADE!



**THE TRANSCRIPTIONAL
PROGRAM OF NATURAL IMMUNITY AND
INFLAMMATION
CONTROLS**

**EXPRESSION AND PRODUCTION OF
MOLECULES INVOLVED IN MULTIPLE
FUNCTIONS SUCH AS:**

...THE BIOLOGY OF INFLAMMATORY AND IMMUNE CELLS WHICH DEGRANULATE.....

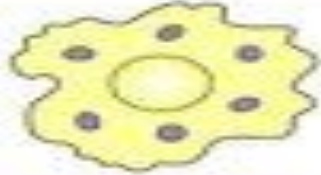
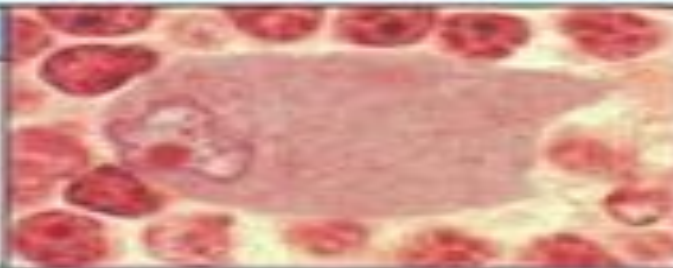



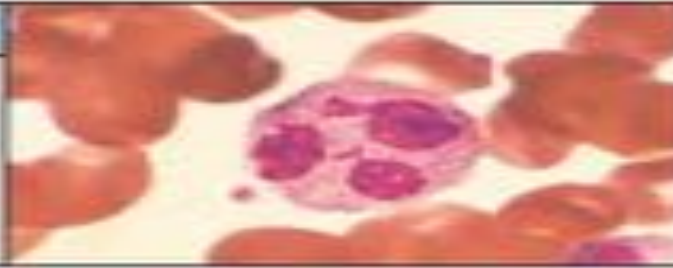

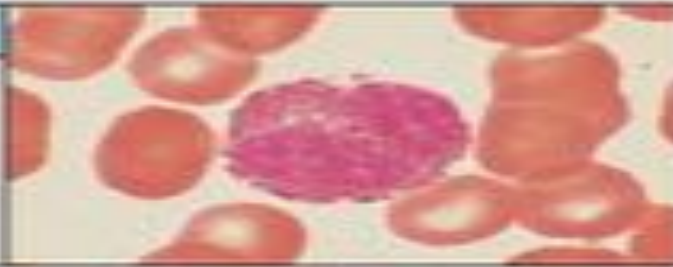

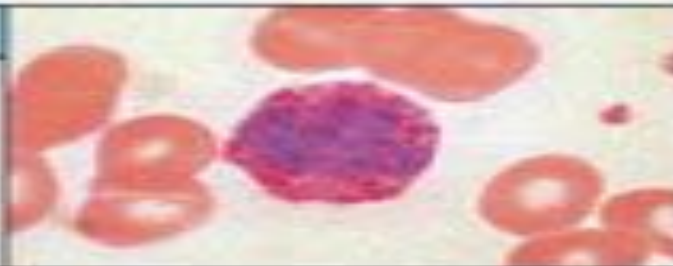


| Cell | | Activated function |
|--|---|---|
| P H A G O C Y T O S I S D E G R A N U L A T I O N | <div>Monocytes/</div> <div>Macrophage</div>  |  <div>Phagocytosis and activation of bactericidal mechanisms</div> <div>Antigen presentation</div> |
| | <div>Dendritic cell</div>  |  <div>Antigen uptake in peripheral sites</div> <div>Antigen presentation in lymph nodes</div> |
| | <div>Neutrophil</div>  |  <div>Phagocytosis and activation of bactericidal mechanisms</div> |
| | <div>Eosinophil</div>  |  <div>Killing of antibody-coated parasites</div> <div>antiviral</div> |
| | <div>Basophil</div>  |  <div>Release of histamine</div> |
| | <div>Mast cell</div>  |  <div>Release of granules containing histamine and other active agents</div> |

Figure 1-4 Immunobiology, 6/e. (© Garland Science 2005)

..... EXCERPT DYNAMICS PHAGOCYTOSIS.....

PHAGOCYTOSIS:

To defend the body against bacteria, human neutrophils (white blood cells) ingest invading pathogens like this *E. coli*

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PHAGOCYTOSIS:

SENSING
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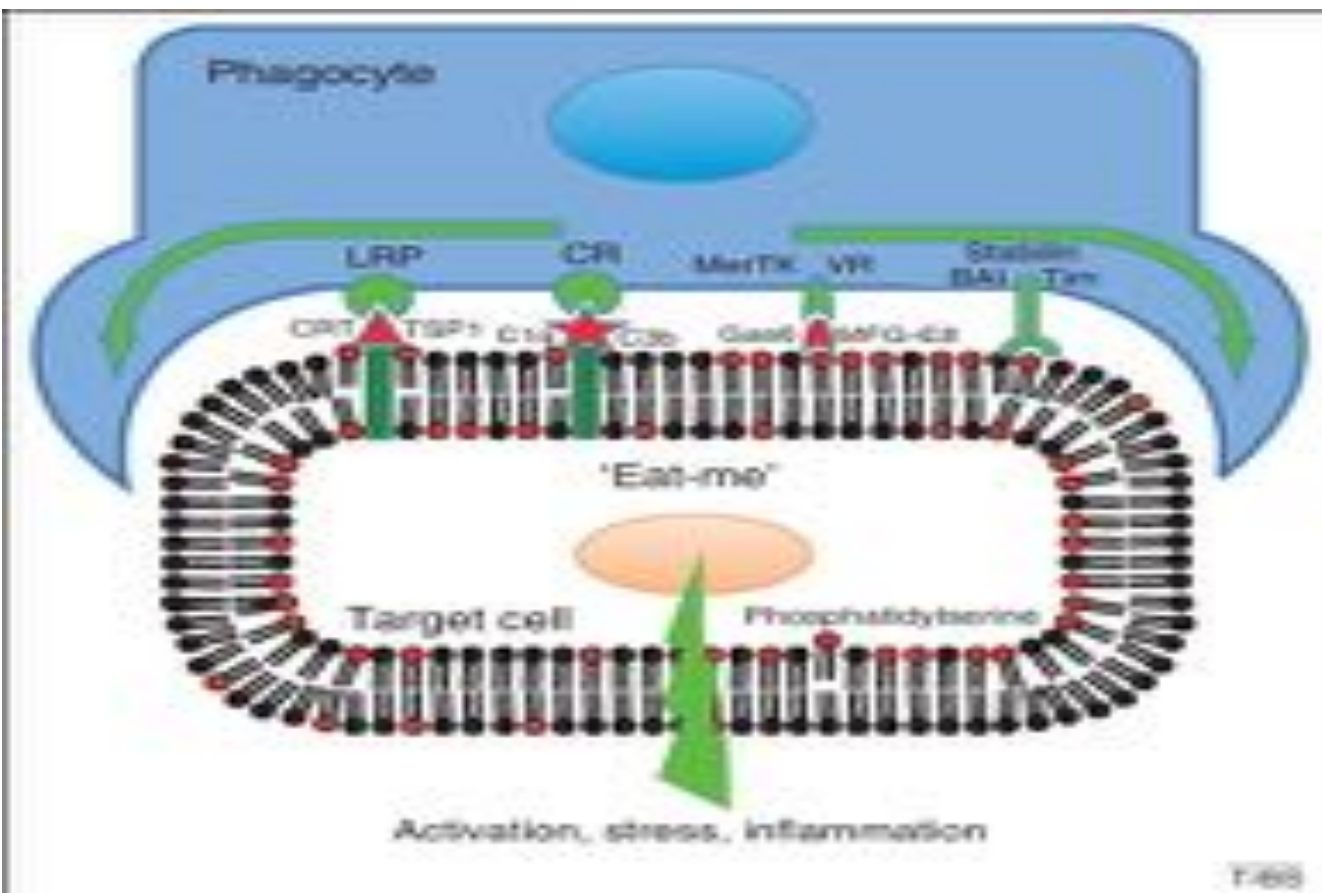
DIGESTING

.....or phagoptosis....

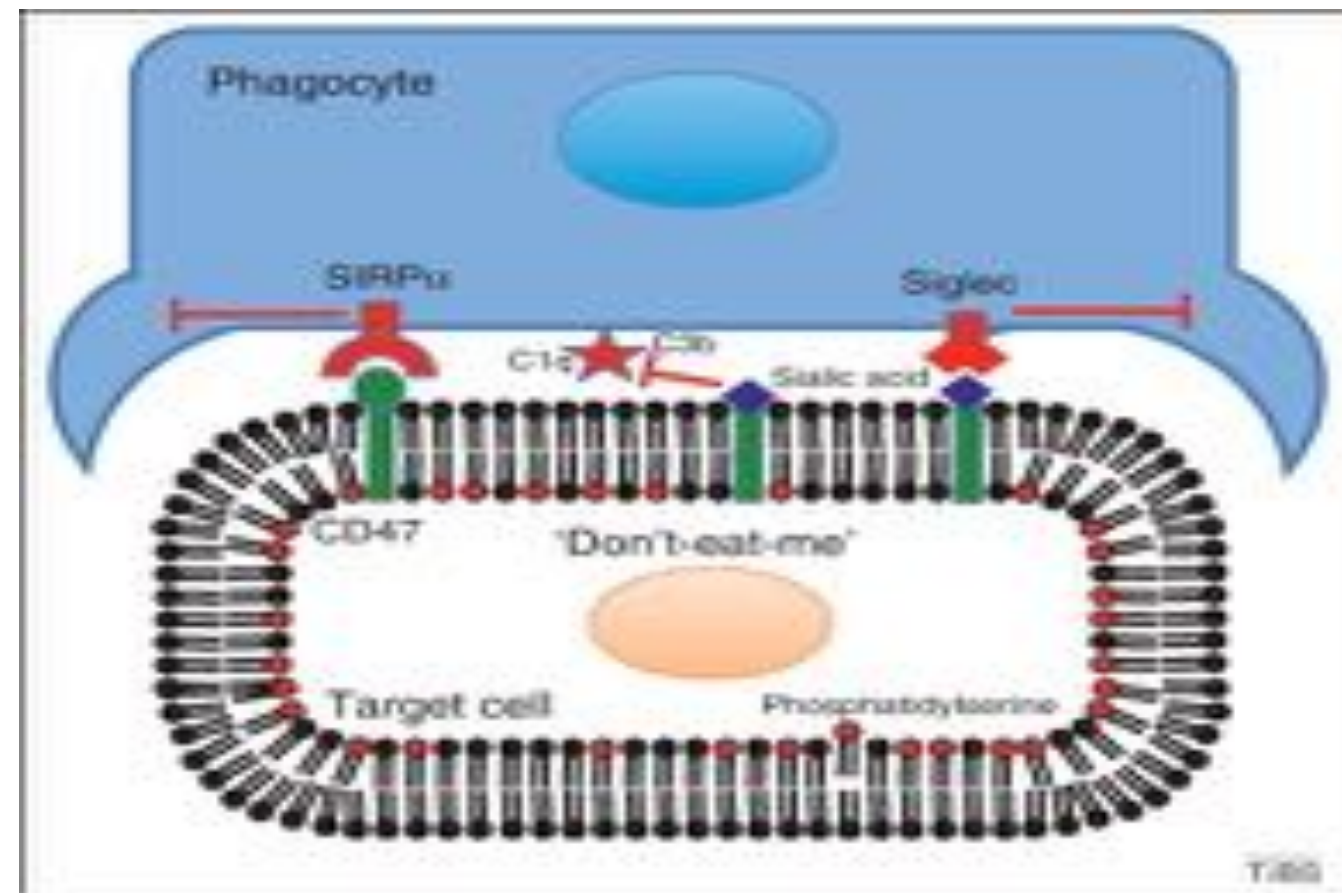
RECENTLY IT HAS BEEN DEMONSTRATED THAT A TYPE OF PHAGOCYTOSIS, CALLED **PHAGOPTOSIS**, ELIMINATES ALSO CELLS ALIVE!

This term is created by combining phago-, which is derived from the ancient Greek 'phagein' meaning to devour, and -ptosis, which is from the ancient Greek 'ptosis' meaning to fall; used here with the connotation of dying; therefore, phagoptosis would connote 'devouring-induced death' or 'death caused by being devoured'.

'Eat-me' signalling!



Don't eat-me' signalling!

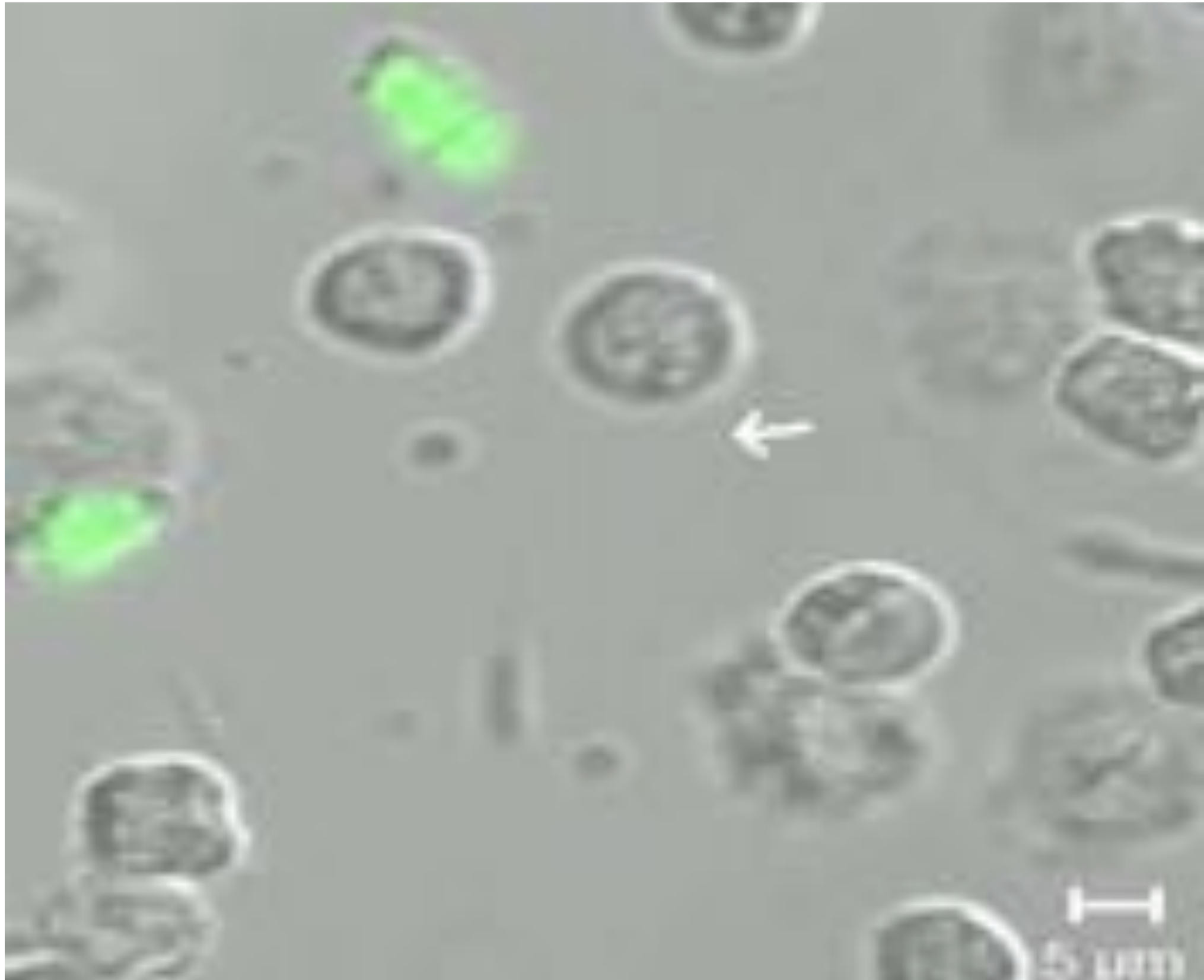


Phagoptosis mediates turnover of erythrocytes, neutrophils and other cells, and thus is quantitatively one of the main forms of cell death in the body!

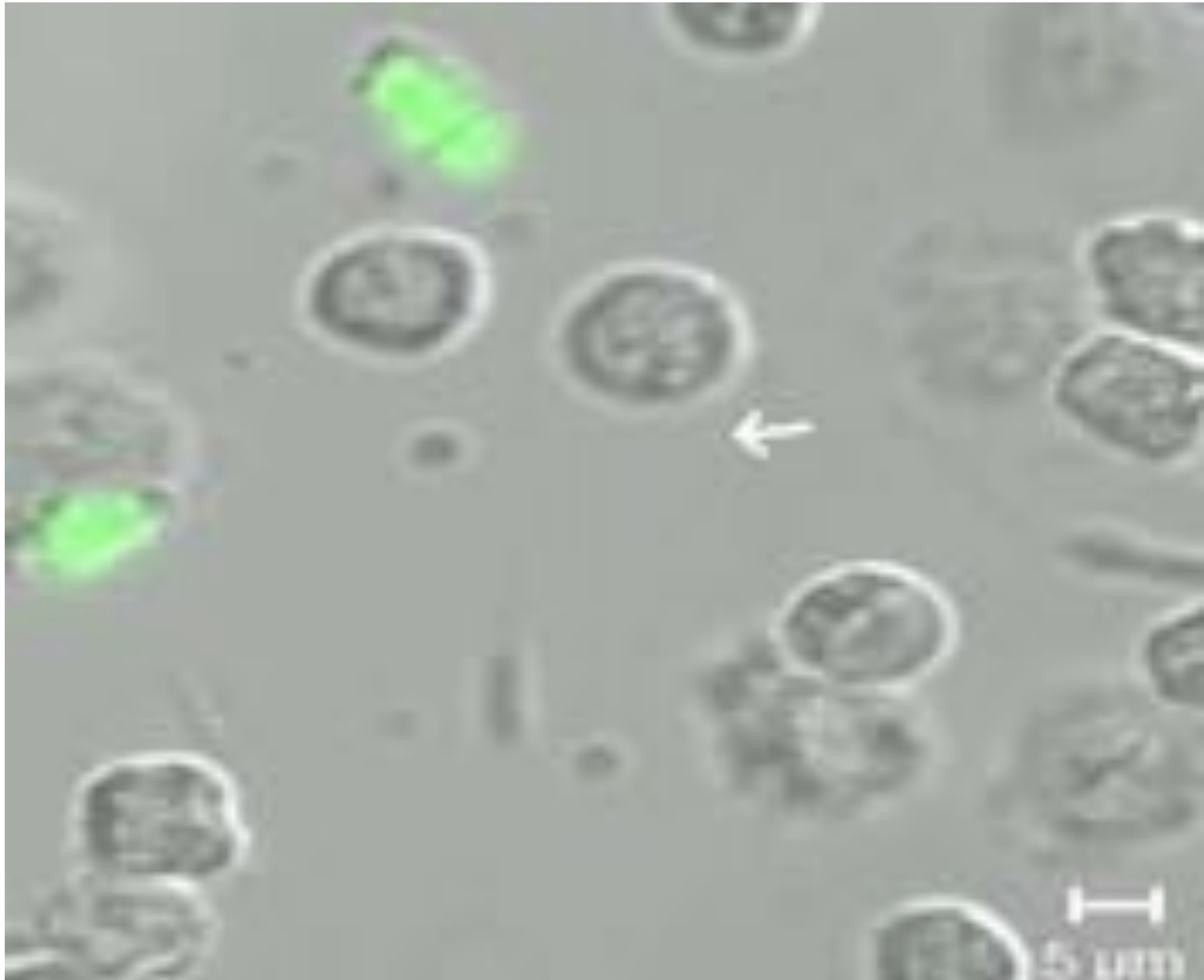
Table I. Rough estimates of the physiological rates of cell turnover by different forms of cell death in humans

| Type of cell death | Cells | Rate (thousands of cells/second) |
|--------------------|---------------------|----------------------------------|
| Phagoptosis | Erythrocytes | 2000 |
| | Neutrophils | 500–1000 |
| Shedding | Enterocytes | 80 |
| Cornification | Keratinocytes | 40 |
| Necrosis | Enterocytes | 10 |
| Apoptosis | T cells and B cells | 1 |
| Autophagy | | None known |

....or NETosis....

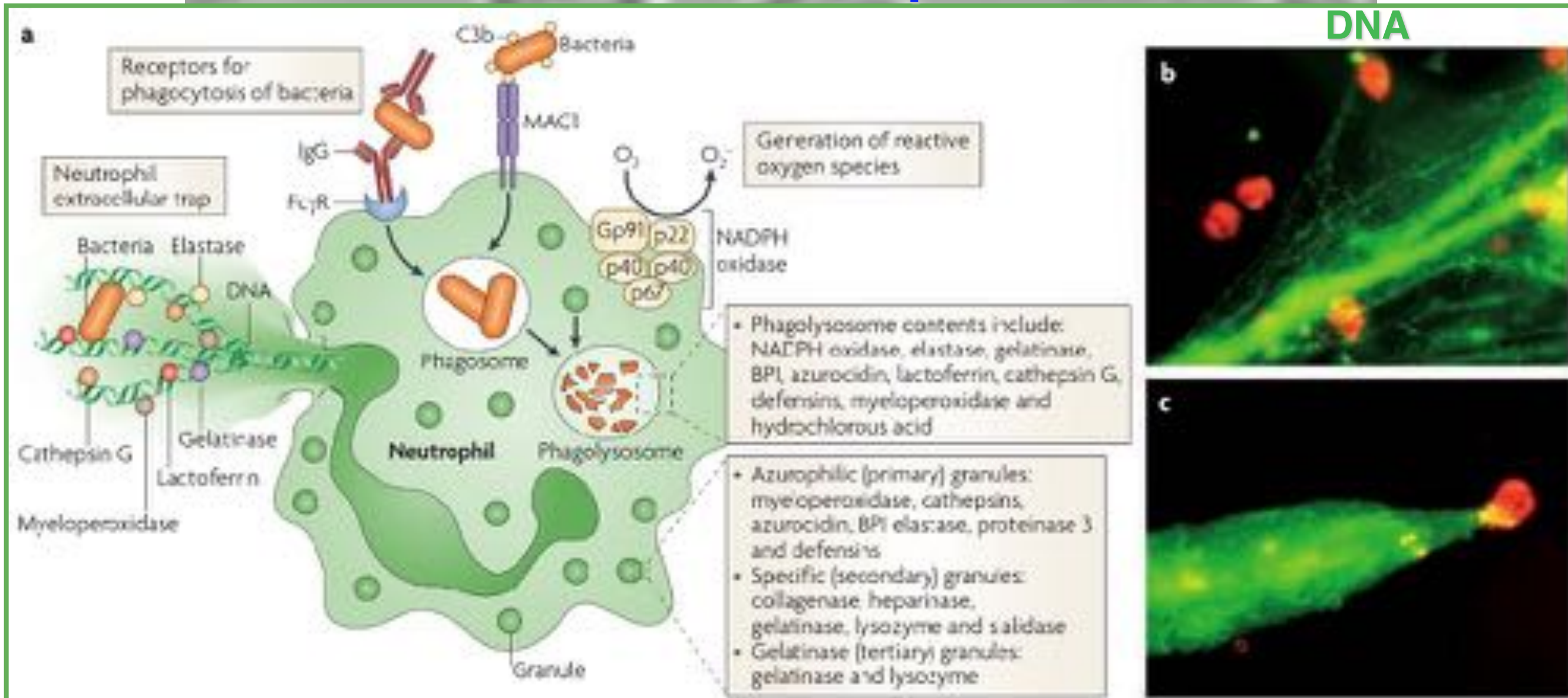


....or NETosis....

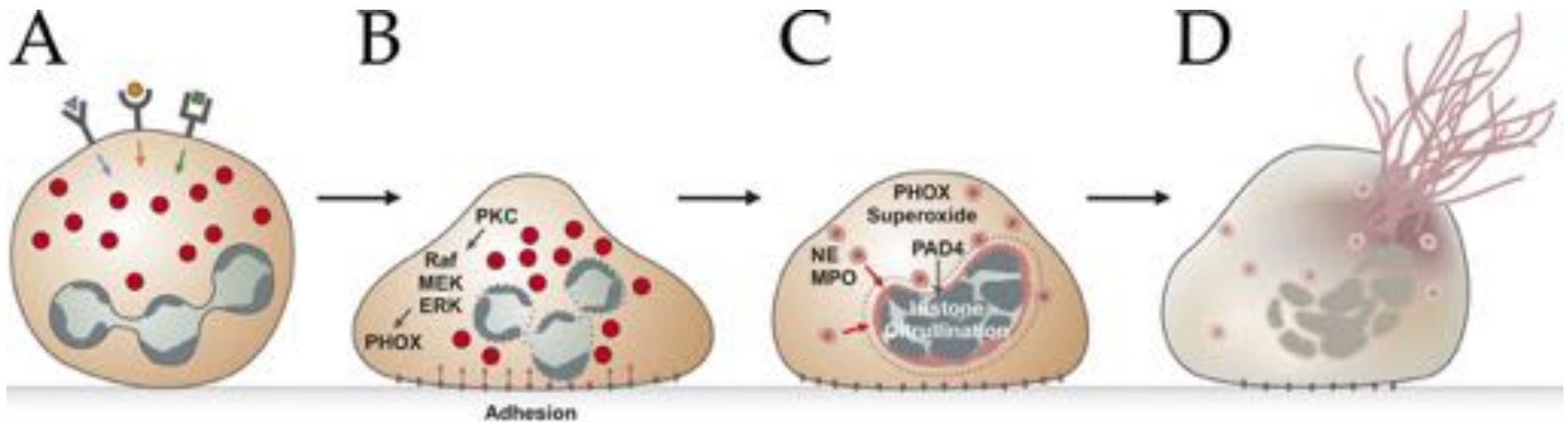


....or NETosis....

Neutrophil Extracellular Traps: an additional antibacterial weapon!



....with particular biochemical pathways leading to Neutrophil Extracellular Traps (NET) formation!



After stimulation of receptors (A), neutrophils adhere to the substrate (B) and mobilize granule components, namely NE and MPO (C). Granules are depicted as red circles. Histones in the nucleus get processed, and the intracellular membranes disintegrate. Finally, the cell membrane ruptures, and the mixture of cytoplasm and nucleoplasm gets expelled to form NETs (D).

It has been reported that peptidylarginine deiminase 4 (PAD4), an enzyme that converts Arg or monomethyl-Arg to citrulline in histones, is essential for NET formation. The areas of extensive chromatin decondensation along the NETs were rich in histone citrullination.

Front Immunol. 2012;3:307.

PAD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures.

Leshner M, Wang S, Lewis C, Zheng H, Chen XA, Santy L, Wang Y.

Department of Biochemistry and Molecular Biology, Center for Eukaryotic Gene Regulation, Pennsylvania State University, University Park PA, USA.

Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity!

Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity!

The pathogenesis of many autoimmune diseases is initially based on a redundant or prolonged activation of the innate immune system. It was suggested that an excessive activation of the innate immunity is often the result of a chronic inflammatory process in the organism. This inflammation can be induced by exogenous and endogenous alarm factors, or alarmins. We believe that the recently discovered neutrophil extracellular traps, or NETs, completely meet the criteria of alarmins.

Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity!

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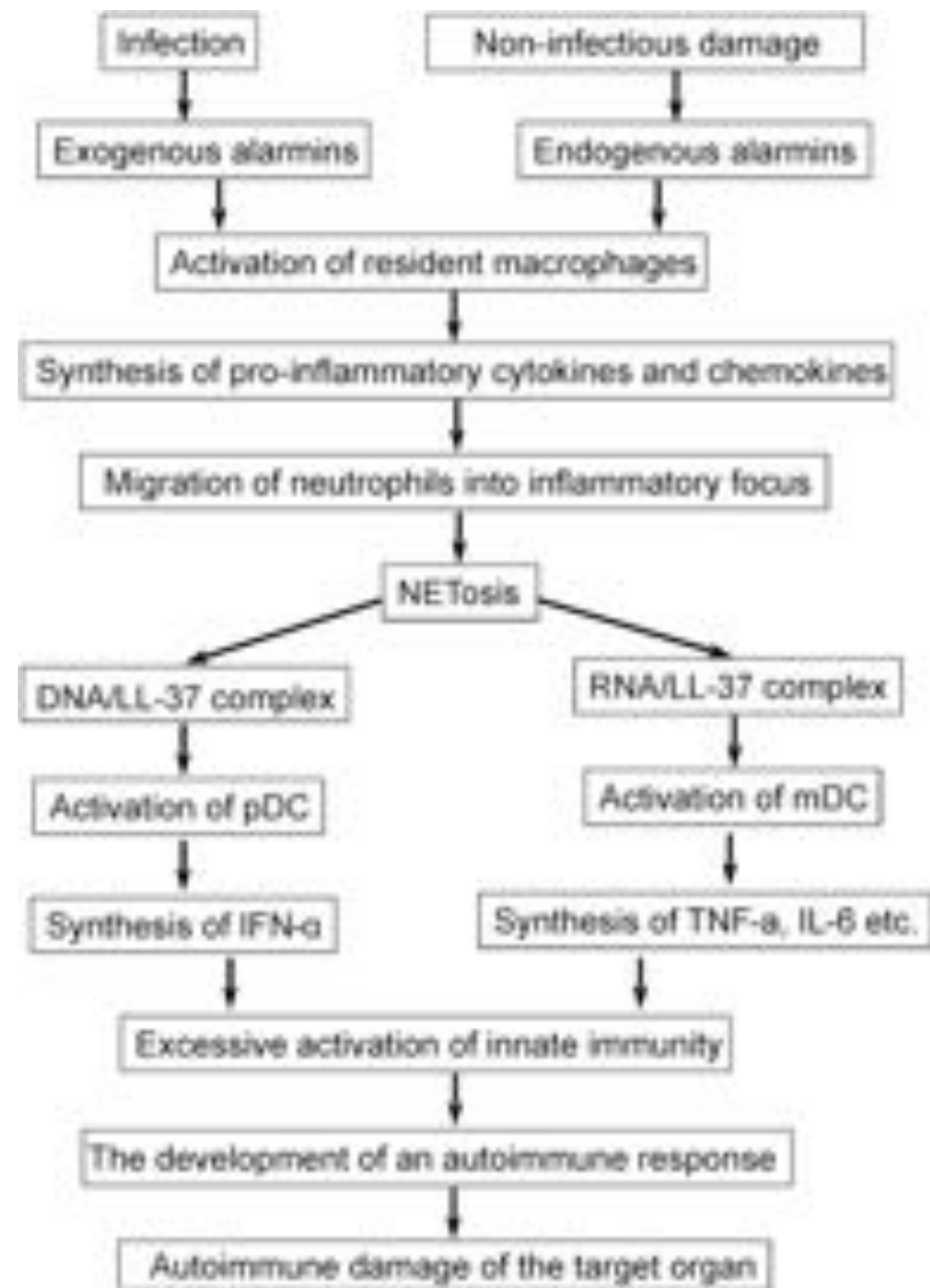
Studies on the NETosis can provide the foundation for developing new diagnostic methods and effective treatment of chronic inflammatory and autoimmune diseases.

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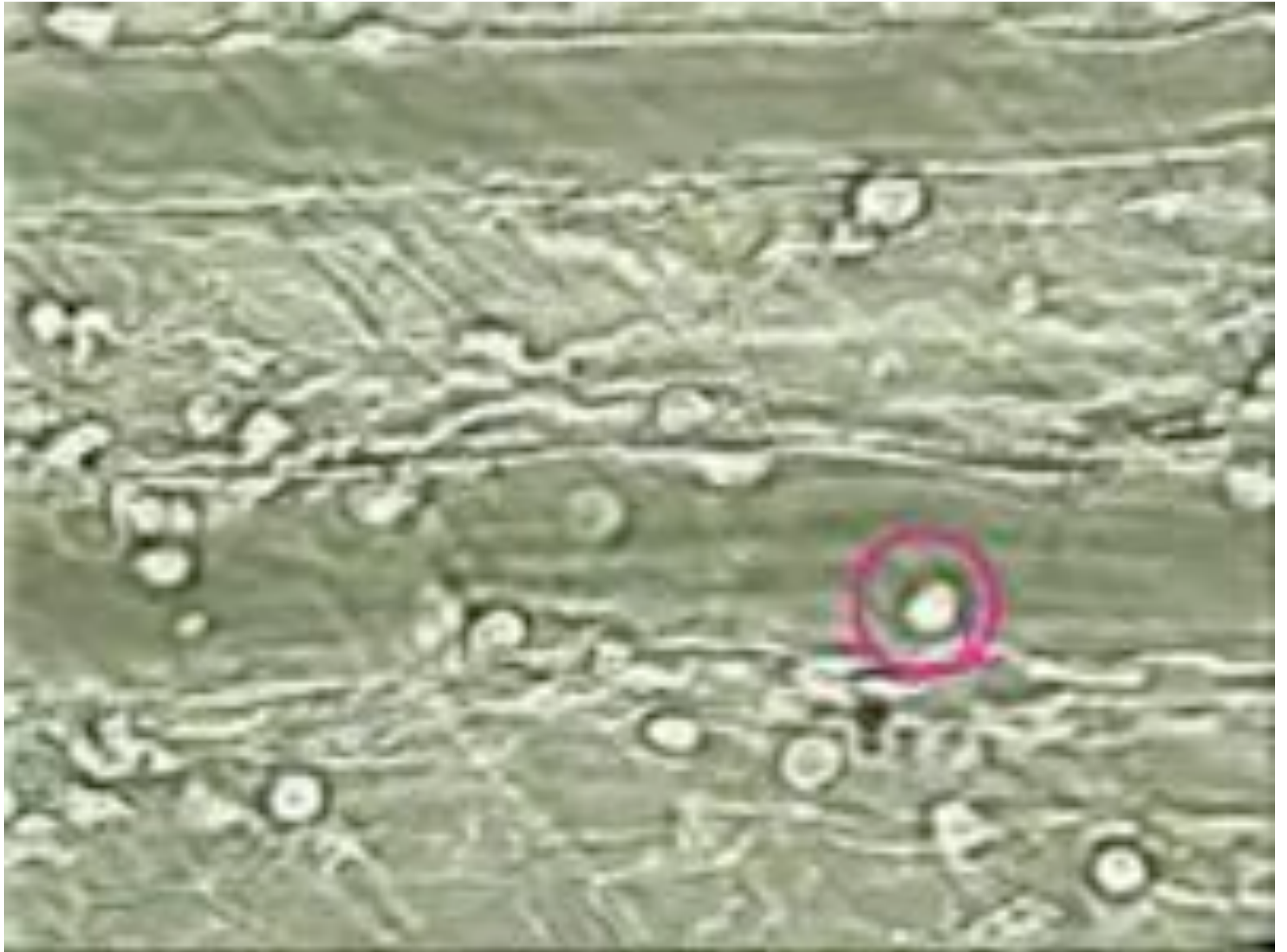
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.....AND MIGRATION.....

.....AND MIGRATION.....

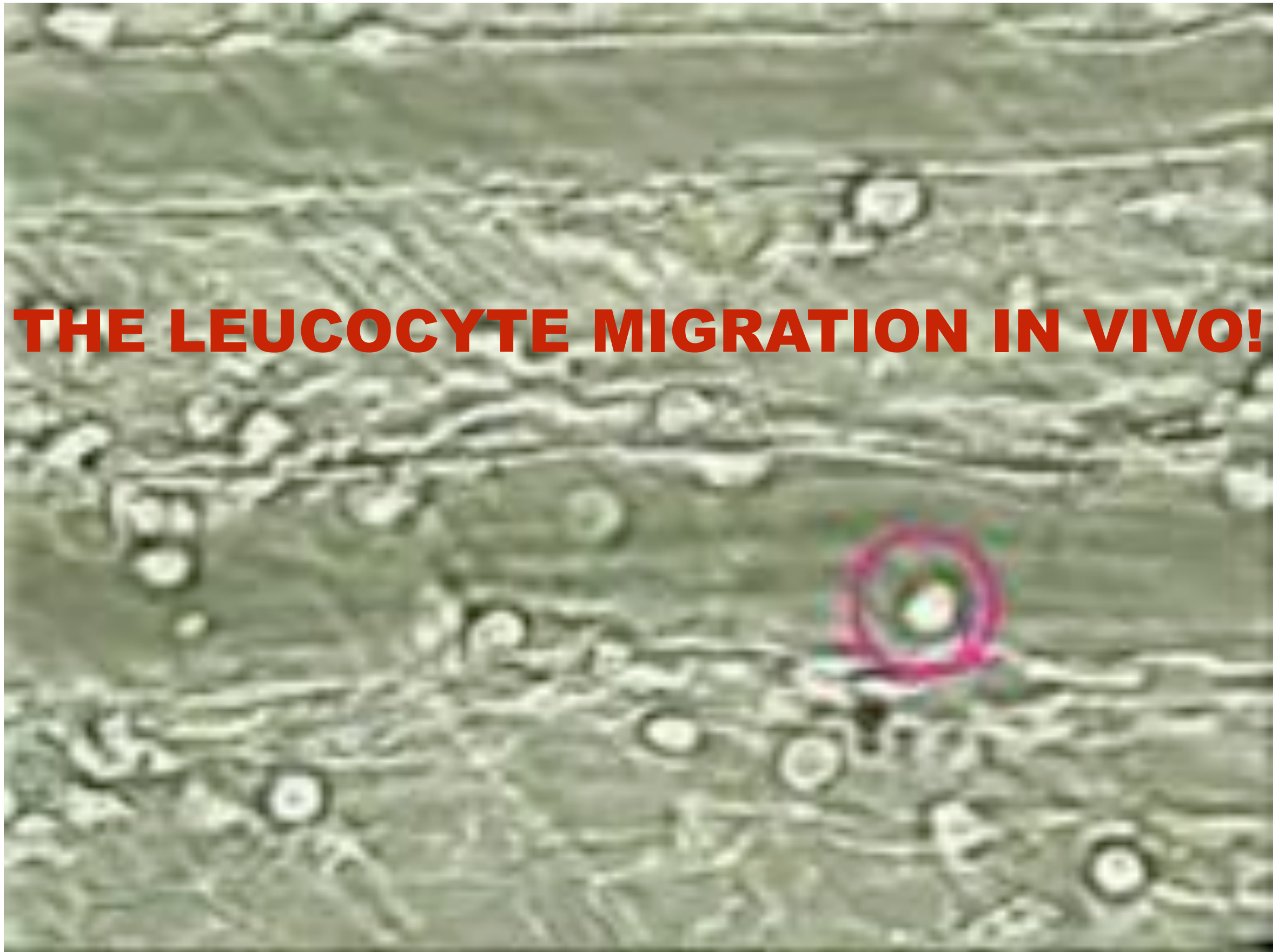


.....AND MIGRATION.....



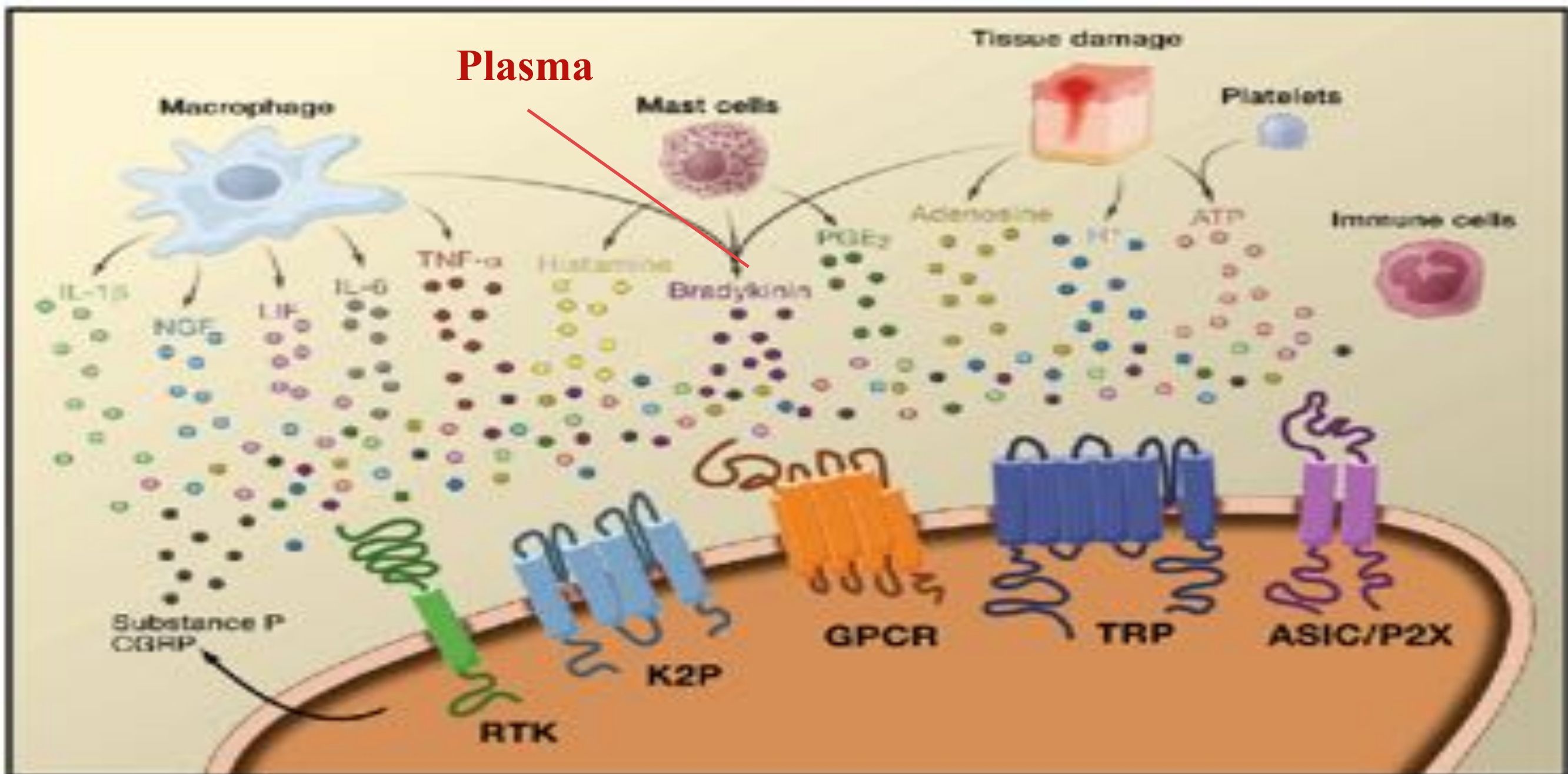
.....AND MIGRATION.....

THE LEUCOCYTE MIGRATION IN VIVO!



...AND PRODUCE INFLAMMATION MEDIATORS....

Tissue damage leads to the release of inflammatory mediators by activated nociceptors or nonneural cells including mast cells, basophils, platelets, macrophages, neutrophils, endothelial cells, keratinocytes, and fibroblasts. This “**inflammatory soup**” of signaling molecules includes **histamine**, **ATP**, **adenosine**, **substance P**, **calcitonin-gene related peptide (CGRP)**, **bradykinin**, extracellular **prostaglandins**, **thromboxanes**, **leukotrienes**, **nerve growth factor (NGF)**, **tumor necrosis factor α (TNF- α)**, **interleukin-1 β (IL-1 β)** etc. These factors act directly by binding to one or more cell surface receptors, including G protein-coupled receptors (GPCR), TRP channels, acid-sensitive ion channels (ASIC), two-pore potassium channels (K2P), and receptor tyrosine kinases (RTK).



...SUCH AS CYTOKINES AND CHEMOKINES..

| Cytokine | Main producer | Acts upon | Effect |
|-----------------|--------------------------------|-------------------------|---|
| IL-1 | Macrophages Keratinocytes | Lymphocytes | Enhances responses |
| | | Liver | Induces acute-phase protein secretion |
| IL-6 | Macrophages Dendritic cells | Lymphocytes | Enhances responses |
| | | Liver | Induces acute-phase protein secretion |
| CXCL8 (IL-8) | Macrophages Dendritic cells | Phagocytes | Chemoattractant for neutrophils |
| IL-12 | Macrophages Dendritic cells | Naive T cells | Diverts immune response to type 1, proinflammatory, cytokine secretion |
| TNF- α | Macrophages Dendritic cells | Vascular endothelium | Induces changes in vascular endothelium (expression of cell- adhesion molecules (E- and P- selectin), changes in cell-cell junctions with increased fluid loss, |

Figure 2-15 Immunobiology, 6/e. (© Garland Science 2005)

...WHICH CAN HAVE VARIOUS EFFECT...

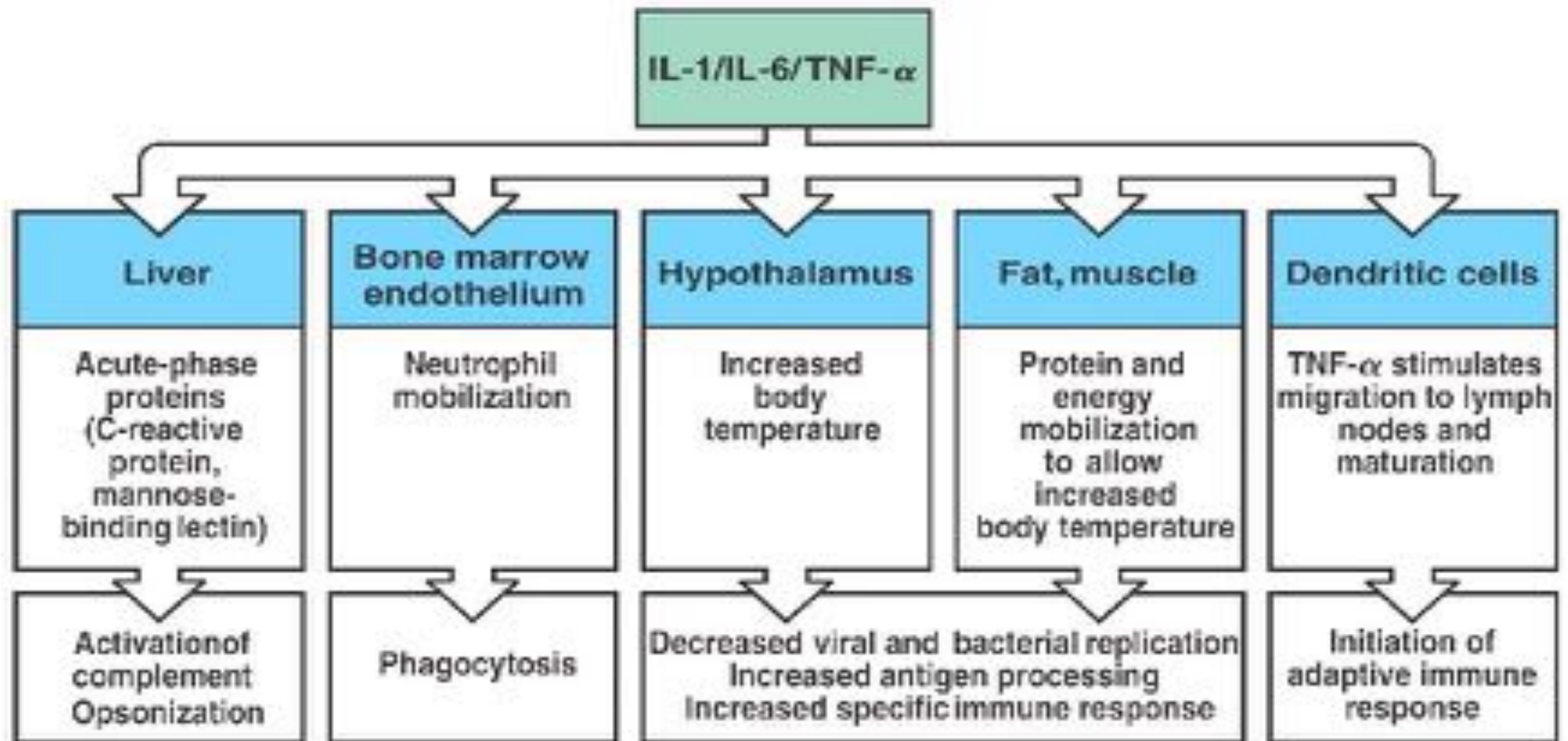
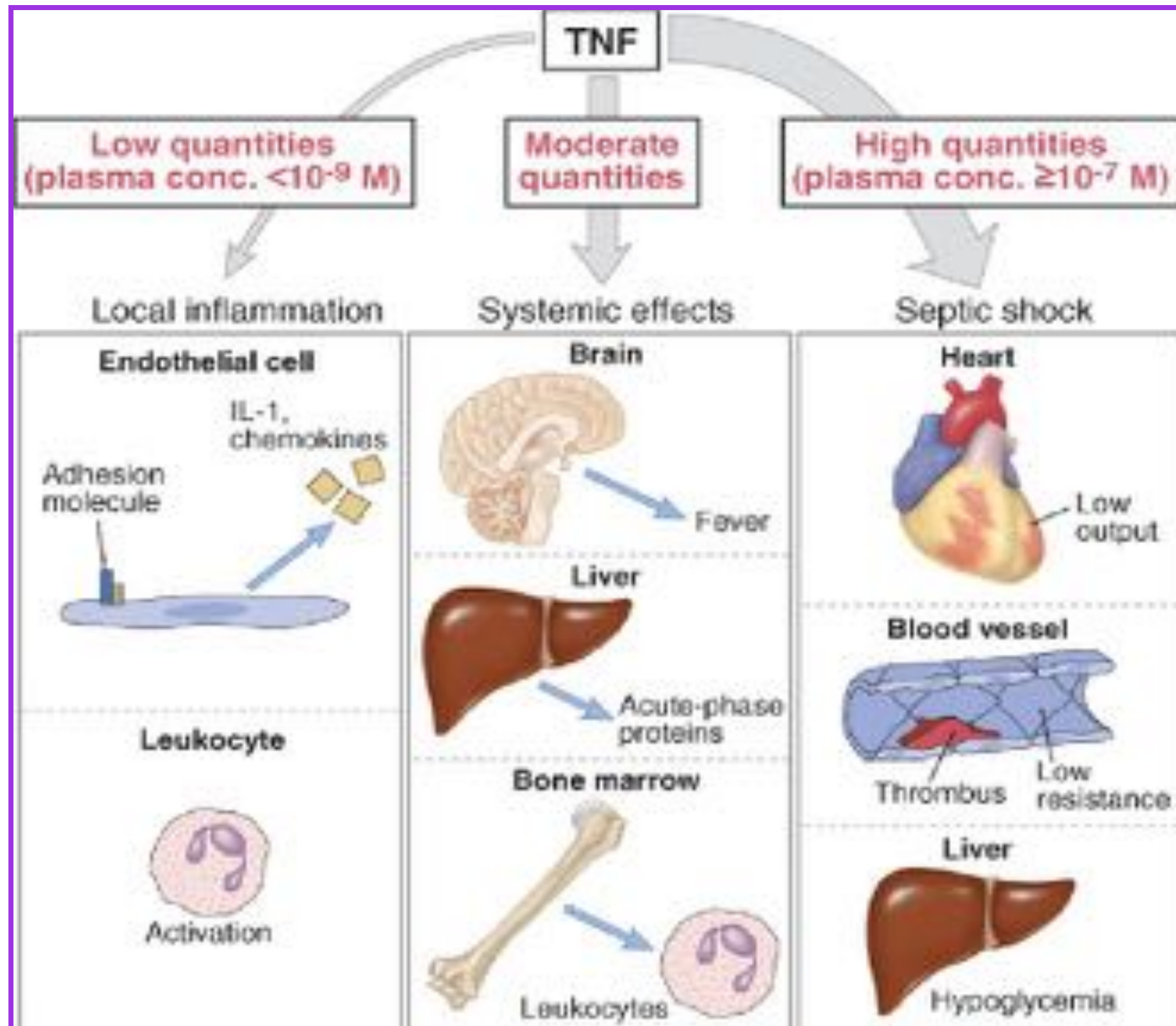
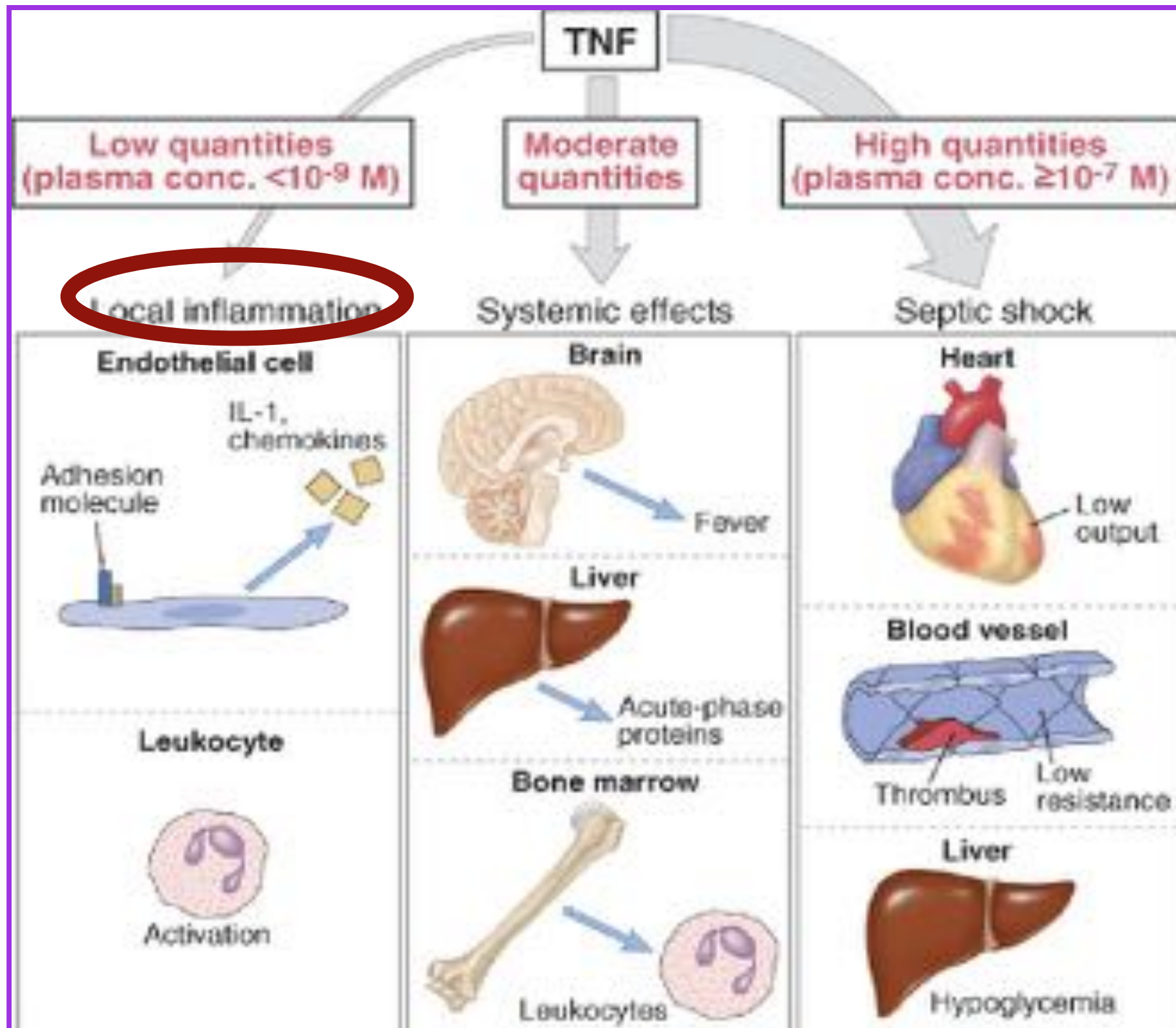


Figure 2-46 Immunobiology, 6/e. (© Garland Science 2005)

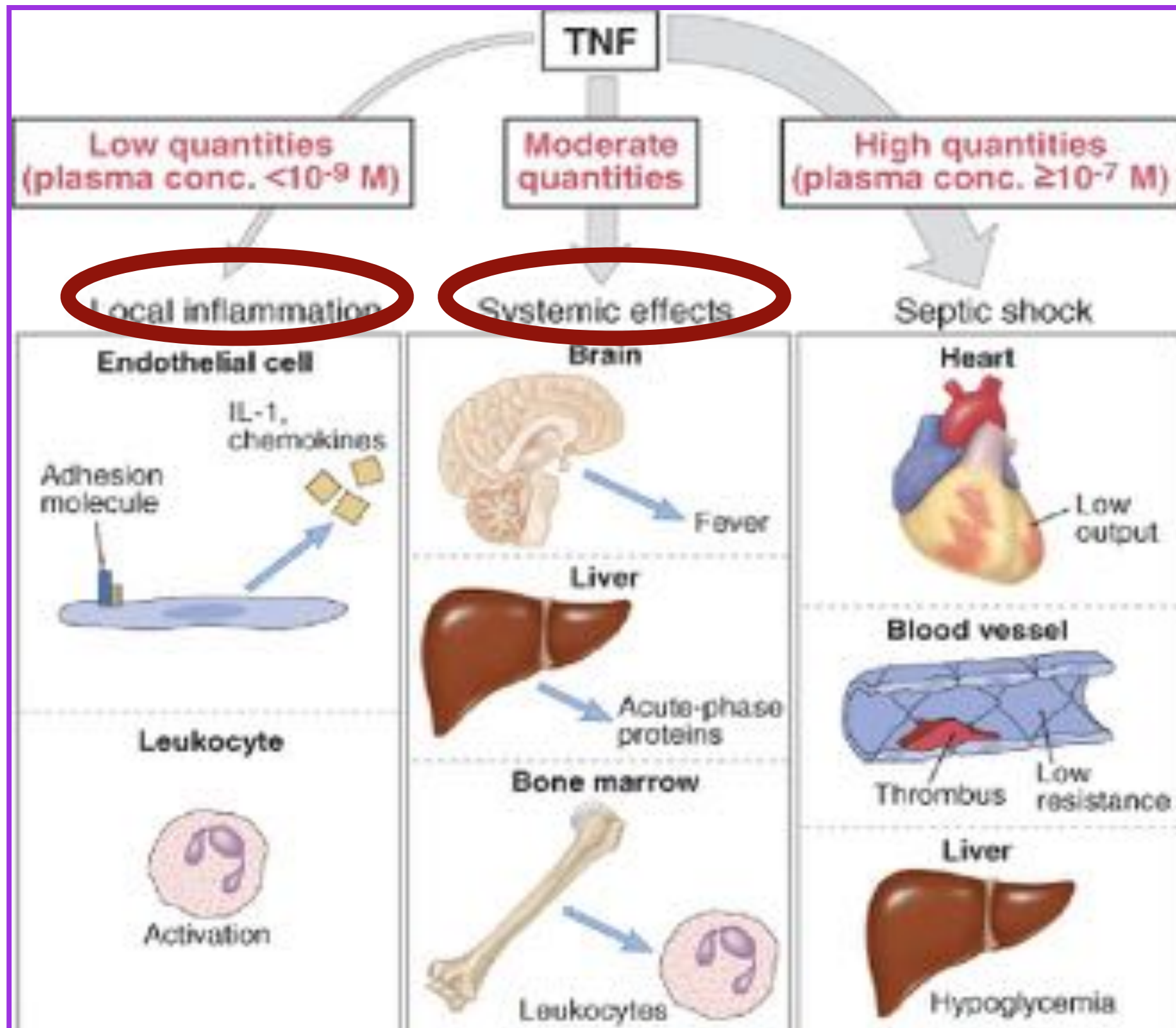
...LOCALLY AND SYSTEMICALLY!!!!!!



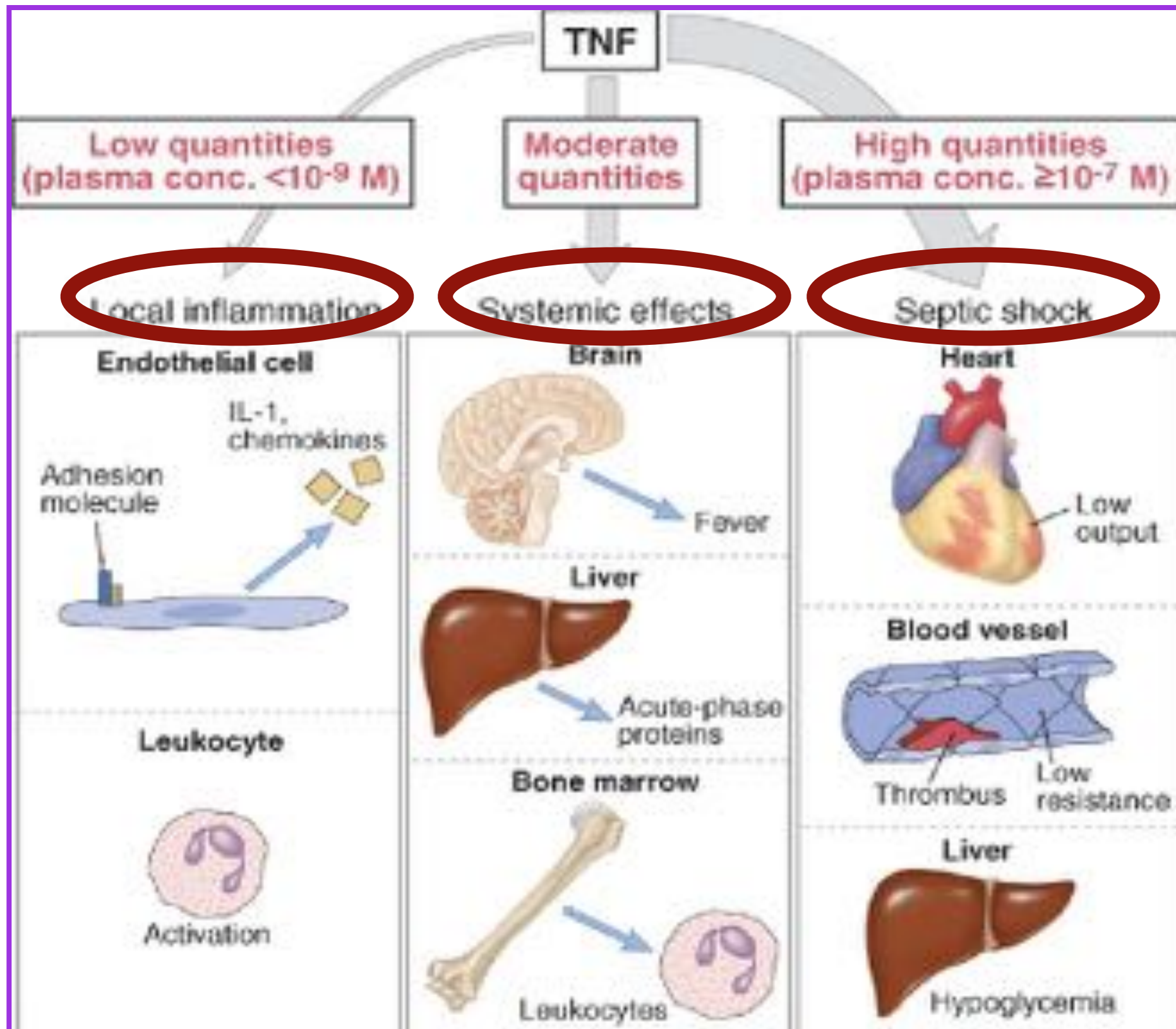
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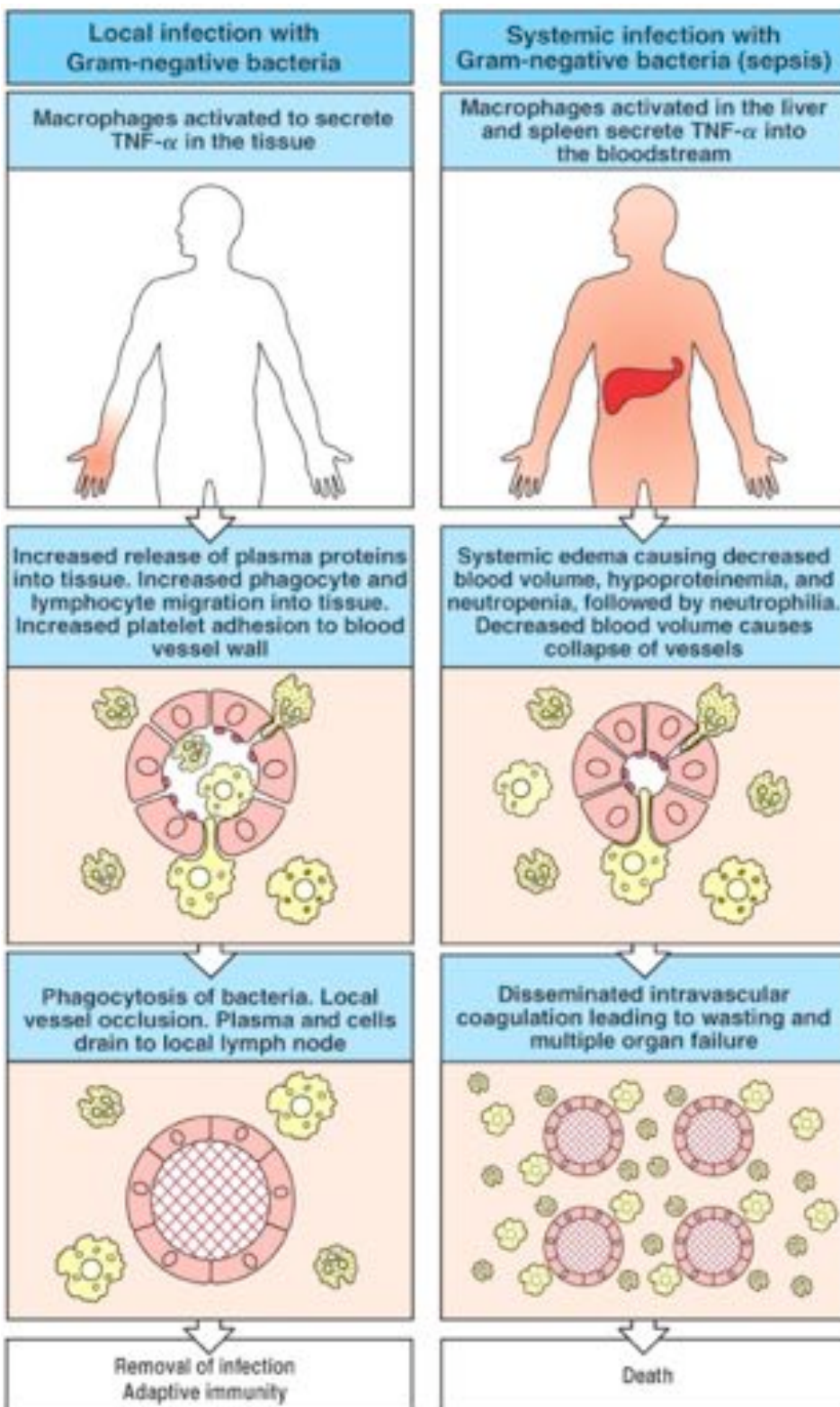
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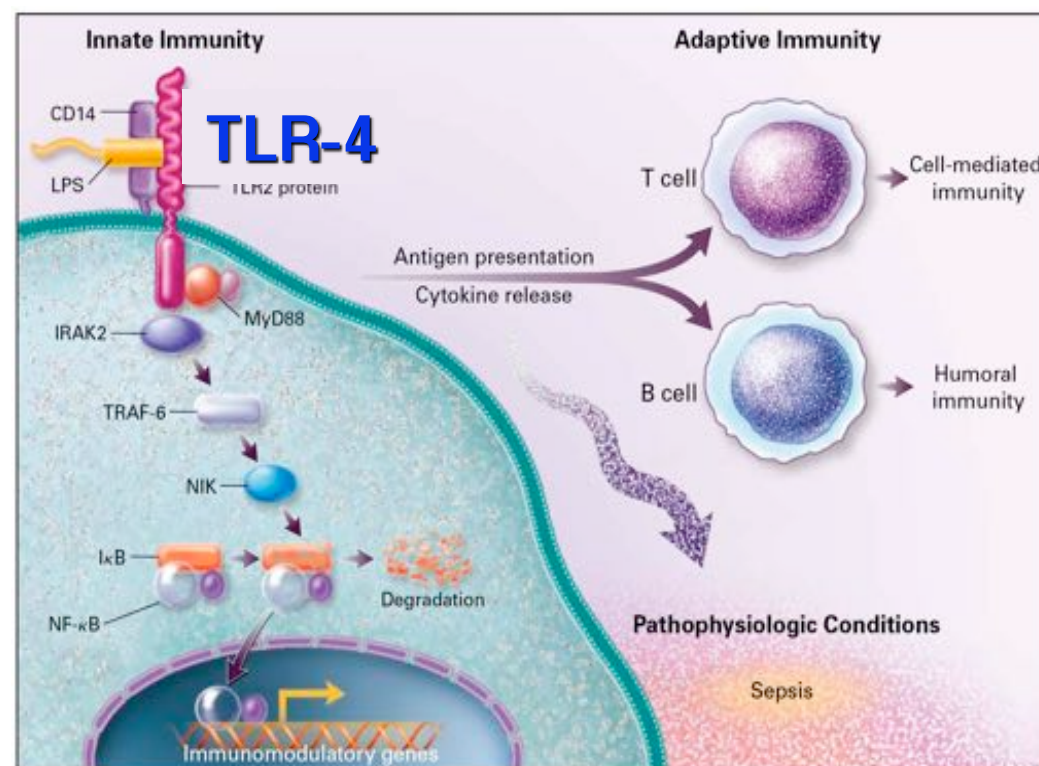
Pathological consequences of Systemic Inflammatory response (SIRS) are:

- **Septic shock;**
- **ARDS;**
- **Multiple organ dysfunction (MOD)
and Multiple organ failure (MOF)!**

THE SEPTIC SHOCK: endototoxic ed esotossic!



Pathological consequences of inflammatory response to systemic LPS: the septic shock



• endototoxic

Septic shock is classically triggered by Gram- bacteria (TLR-4/LPS); Gram+ bacteria too can induce a systemic inflammatory response (SuperAg, TLR2/ lipoproteins)!

• esotossic

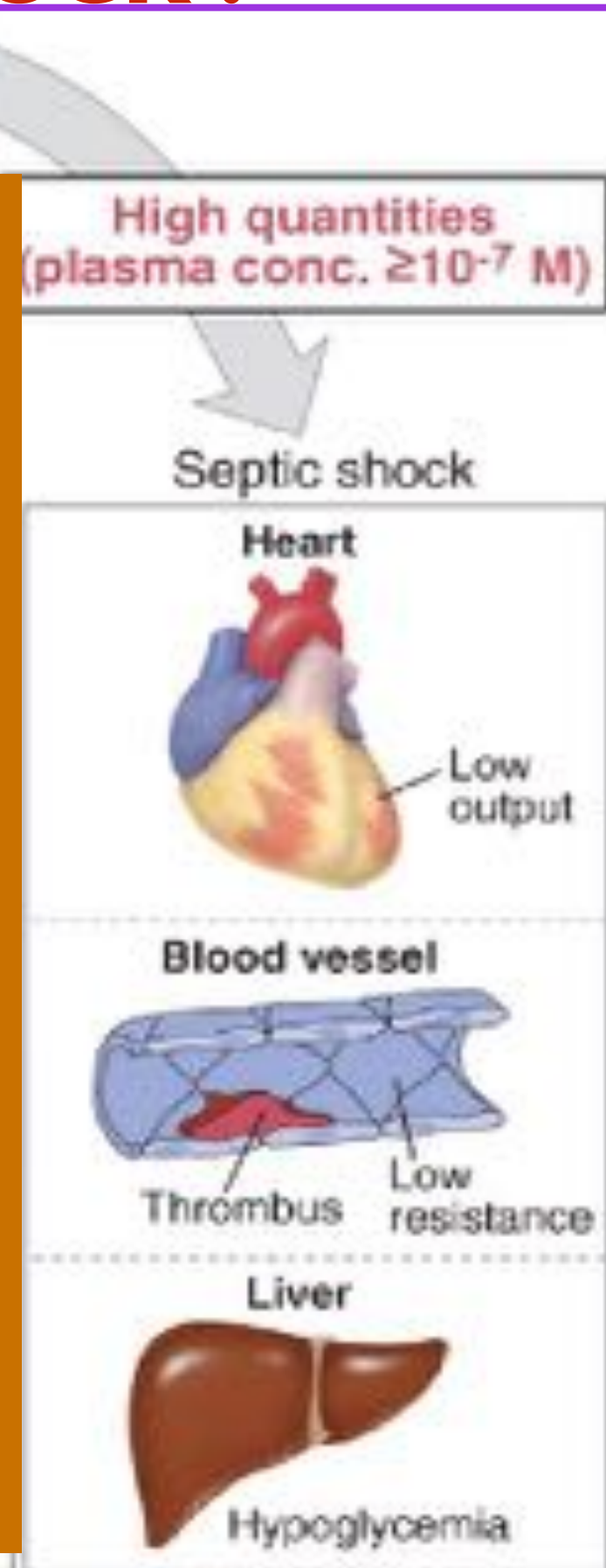
THE TOXIC SYSTEMIC EFFECTS of $\text{TNF}\alpha$ and $\text{IL1}\beta$: THE SEPTIC SHOCK !

vasodilation

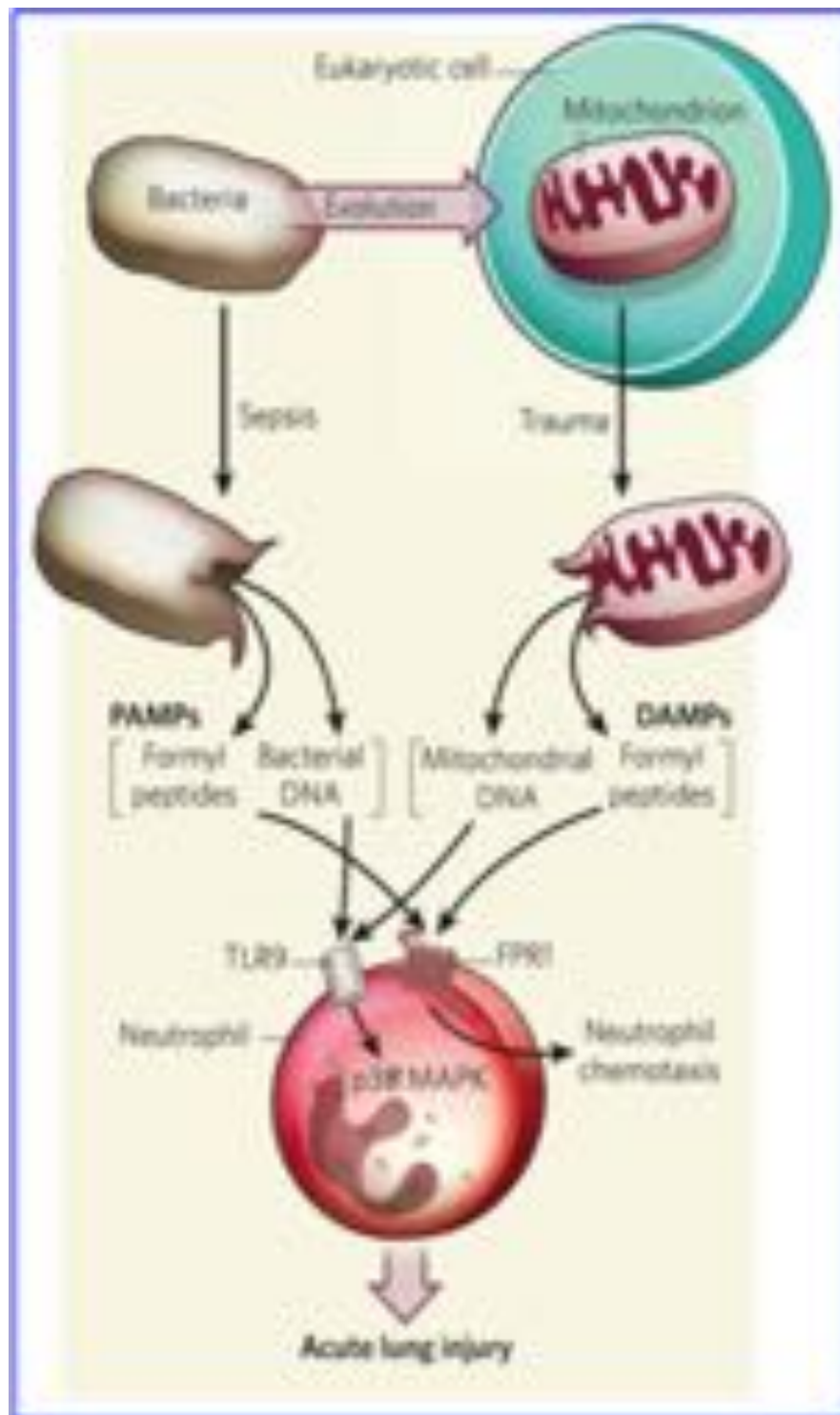
low cardiac output

**formation of
thrombi**

**intravascular
coagulation**



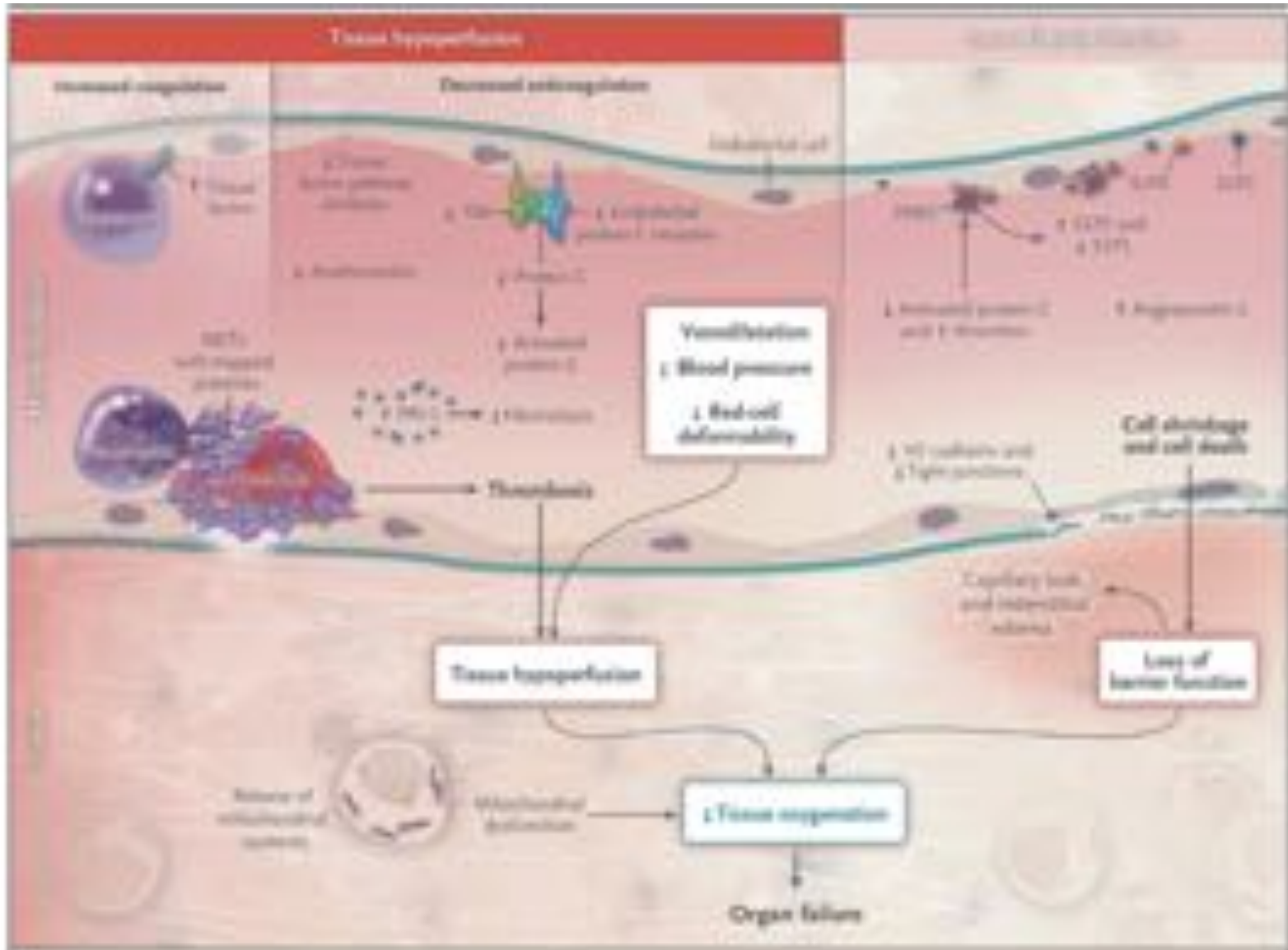
SEVERE SEPSYS CAN ACTIVATE ALSO ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)!



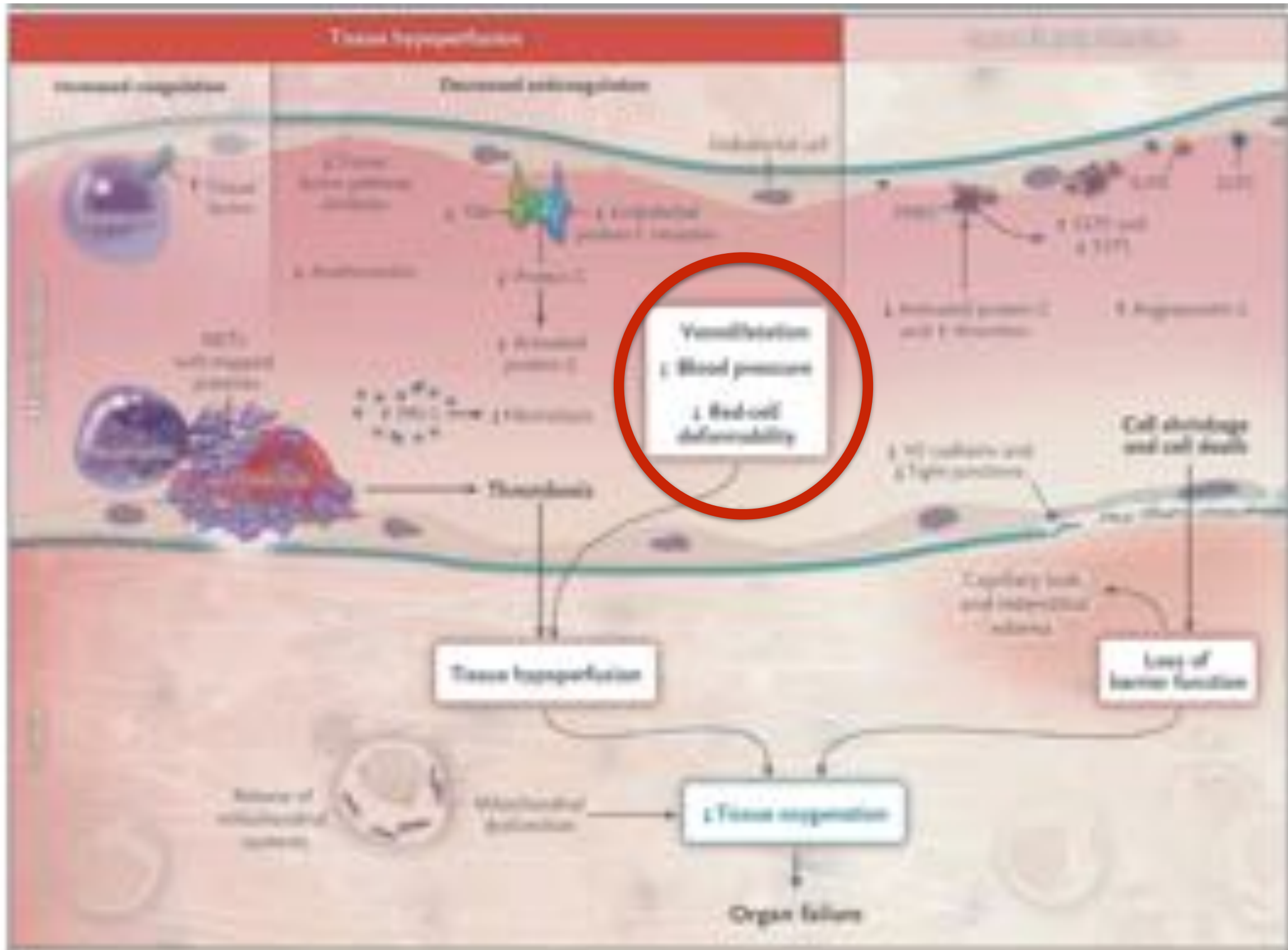
| | ARDS | | |
|---|---|---|---|
| | Mild | Moderate | Severe |
| Timing | Acute onset within 1 week of a known clinical insult or new/worsening respiratory symptoms | | |
| Hypoxemia | $\text{PaO}_2/\text{FiO}_2$ 201–300 with PEEP/CPAP \geq 5 | $\text{PaO}_2/\text{FiO}_2 \leq 200$ with PEEP \geq 5 | $\text{PaO}_2/\text{FiO}_2 \leq 100$ with PEEP \geq 10 |
| Origin of Edema | Respiratory failure associated to known risk factors and not fully explained by cardiac failure or fluid overload. Need objective assessment of cardiac failure or fluid overload if no risk factor are present | | |
| Radiological Abnormalities | Bilateral opacities* | Bilateral opacities* | Opacities involving at least 3 quadrants* |
| Additional Physiological Derangement | N/A | N/A | $\text{V}_{\text{E}}/\text{C}_{\text{DR}} > 10 \text{ L/min}$ or $\text{C}_{\text{DR}} < 40 \text{ ml/cmH}_2\text{O}$ |

*Not fully explained by effusions, nodules, masses, or lobar/lung collapse; use training set of CXRs; $\text{V}_{\text{E}}/\text{C}_{\text{DR}} = \text{V}_{\text{E}} \times \text{PaCO}_2/40$ (corrected for Body Surface Area)

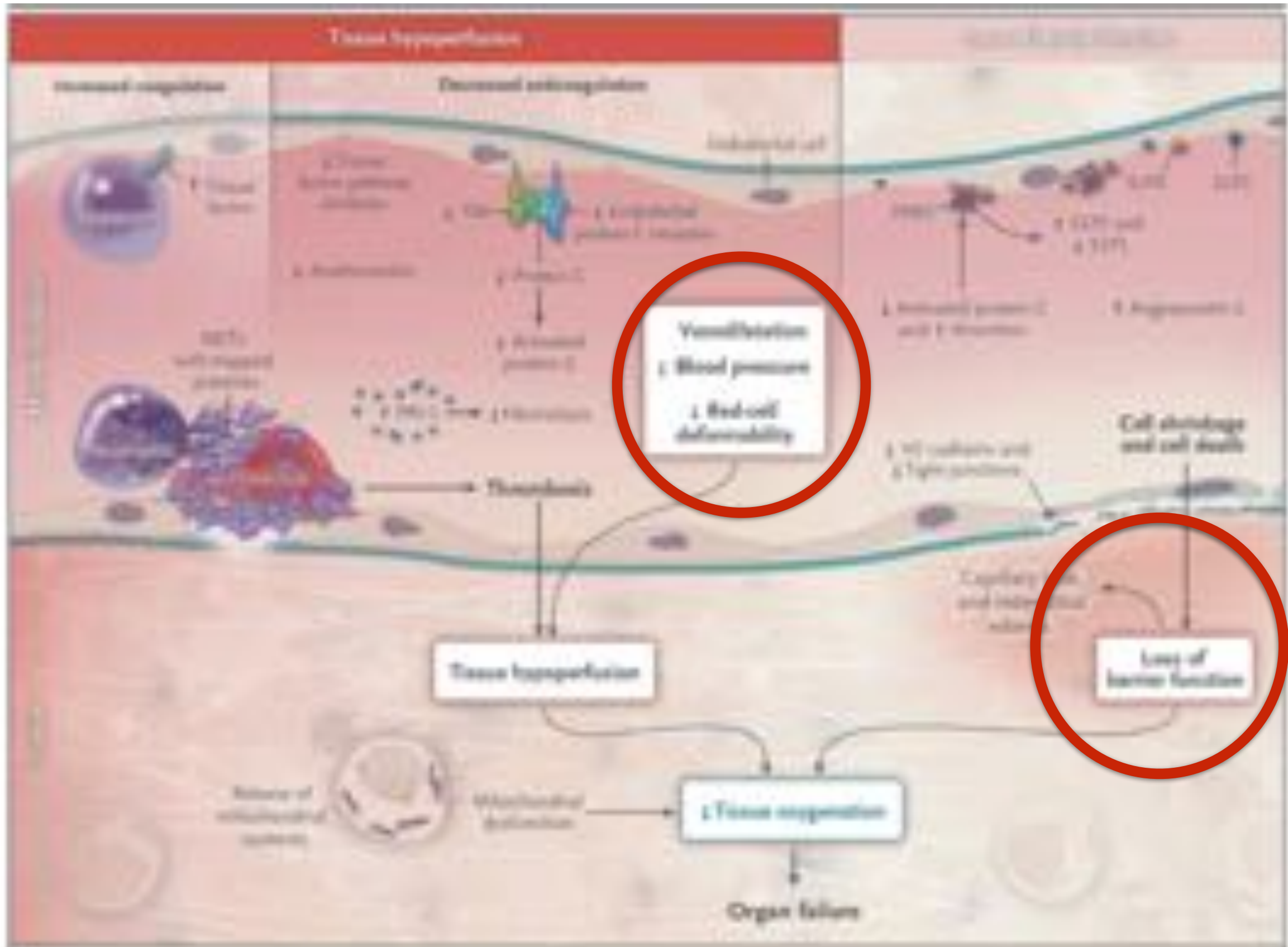
Dysfunction of the vascular endothelium in severe sepsis leads to Multiple organ dysfunction (MOD) and Multiple organ failure (MOF)!



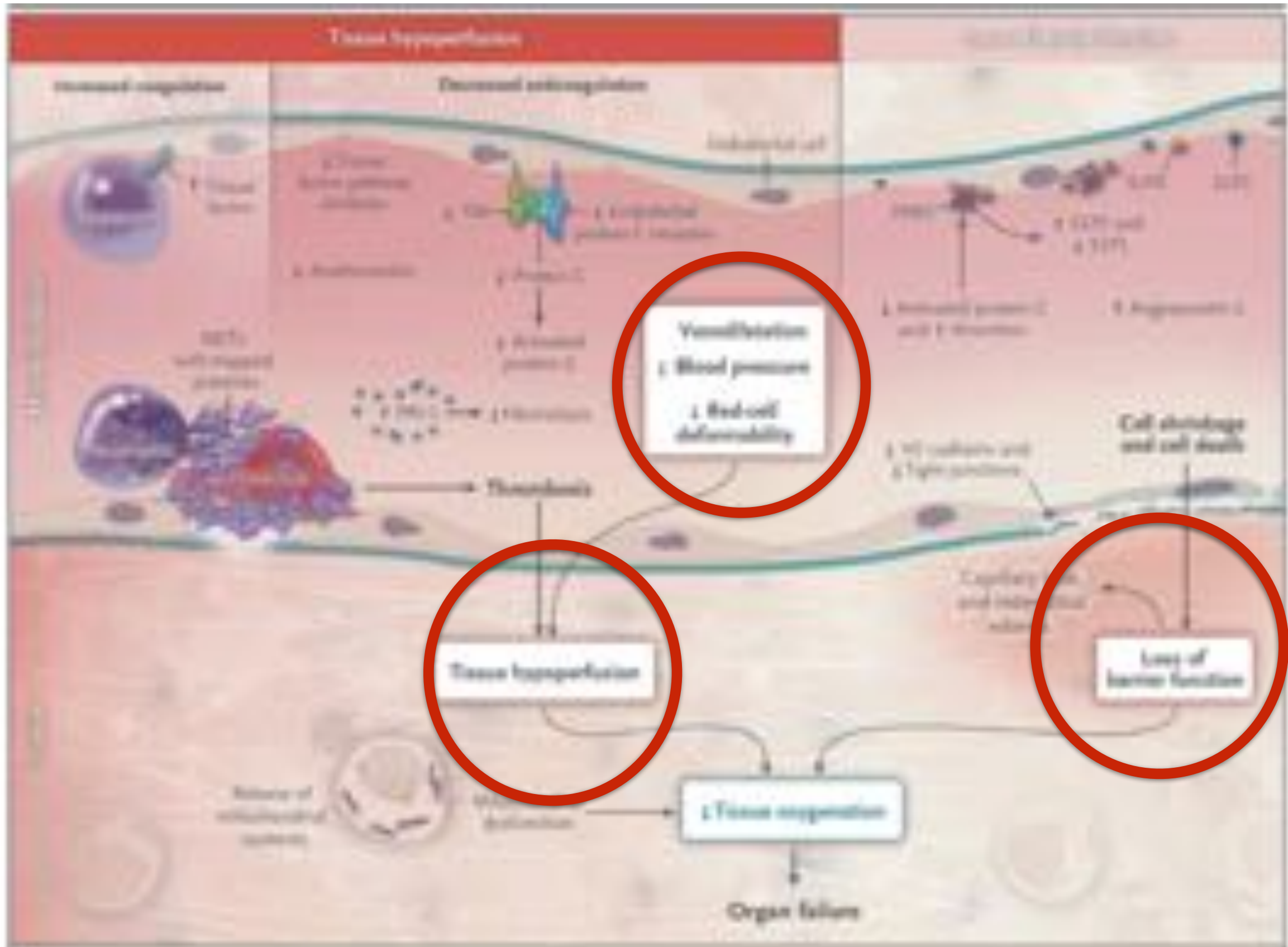
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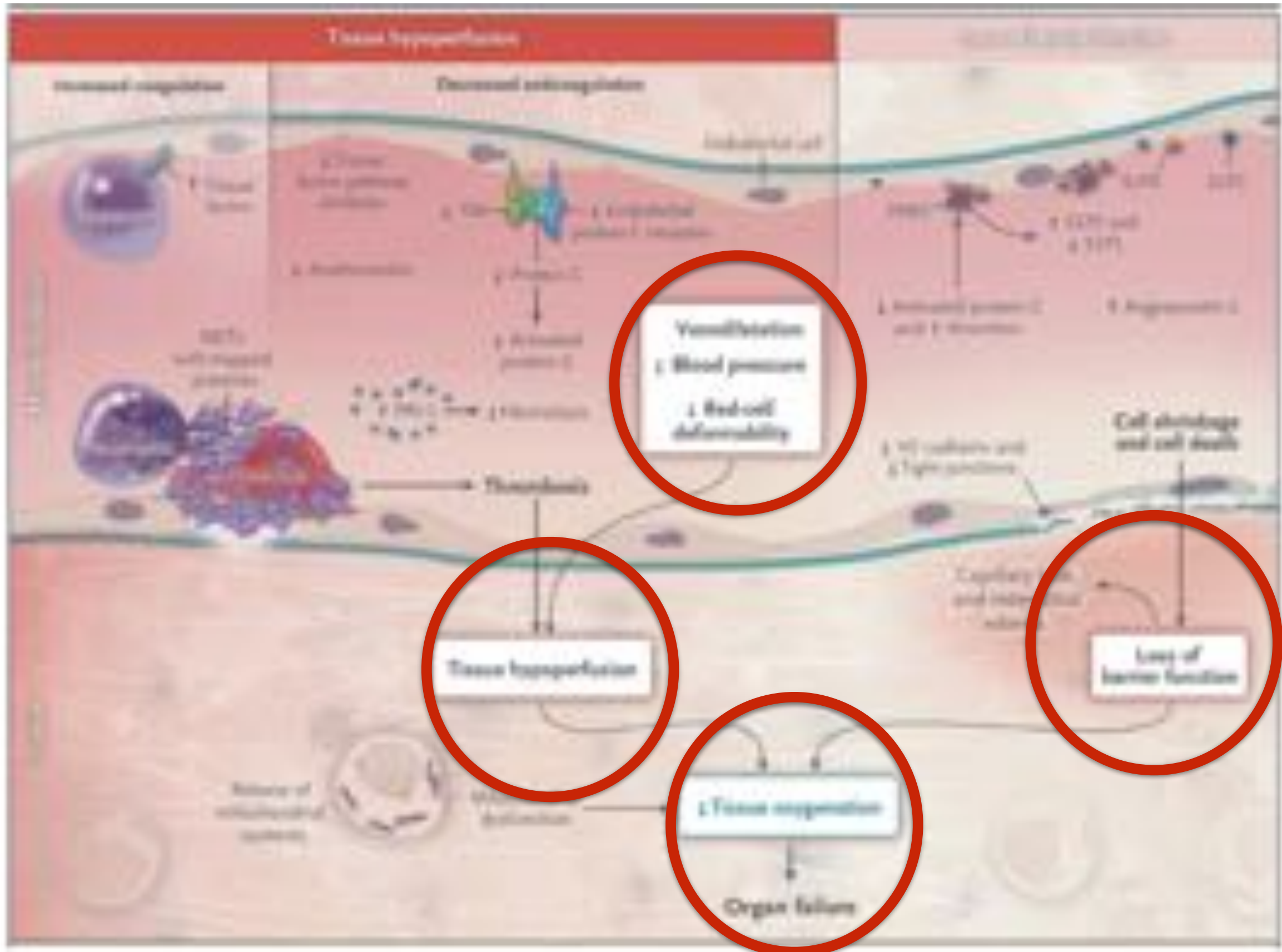
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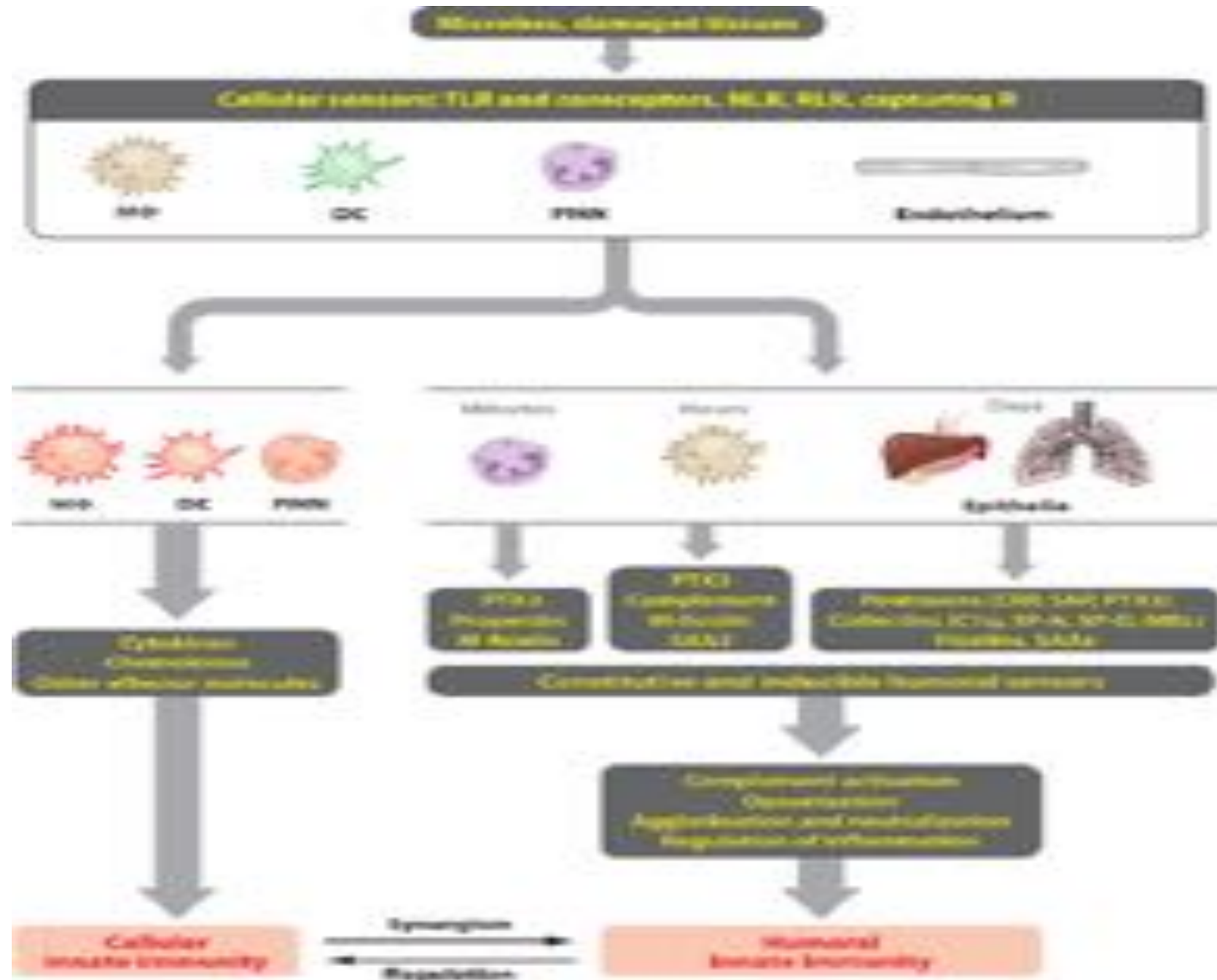
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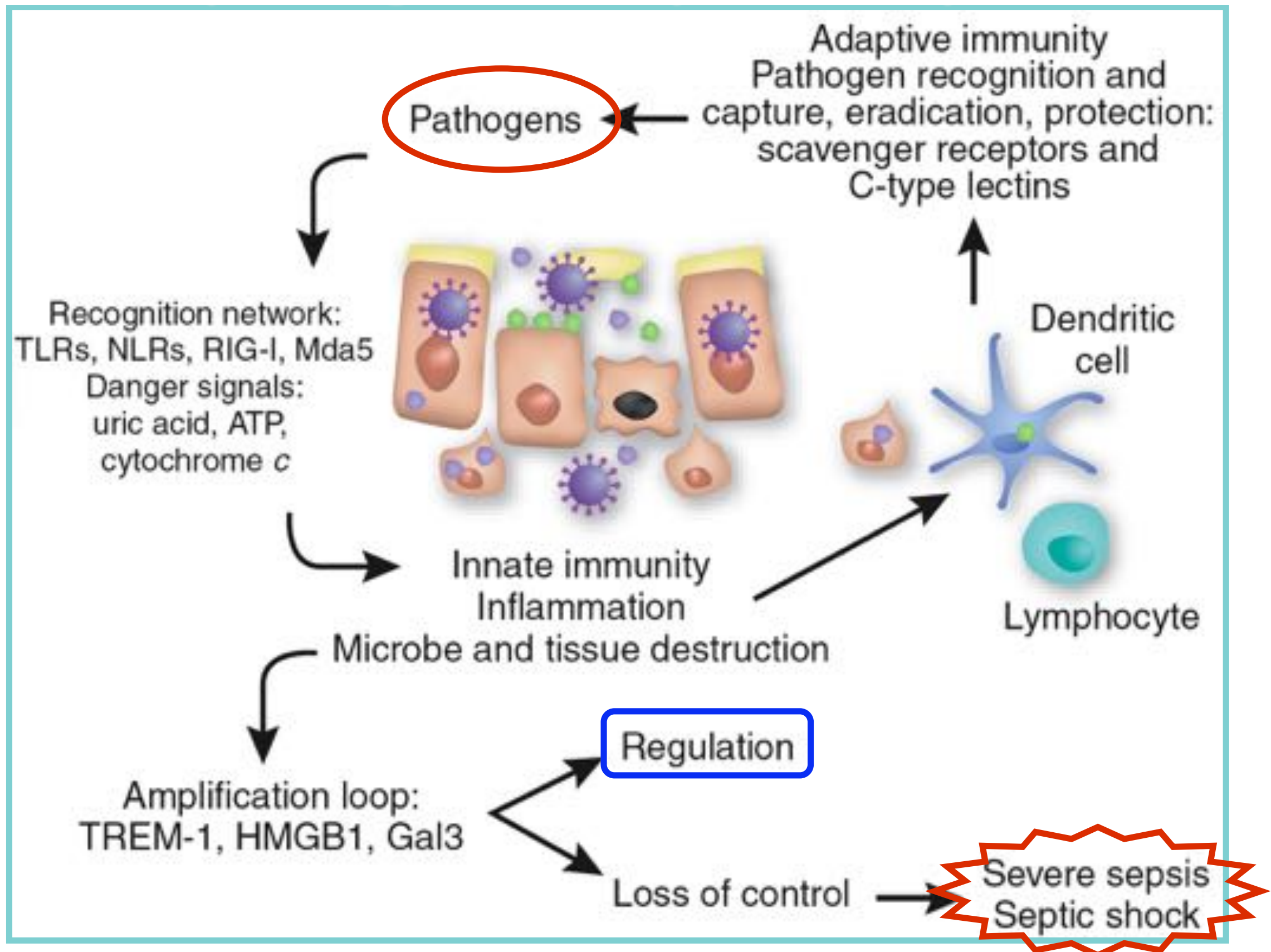


SEQUENCE OF RECRUITMENT OF DAMAGE SOLUBLE MEMBRANE AND CYTOPLASMIC RECEPTORS.....



Humoral and Cellular sensors share fundamental mechanisms of effector function: complement activation and regulation, opsonization, agglutination, virus neutralization, and regulation of inflammation!!!!

.....OF NATURAL IMMUNITY AND INFLAMMATION UNDER NORMAL CONDITIONS AND SEPSIS!



The humoral and cellular arms of innate immunity form an integrated system with synergism in deciphering pathological patterns and regulating the innate and inflammatory response!