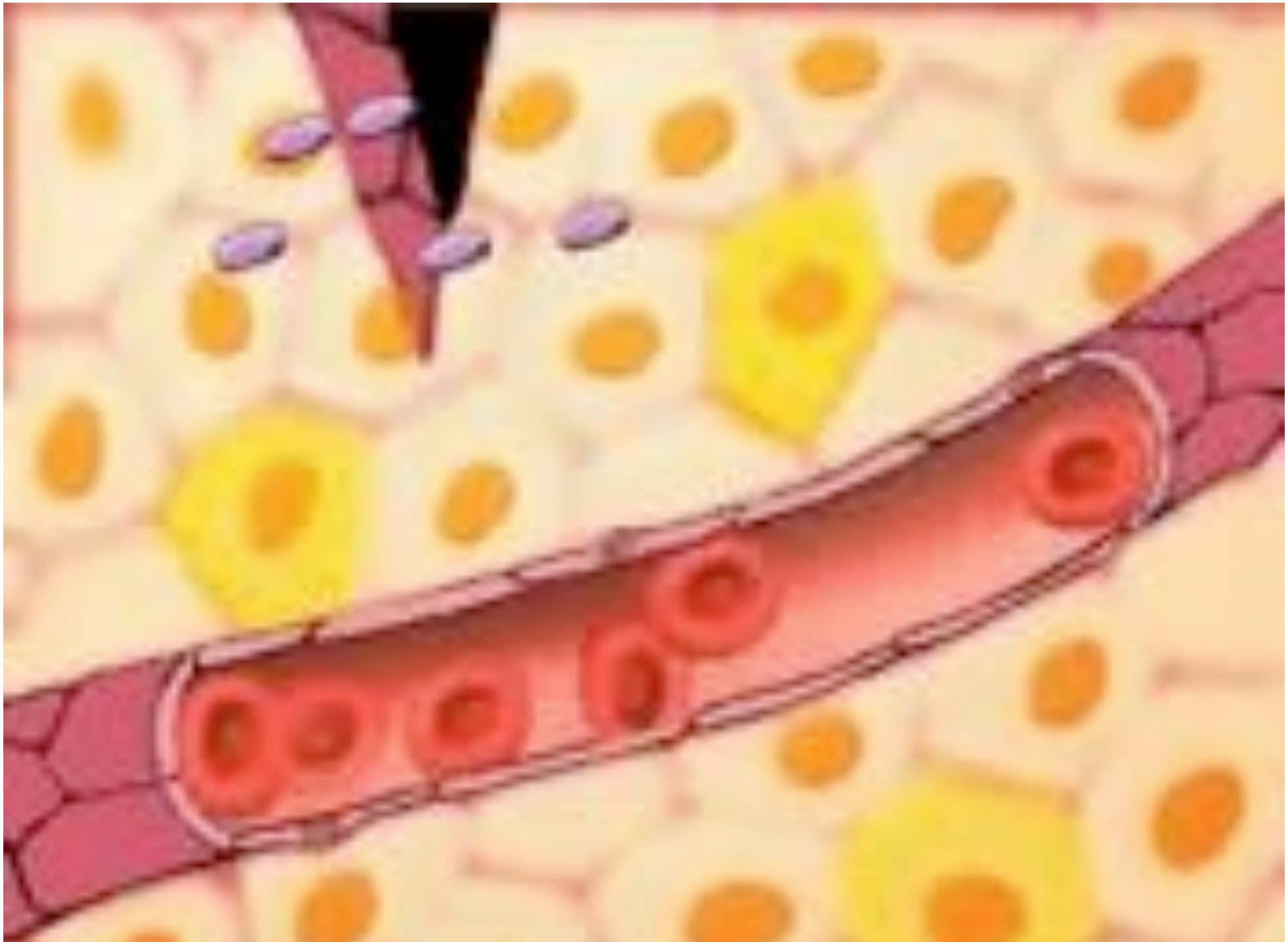
INFLAMMATION RESPONSE!



THE INNATE IMMUNITY AND
NFLAMMATION RESPONSE BIOLOGY
THE ACUTE PHASE PROTEINS (APP)

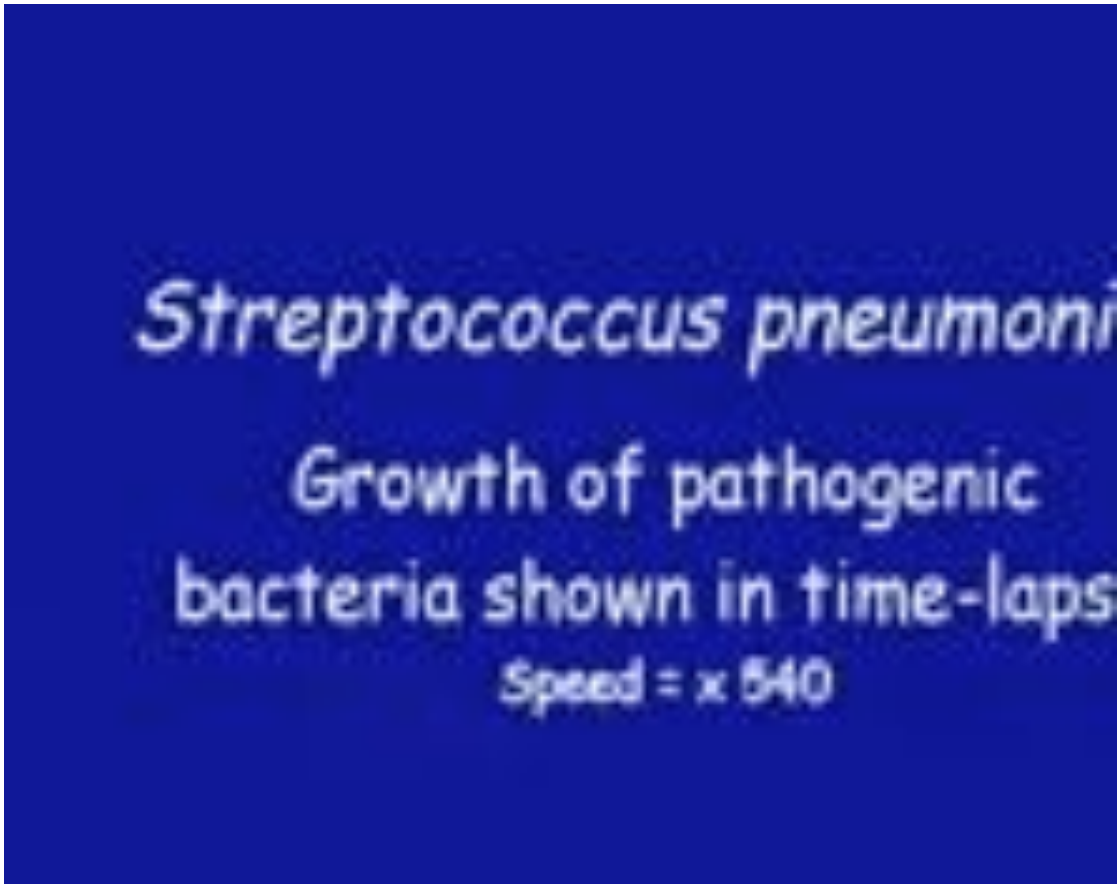
Prof. Fabrizio Mainiero

Professor of General Pathology and Physiopathology and Immunolog
Immunopathology

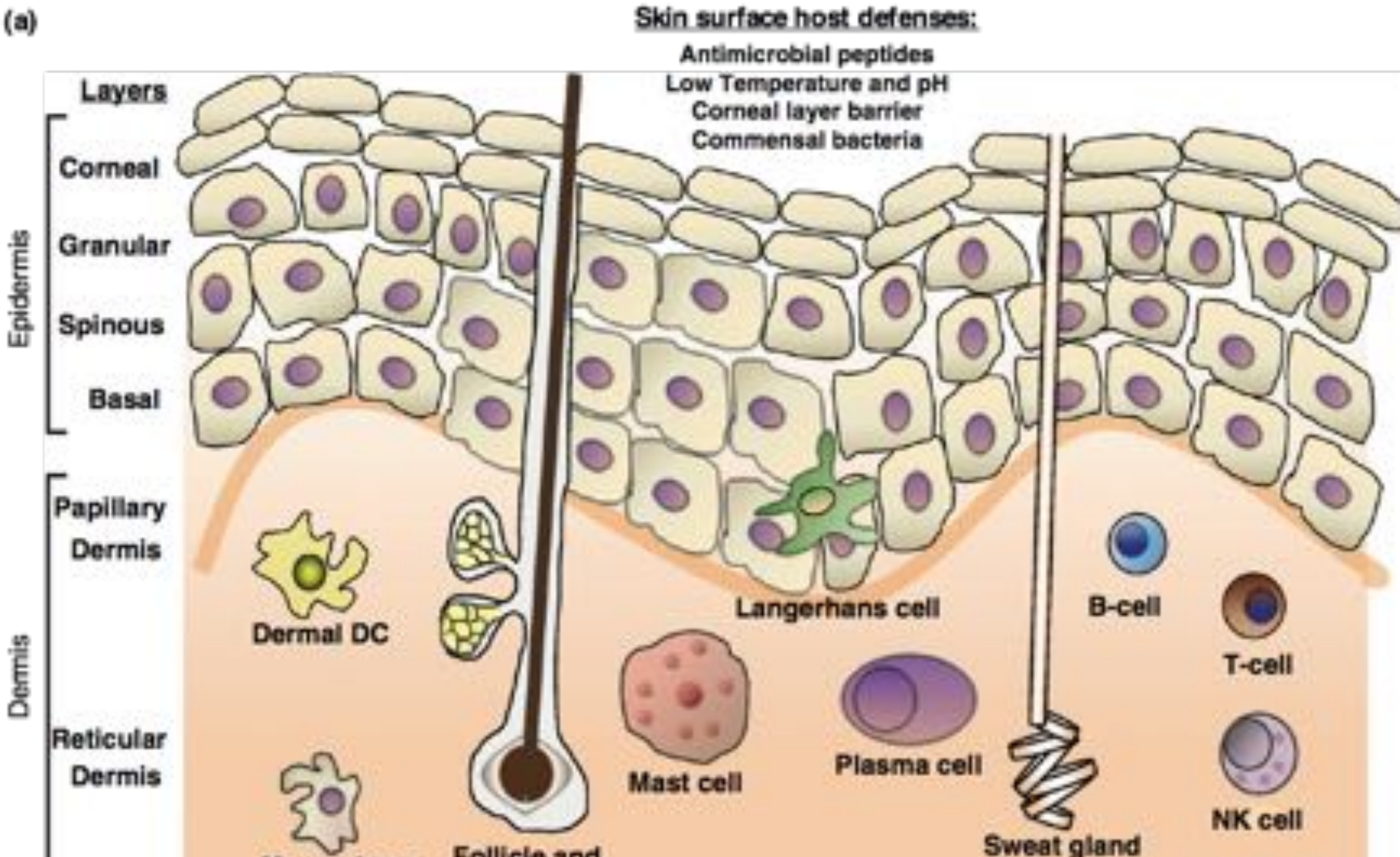
artment of Experimental Medicine
ersità degli Studi "La Sapienza"
e Regina Elena 324
61 Roma

izio.mainiero@uniroma1.it

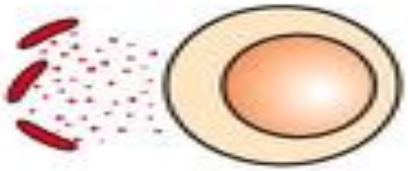
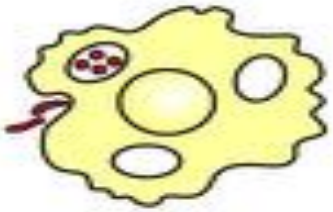
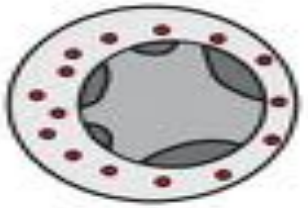
esponse are microbial, such as viruses and bacteria
which are the major
extracellular DAMPs or Danger-Associated Molecular
Patterns
and contain
AMPS or Pathogen Associated Molecular Patterns...



.upon the escape from the potent immunologic tissue barriers!



DAMAGE WITH THREE MAIN DIRECT MECHANISMS.....

	Direct mechanisms of tissue damage by pathogens		
	Exotoxin production	Endotoxin	Direct cytopathic effect
Pathogenic mechanism			
Infectious agent	*Streptococcus pyogenes* *Staphylococcus aureus* *Corynebacterium diphtheriae* *Clostridiumtetani* *Vibrio cholerae*	*Escherichiacoli* *Haemophilus influenzae* *Salmonella typhi* *Shigella* *Pseudomonas aeruginosa* *Yersinia pestis*	Variola Varicella-zoster Hepatitis B virus Polio virus Measles virus Influenza virus Herpes simplex virus Human herpes virus 8 (HHV8)
Disease	Tonsilitis, scarlet fever Boils, toxic shock syndrome, food poisoning Diphtheria Tetanus	Gram-negative sepsis Meningitis, pneumonia Typhoid Bacillary dysentery Wound infection	Smallpox Chickenpox, shingles Hepatitis Poliomyelitis Measles, subacute sclerosing

HEY CAN ENTER OUR CELLS, USING N ONLY SPECIFIC RECEPTORS ....

Trends in Biotechnology June 2012, V

athogenic microbes and their membrane receptor targets

	Species	Virulence factor	Cell receptor[a]
	E. coli	Heat-labile enterotoxin, endotoxin	Ganglioside
	V. cholera	Cholera toxin	Ganglioside
	Streptococcus, Staphylococcus	Lipoteichoic acid, hemolysin	Phospholipid
virus	Influenza	Hemagglutinin, neuraminidase	Ganglioside
	HIV	GP120 protein	Galactosyl ceramide
	Paramyxovirus	Attachment protein G	EphrinB2 protein
oped virus	Polyomavirus, rhinovirus	Capsid coat protein	Ganglioside, ceramide, ICAM-1 and LDLR proteins
	Adenovirus	Capsid protein knob domain	CAR and LDLR proteins

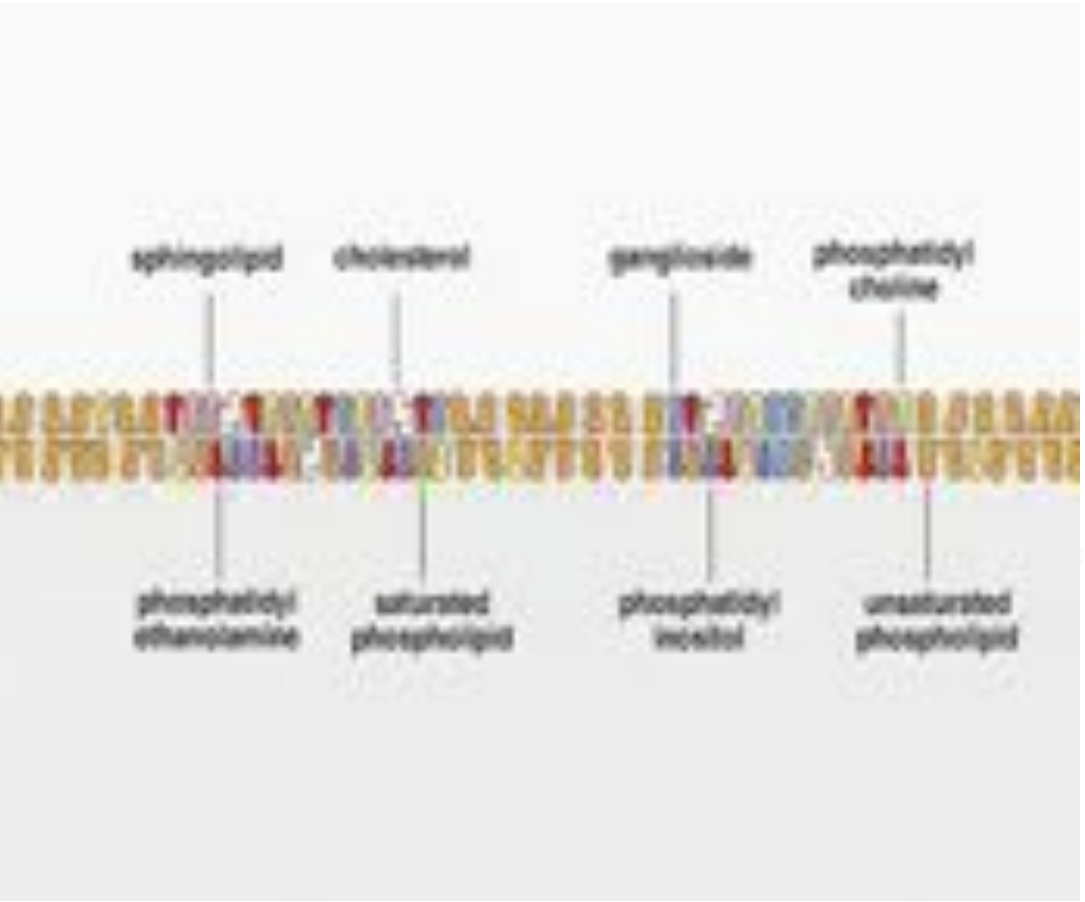
ns: ICAM-1, intercellular adhesion molecule 1; CAR, coxsackie virus and adenovirus receptor; LDLR, low-density lipoprotein receptor.

ting host–pathogen interactions membrane-based nanostructures

icarello[1,4], Mira A. Patel[2] and Atul N. Parikh[2,3,4,5]

Santos Mañes, Gustavo del Real & Carlos Martínez-A

Nature Reviews Immunology **3**, 557-568 (2003)

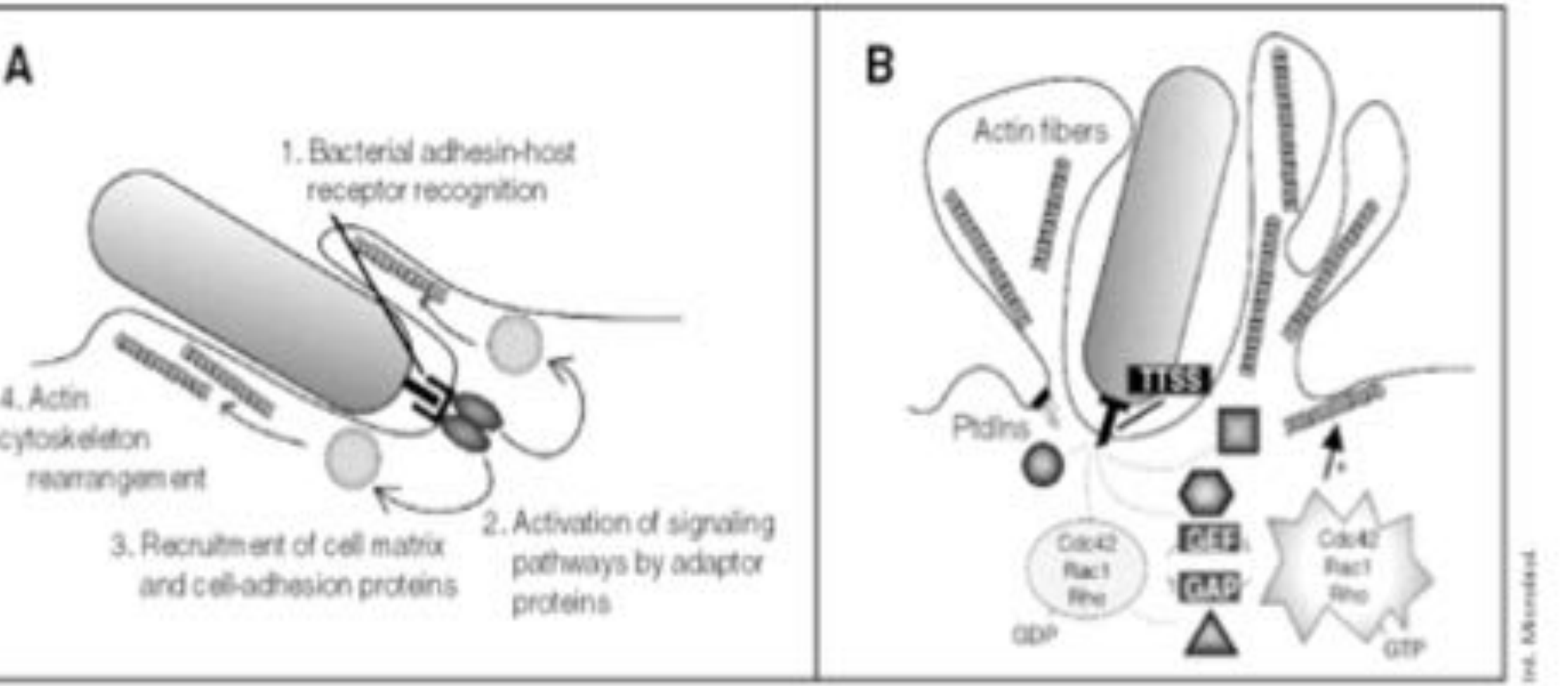


I rafts lipidici sono de lle strutture di mem
eterogenee, insolubili in detergenti non ionici c
Triton X-100 ed arricchite in colesterolo, glicosfin
come GM1 o GM3 e proteine come le caveolin
flotillin e

iruses	
mian virus 40	Entry/trafficking
chovirus 11	Entry/trafficking
chovirus 1	Entry
vian sarcoma and leukosis virus	Entry
emliki-forest virus	Entry/budding
cotropic mouse leukaemia virus	Entry/budding
uman T-cell leukaemia virus type 1	Entry/budding
V-1	Entry/budding/transcytosis
bola and Marburg viruses	Entry/budding

Bacteria	
Campylobacter jejuni	Intracellular survival
Legionella pneumophila	Intracellular survival
Pseudomonas aeruginosa	Host response, signalling
Brucella spp.	Entry/intracellular survival
FimH and Dr+ *Escherichia coli*	Entry/intracellular survival
Salmonella typhimurium	Entry/intracellular survival
Shigella flexneri	Entry/intracellular survival
Chlamydia spp.	Entry/intracellular survival
Mycobacterium spp.	Entry/intracellular survival
Vibrio cholerae (cytolysin)	Toxin binding/oligomeriza
Aeromonas hydrophila (aerolysin)	Toxin binding/oligomeriza
Clostridium spp.	Toxin binding/oligomeriza
Streptococcus pyogenes (streptolysin O)	Toxin oligomerization
Bacillus anthracis (anthrax toxin)	Toxin oligomerization
Bacillus thuringiensis (Cry1A toxin)	Toxin binding/oligomeriza

ZIPPER MECHANISM TRIGGER MECHANISM



bacteria to enter the cells, move and pe the cytoplasm and modulate the ions using proteins that mimic the ons of the structural and signaling ns (such as small G proteins Rho, Rac dc42) and their effectors (such as Wasp,

Microbial pathogenesis cytoskeletal functio

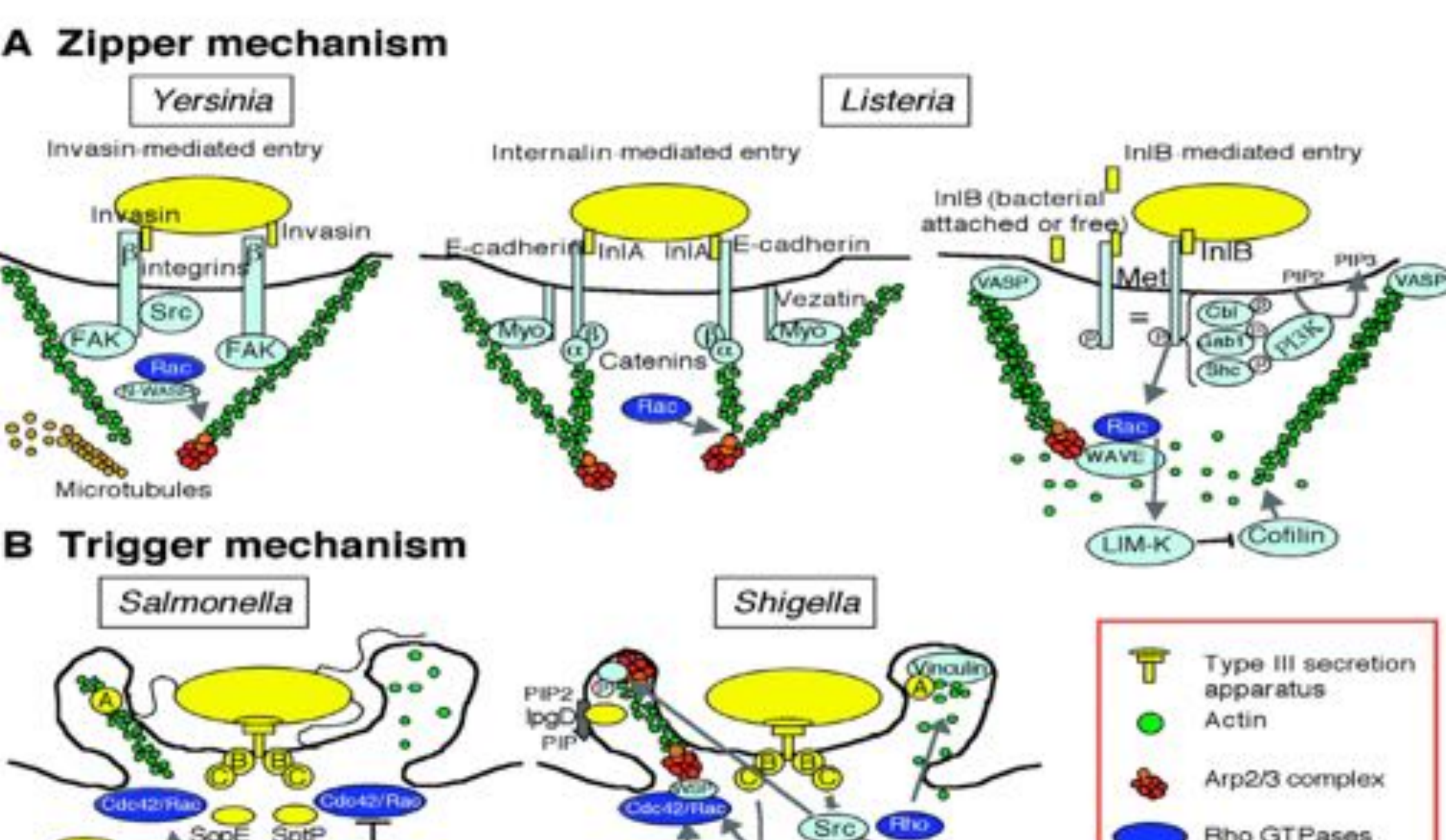
Samantha Gruenheid a Brett Finlay

Nature 422, 775-781 (17 2003)

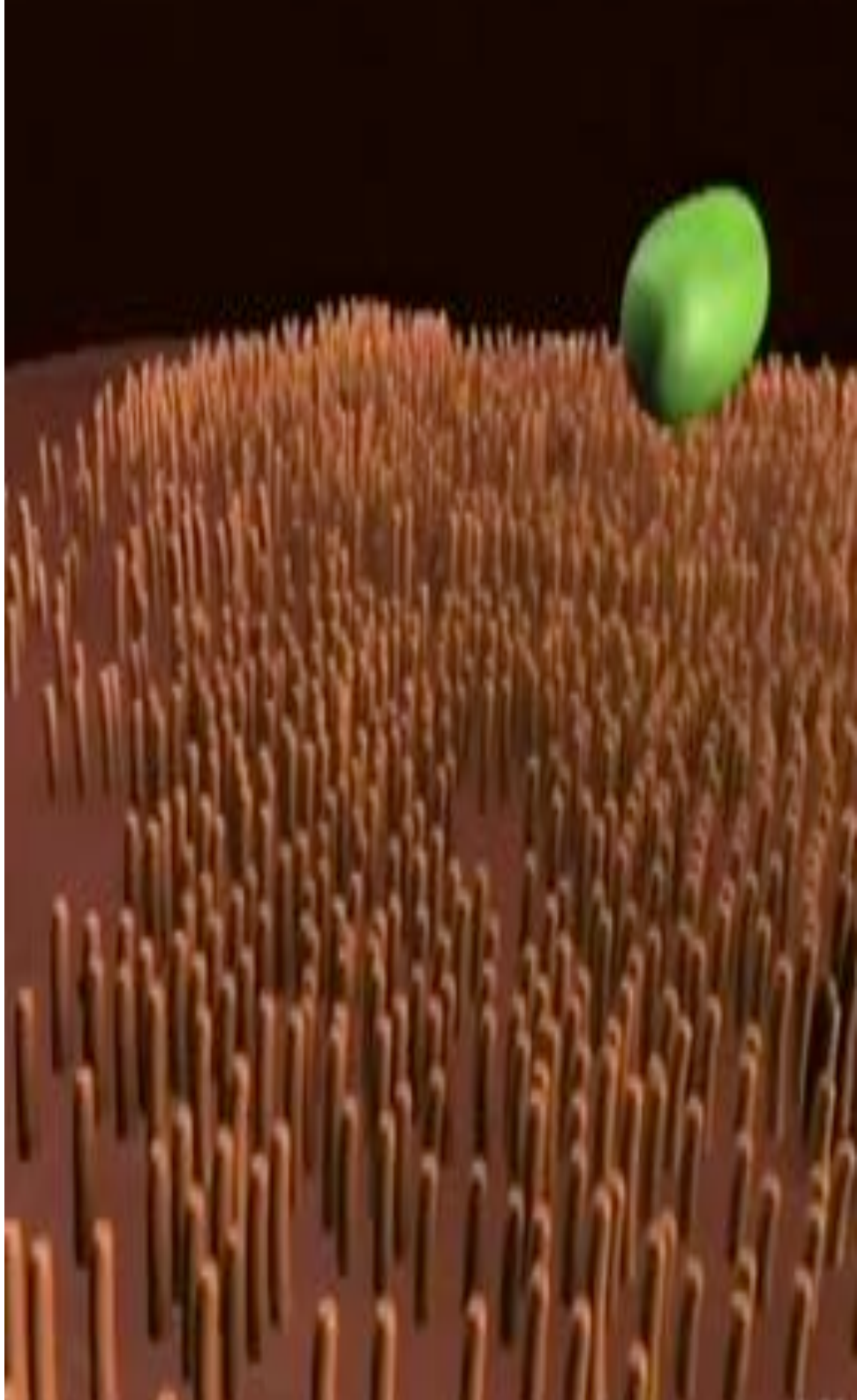
ale Cossart and Philippe J. Sansonetti

chanisms used by bacteria to enter cells.

he zipper mechanism used by Yersinia and Listeria.
he trigger mechanism used by Salmonella and Shigella.

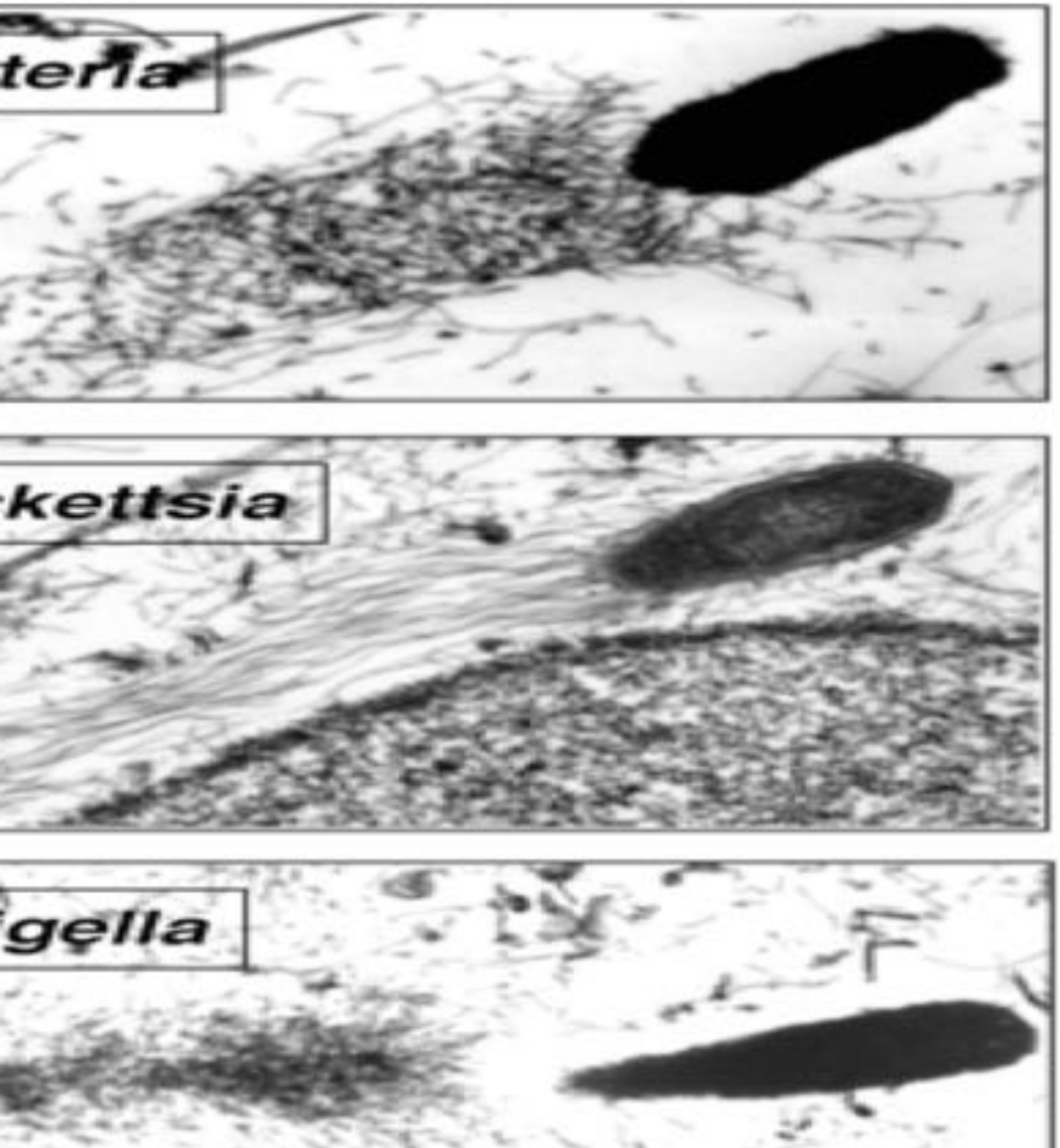


The invasion and cell migration of Salmonella!

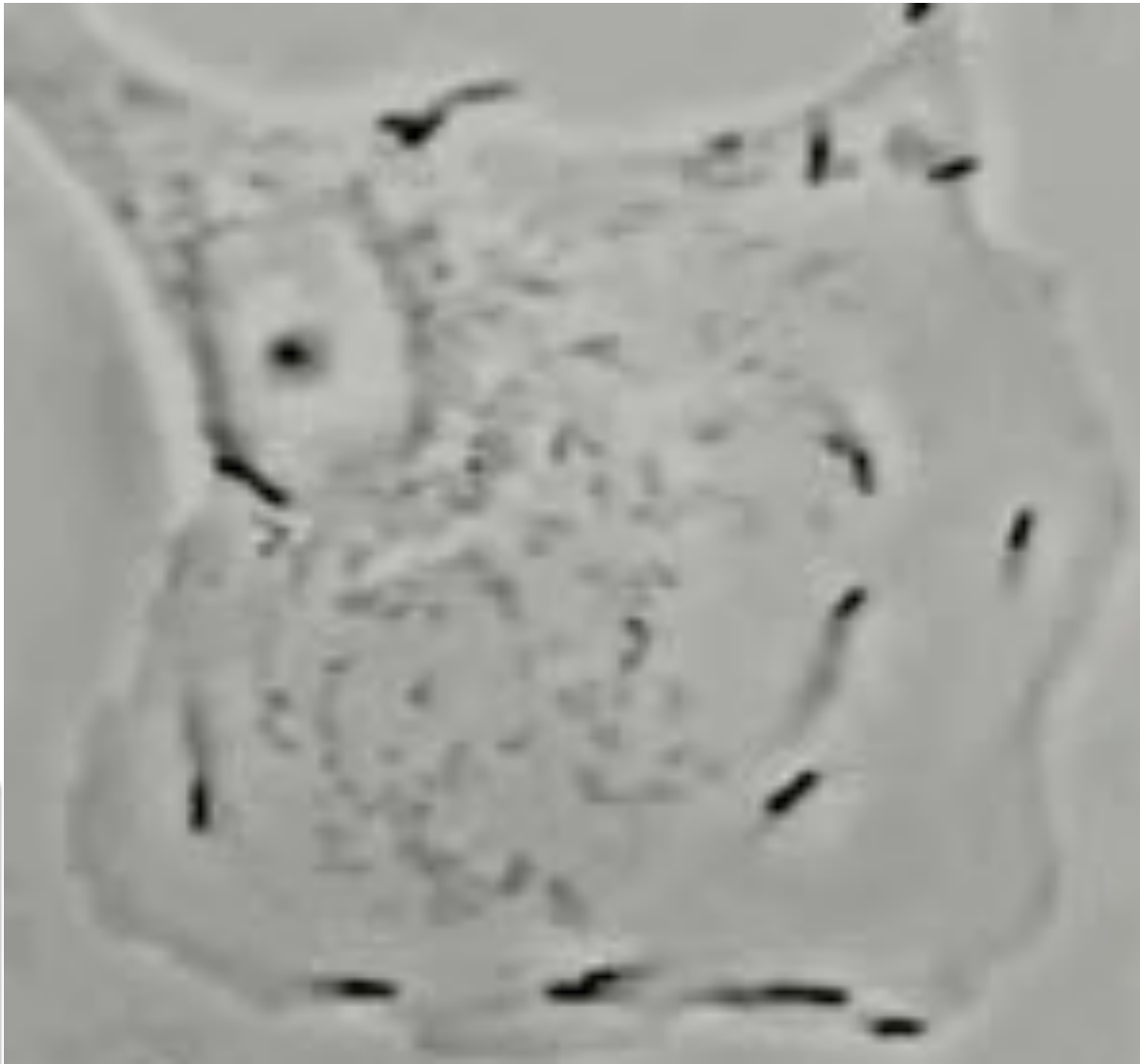


-based motility of Listeria,
ickettsia, and Shigella.

ron micrographs of actin tails
ed with fragment S1 of myosin!



The invasion and ce
migration of LISTER



Many endogenous inducers activate or are "allarmins" or DAMP, danger-associated molecular patterns!

rrently known alarmins include **defensins, cathelicidins, eosinophil-deriv**

urotoxin, lactoferrin, some high-mobility group (HMG) proteins, granulysin, a

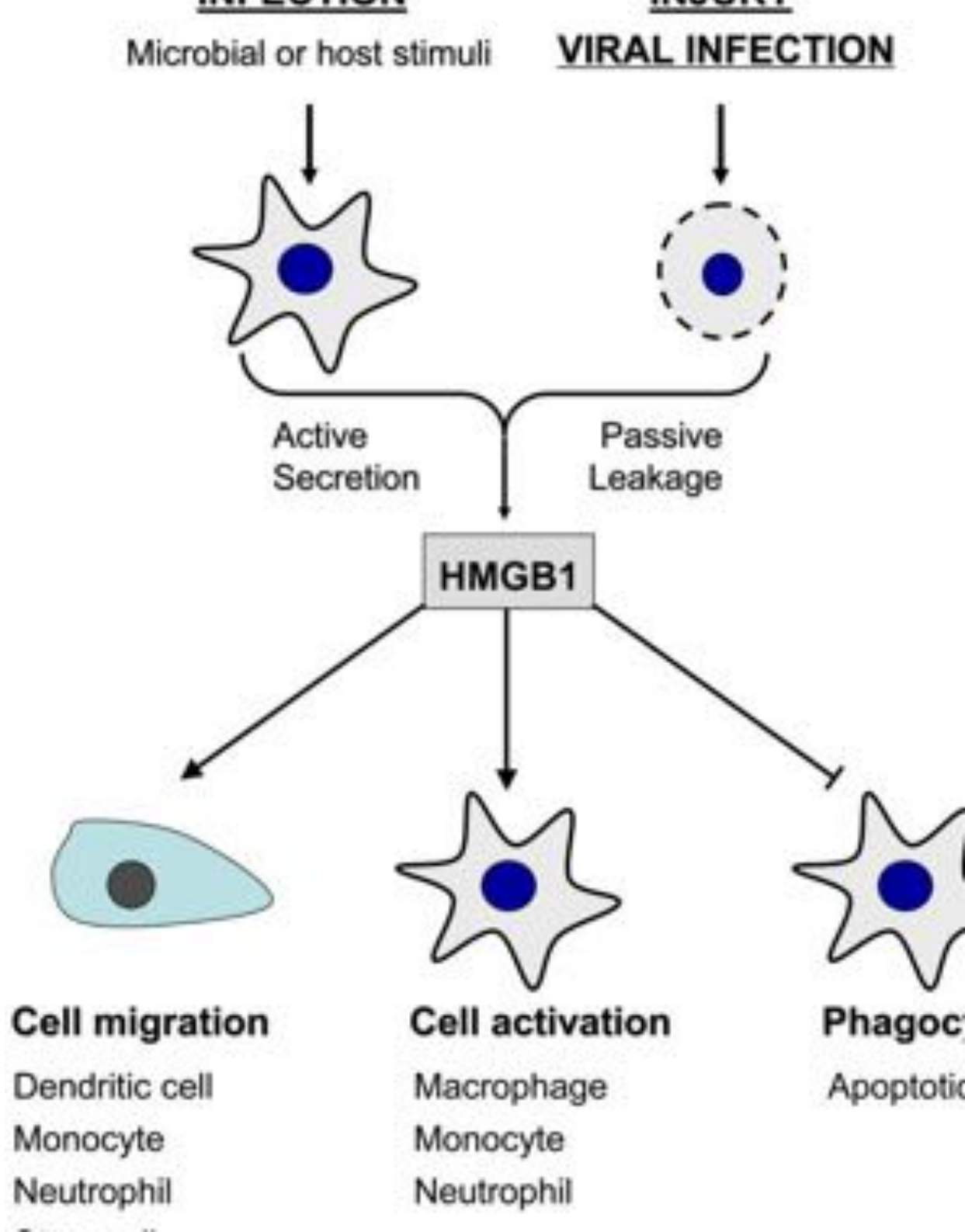
bably also **ATP** and **histamine**, while endogenous mediators that may eventu

ve to be alarmins include some members of the **S100 family proteins, heat-sho**

oteins, and certain degraded products of extracellular matrix (e.g. **hyaluronan a**

paran sulfate).

B1 is actively secreted by immune cells in response to ous microbial products (e.g., r CpG-DNA) or endogenous stimuli (TNF, IFN-γ, or gen peroxide), and passively ed by damaged or virus-ted cells. Extracellular B1 sustains an inflammatory se by stimulating migration ate immune cells, facilitating recognition of bacterial cts, activating various innate ne cells, and suppressing cytosis of apoptotic cells. HMGB1 can function as an n signal to recruit, alert and te various innate immune



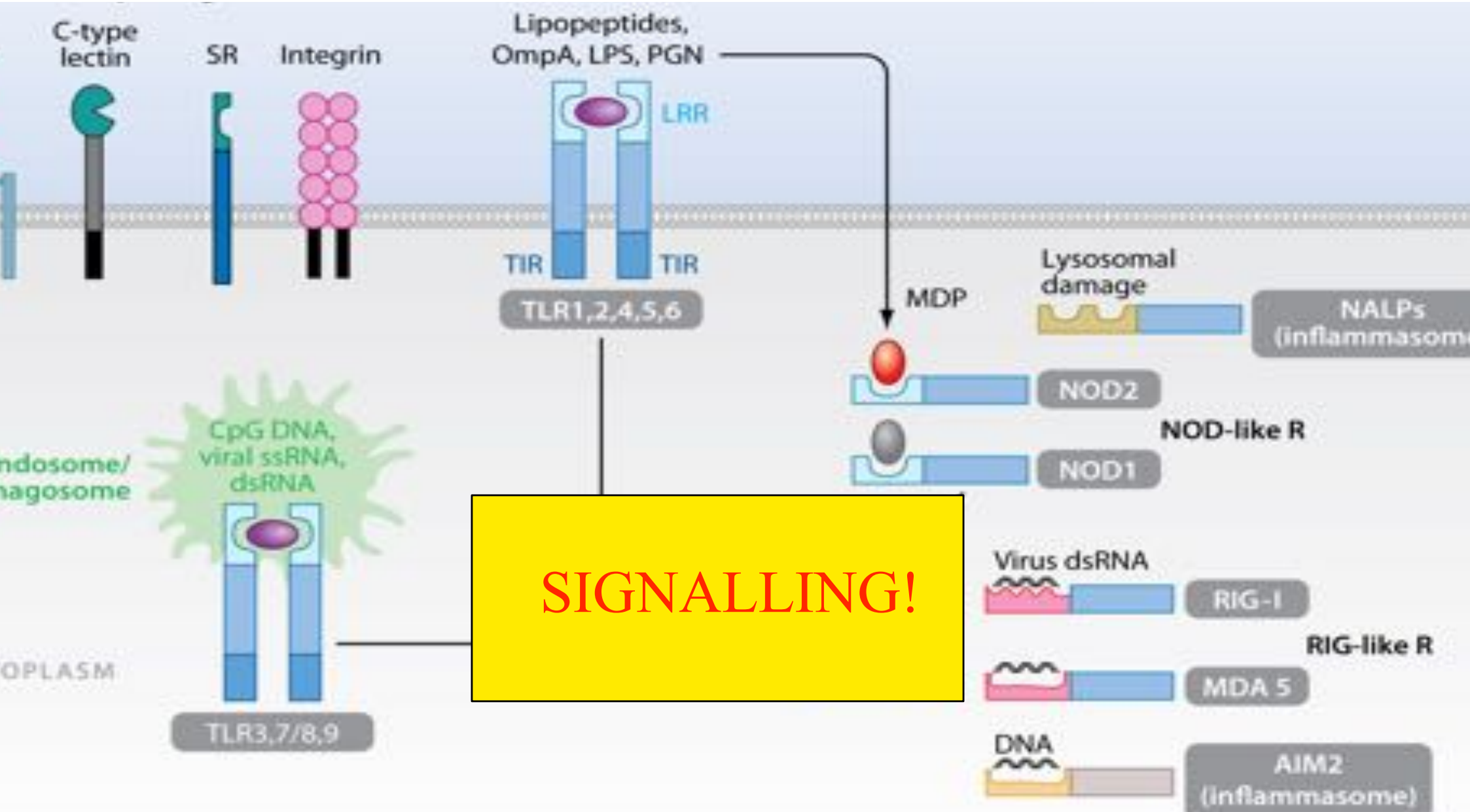
can activate inflammatory and the immune respon through RECEPTORS!

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**

DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION!



INFLAMMATIOIN!

respiratory innate immunity!

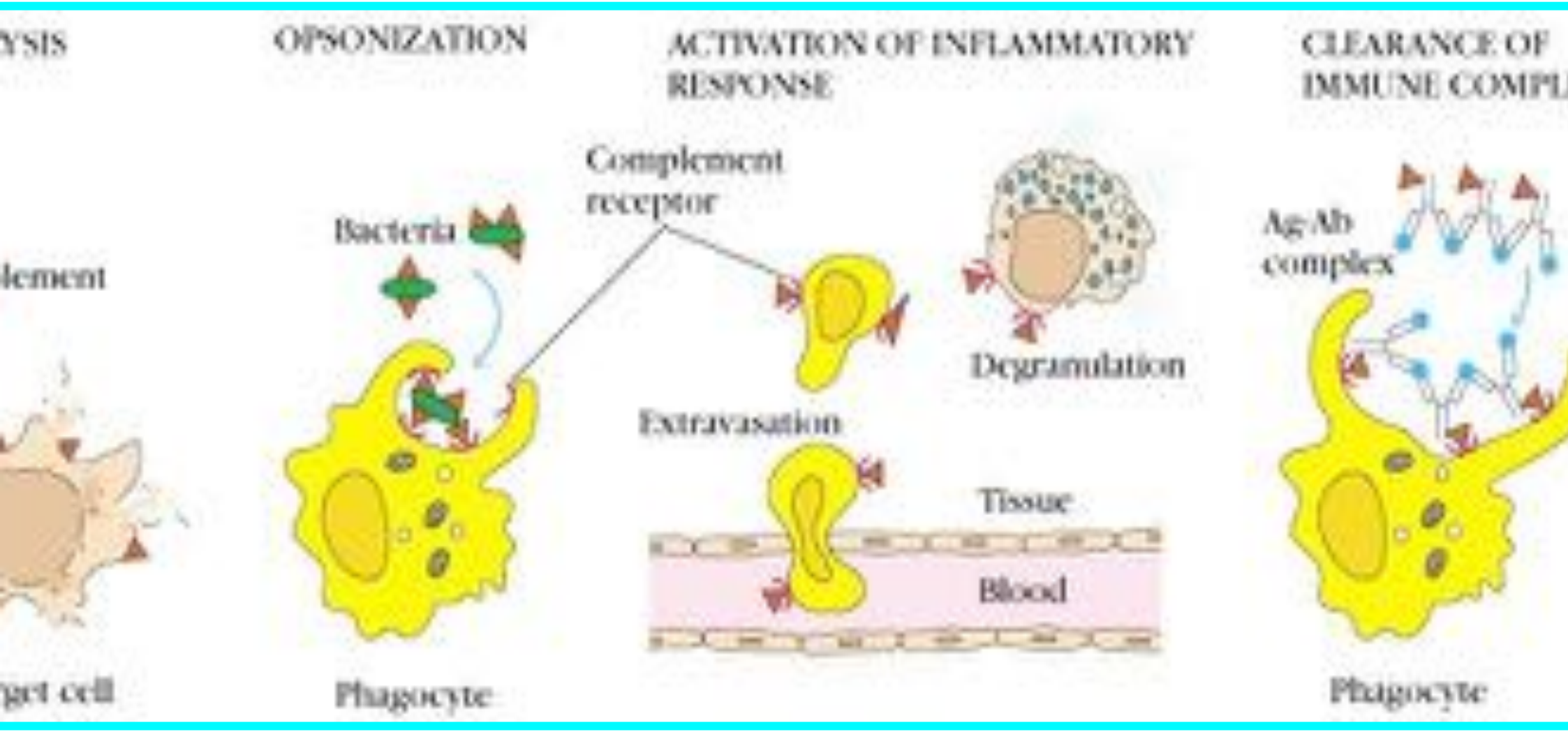
aste receptors (T2Rs) are emerging as novel regulators of innate immunity in the respirato
re expressed in respiratory ciliated cells and in solitary chemosensory cells (SCCs), wh
the T1R2 and T1R3 subunits comprising the human sweet taste receptor. Activation of t

Rs are expressed in ciliated cells a
mosensory cells of the respirato
ct: bitter chemicals released
robes during upper respiratory tr
ections activate T2Rs and indu
thelial secretion of antimicrob
tides, such as β-defensins 1 and 2!

dependent upon Gα-gustducin and IP_3R

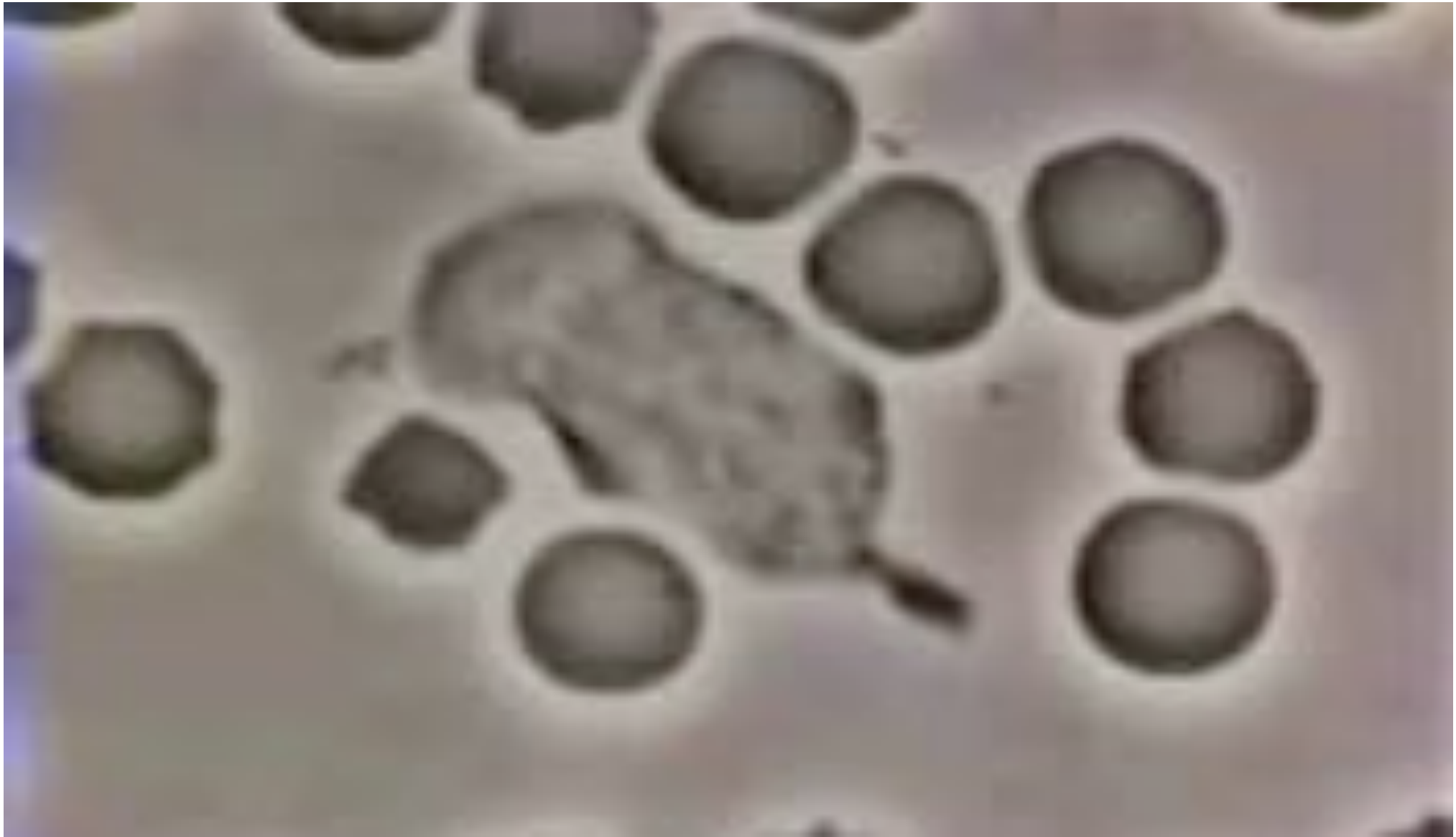
NFLAMMATION ACTIVATE AND REGULATE:

- **THE COMPLEMENT SYSTEM!**



D INFLAMMATION ACTIVATE AND REGULAT

- **THE PHAGOCYTOSIS!**



ID INFLAMMATION ACTIVATE AND REGULAT

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

The acute phase RESPONSE!

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) hematopoietic 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;

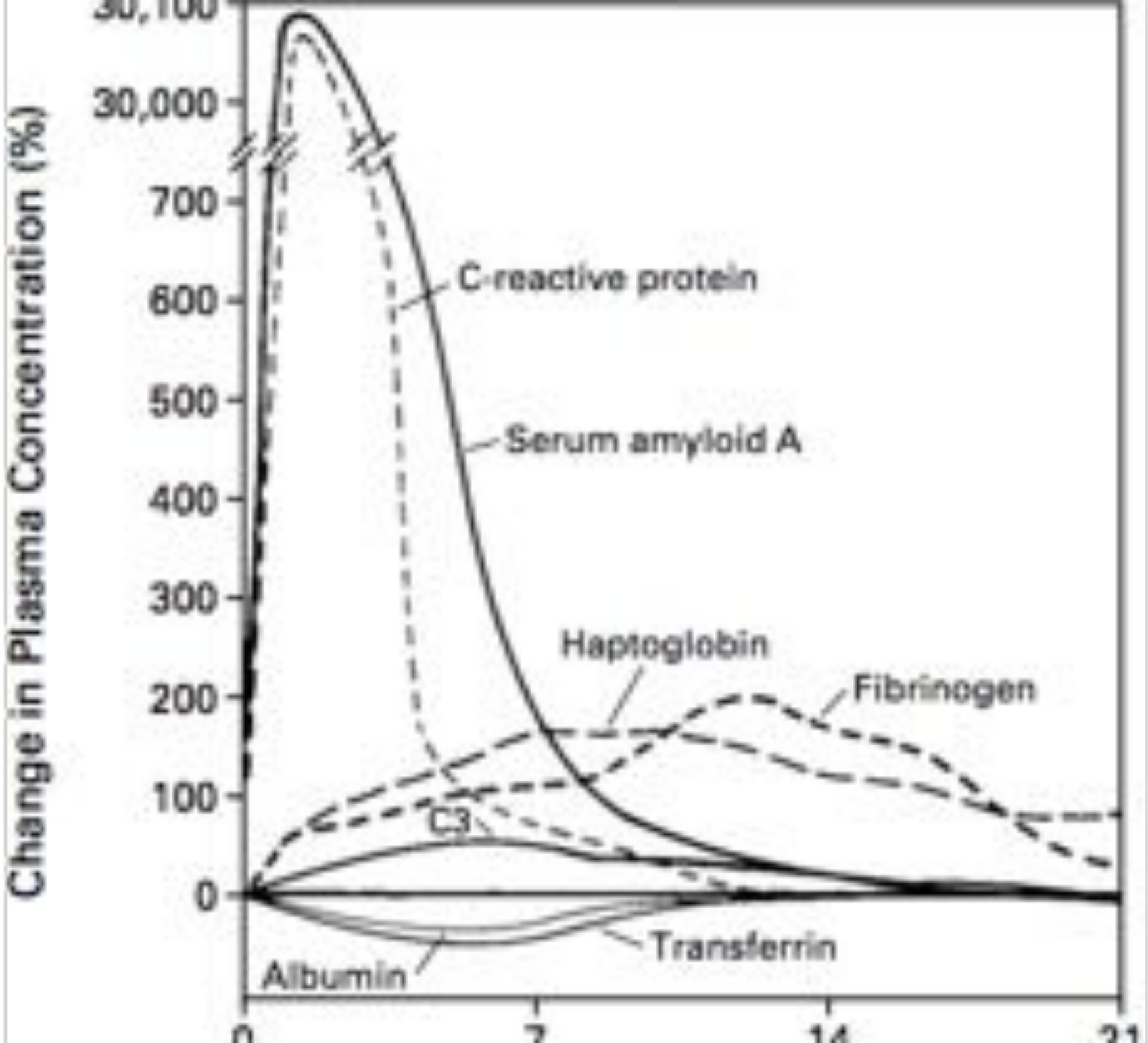
d) plasma modification of some metals such as calcium, iron and zinc, and vitamins, but mostly of some proteins and lipoproteins!

The acute phase proteins or APP!

e study of APP was born with the identification
e first APP, the C-reactive protein, or CRP, with
vent resurgence of research activities from the
til today. The discovery of PCR was made by Till
d Francis in 1930 with the publication of a pa
titled "Serological Reactions In Pneumonia With
matic Nonprotein Fraction Of Pneumococcus" in
rnal of Experimental Medicine:

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

er was observed, in addition to the CPR, t
allel increase in concentration of other plasm
teins including the Amyloid Protein A or SAA, t
rinogen, the C3 complement component, the α
itrypsin and more proteins!


Change in Plasma Concentration (%)
30,100
30,000
700
600
500
400
300
200
100
0
C-reactive protein
Serum amyloid A
Haptoglobin
Fibrinogen
C3
Transferrin
Albumin
0
7
14
21

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!

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)

positive and negative APP!!!

Positive APP:

Short pentraxins;
Collectins;
Proteins of the complement system;
LPS binding protein or LBP;
Proteins of the coagulation system and fibrinolysis;
Antiproteases;

Negative APP:

a) Albumin;
b) Transferrin;
c) Transthyretin;
d) Alpha-fetoprotein;
e) Thyroxin-binding globuli
f) Factor XII.

Negative APP:

bumin;
ansferrin;
ansthyretin;
pha-fetoprotein;
yroxin-binding globulin;
ctor XII.

The negative APP do
diminish just because
hyper-production of pos
APP which are limiting a
acid reserve or beca
escaping from the vesse
the inflammatory exudate
also for **anorexia**
increased energy and **pr**
catabolism that arise in
course of inflammation.
Among the negative APP

nin is a single polypeptide which consists of 585 amino acids with a mole
t of about 69 kDa. **The total pool of albumin is 4-5 g/kg of body weig**
n 40-45% is in the intravascular space and the other 60 % is in the inter
.

iological functions of albumin are impressive:
ins 75-80 % of the plasma colloid osmotic pressure;
ls and transports not only free fatty acids, calcium, certain steroid horm
xine, bilirubin, copper and tryptophan, but also drugs, such as penicilli
non-steroidal antiinflammatory drugs (NSAIDs);
important source of sulfhydryl groups, which remove nitrogen and oxyge
als and other toxins; in this context, the antithrombotic and anticoag
ts of albumin may be due to the uptake of the free radical nitric oxide (NO)

oncentration of albumin in the blood (serum albumin) varies between 3.
/dl and its decrease during inflammation can be significant even if it is l
pecific, as the hypoalbuminemia may occur in various physiopathol
ions, such as **rheumatoid arthritis, cholecystitis acute ulcerative c**

nsferrin is the major β-globulin that transports iron (siderofilli
e transferrin contains 687 amino acids and has a calculat
lecular weight of approximately 79 kDa. The transcription of t
NA for the synthesis of transferrin in the liver is regulated by t
ncentration of iron is that hepatic plasma.

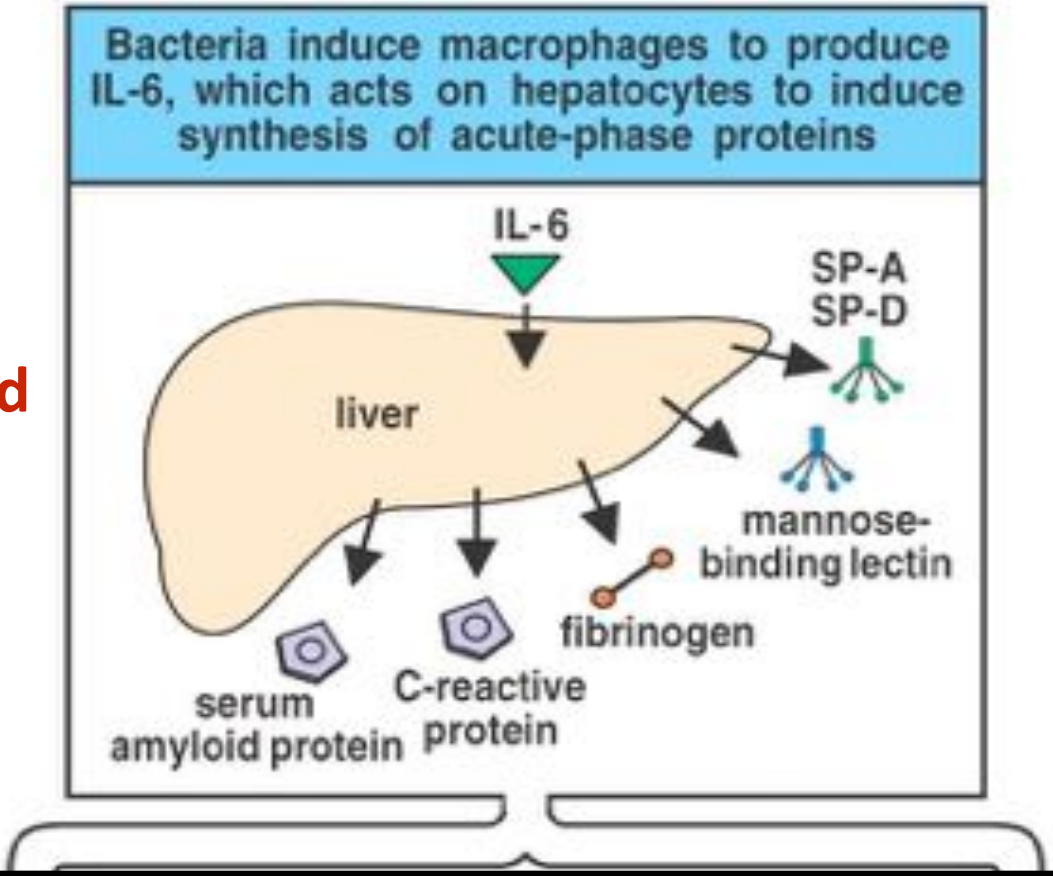
e transferrin levels in the blood are 200-360 mg/dL.

e transferrin levels increase during the use of birth control pi
ring pregnancy and in cases of insufficient levels of iron and **a**
creased by acute and chronic inflammatory diseases, b
pecially for malnutrition, treatment with iron or with steroids, liv
ease and nephrotic syndrome.

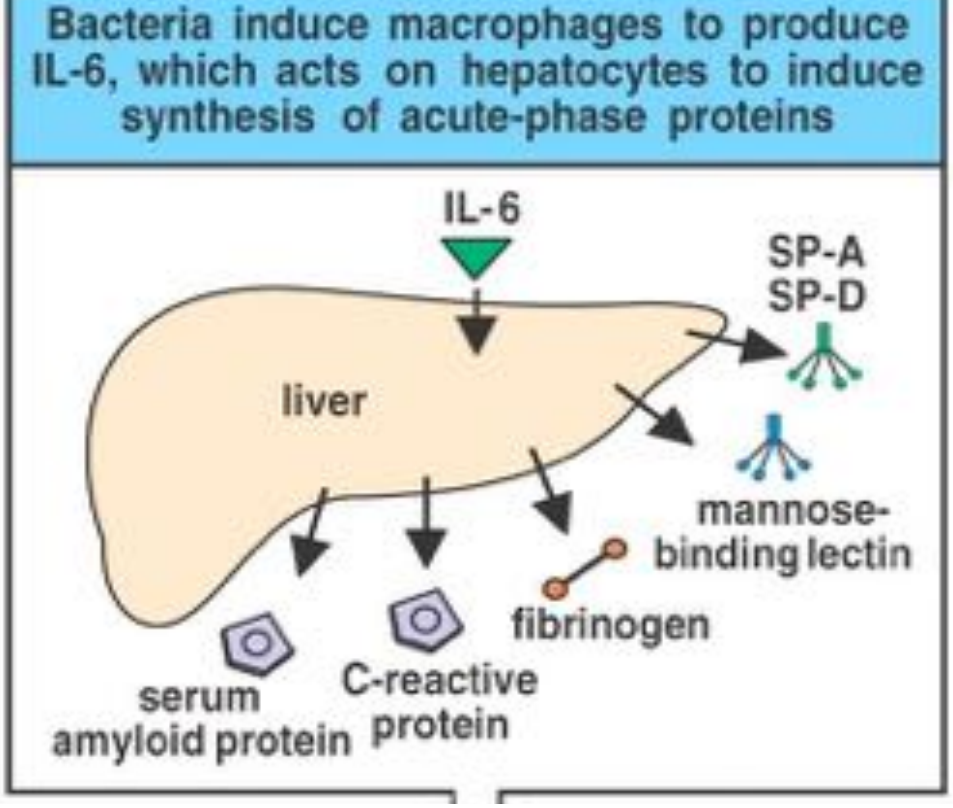
ctrophoretic variants of transferrin in serum are found occasiona

Induction from the LIVER:

ort pentraxins;
llectins;
oteins of the complement system;
S binding protein or LBP;
oteins of the coagulation system and
rinolysis;
tiproteases;
ansport proteins.



nmatory cytokines bind to specific receptors on hepatocytes and induce the activ
nus family kinases (JAK), the **STAT (signal transducers and Activators of transcript**
flammatory transcription factors such as **NF-kB.** Transcription factors most chara
regulate the synthesis of APP belong to the family of leucine zipper, C/EBPA
er of protein binding), C/EBPd and NF-IL6 (nuclear factor associated with IL-6), th
cription by binding to a site called **bZIP1**. **It is also been shown that the induction**
e proteins is correlated with a decreased synthesis of C/EBPA and an increase of



Recently, it has been proposed a new classification of APP positiv according to the cytokines that induce them from the LIVER!

APP positive were therefore divided into two classes:

PP Type-1 induced by IL-1α and β, TNF-α and β, whose prototype is AA;

PP-type 2, induced by IL-6, but also by IL-11, Leukemia inhibitory f

ort pentraxins such as CRP and SAA or amyloid protein A, also indicat
otein serum amyloid or SAP;

llectins, structurally related to C1q, such as mannose-binding lectin o
BL, proteins A and D of the pulmonary surfactant or SPA and SPD and
olins, which include L-, M- and H-ficolins;

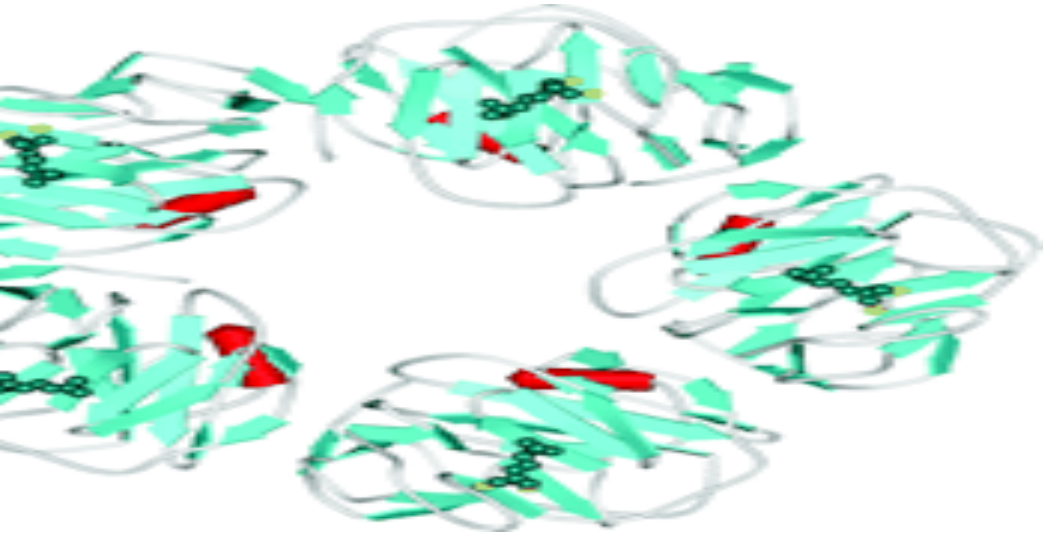
oteins of the complement system, such as C3, C4, C9, Factor B, the
nibitor of C1q, C4b-binding protein;

S binding protein or LBP;

oteins of the coagulation system and fibrinolysis, such as fibrinogen,
asminogen, tissue plasminogen activator, Protein S, vitronectin;

tiproteases, such as α1-antitrypsin (AAT) and the α1 anti-chymotryps

- **SHORT PENTRAXINS:**



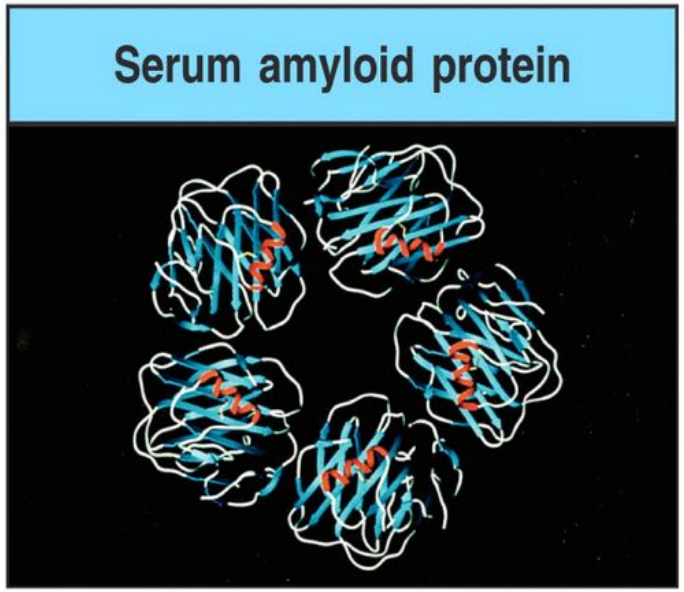
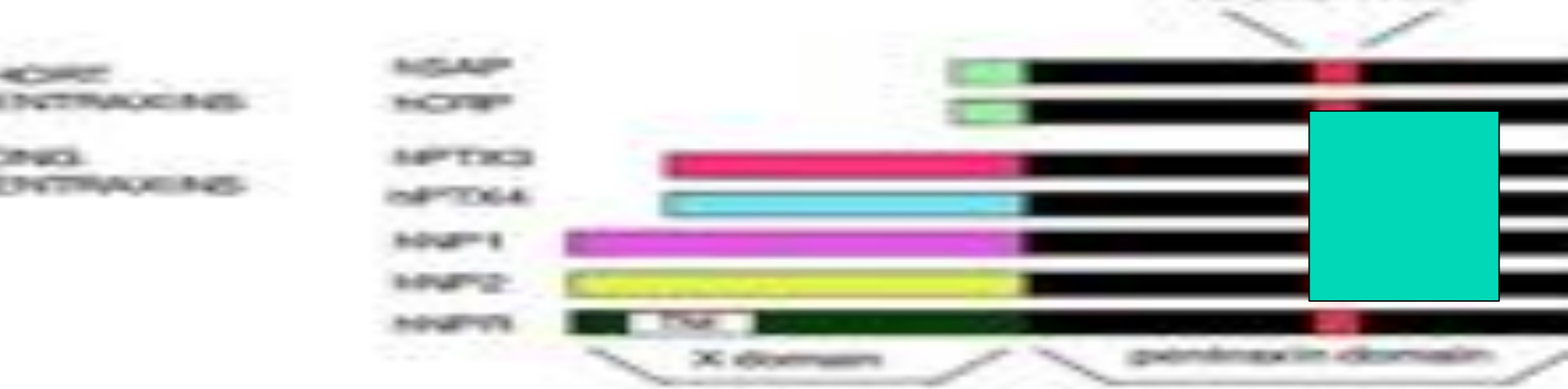


Figure 2-47 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

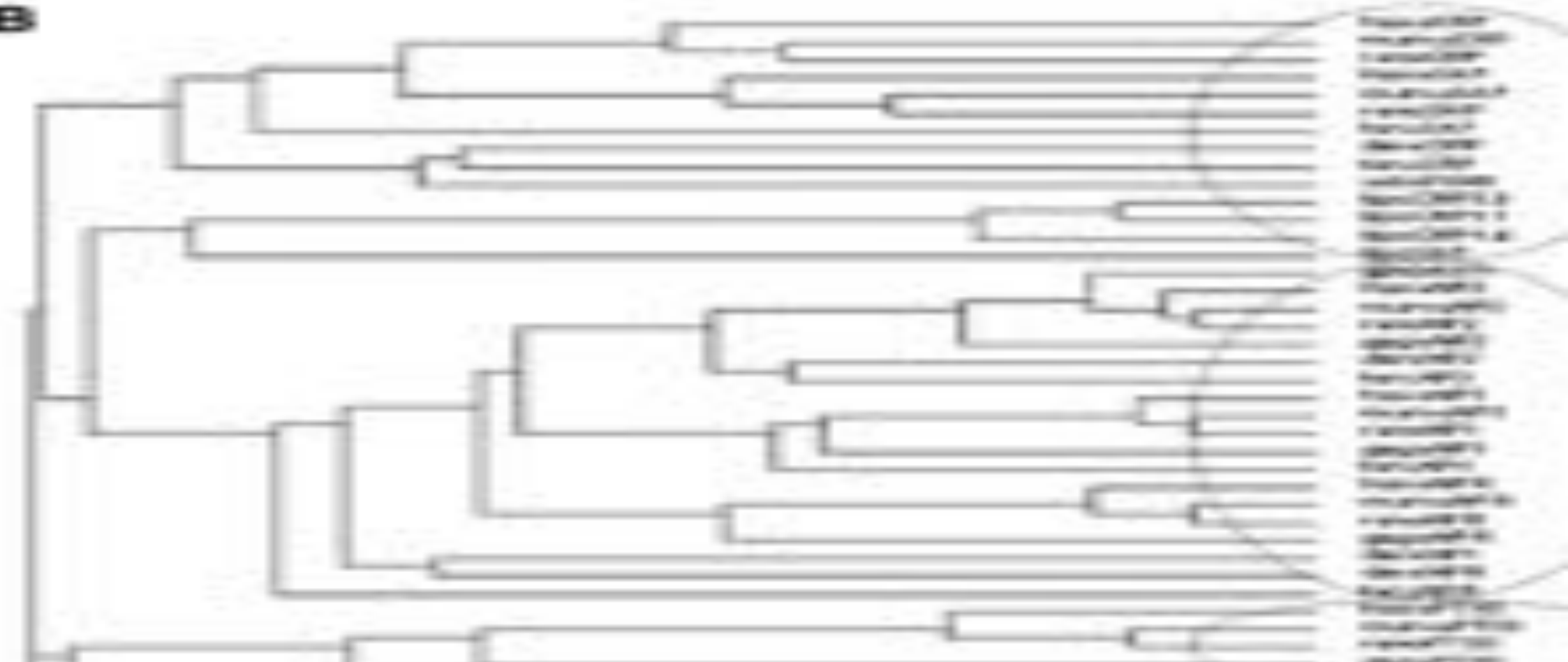
CPR

SAP

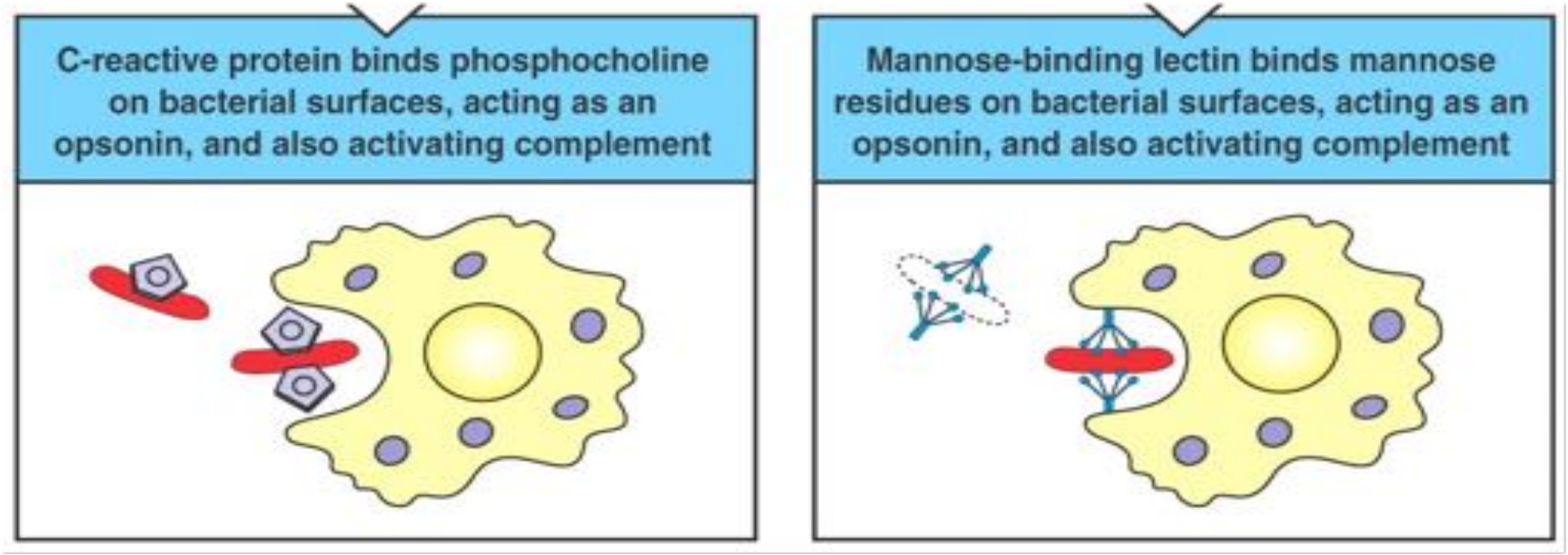
- **LONG PENTRAXINS: PTX3-PTX4**


hGAP
hOTP
hPTX3
hPTX4
hNP1
hNP2
hNP3
TM
X domain
pentraxin domain

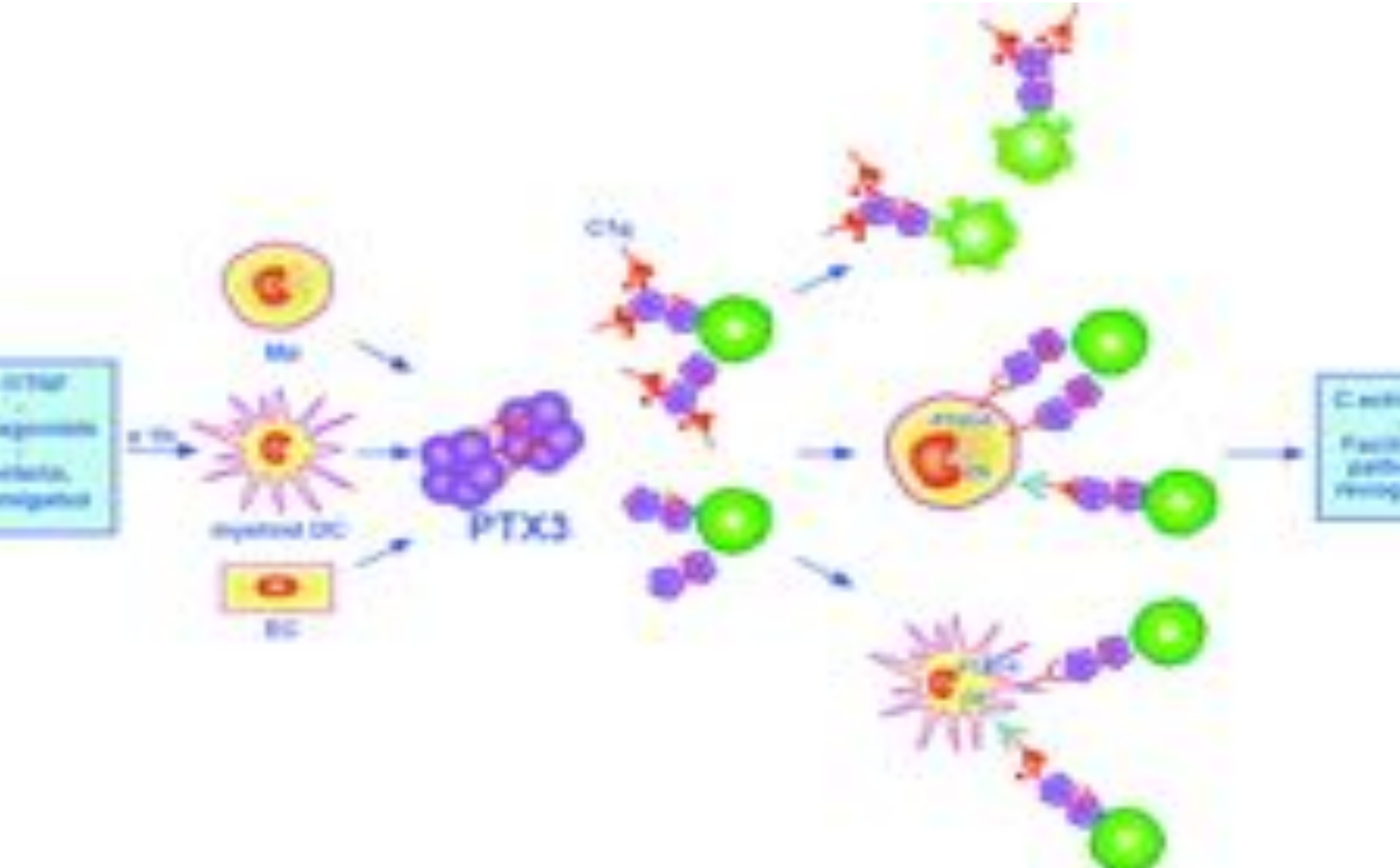
B



of bacteria
and phosphoethanolamine
of apoptotic cells
and activate complement and phagocytosis!



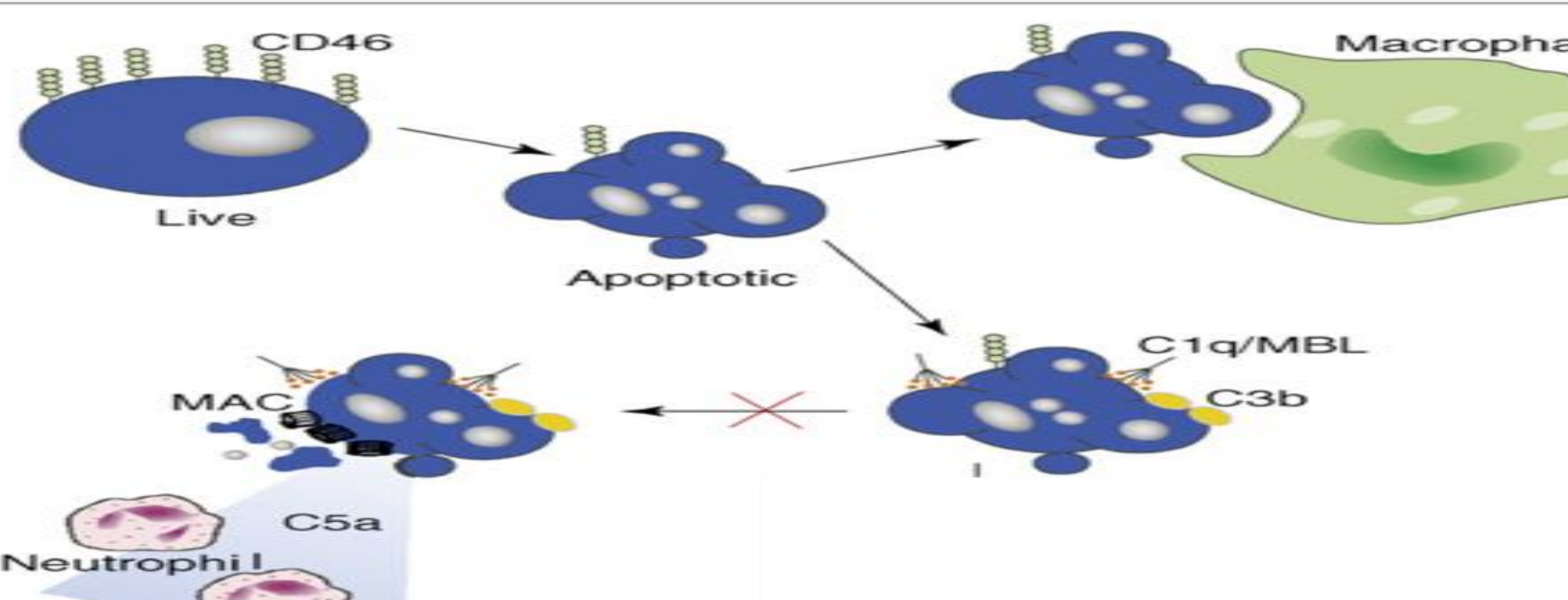
e of the long pentraxin PTX3 in antimicrobial resistan



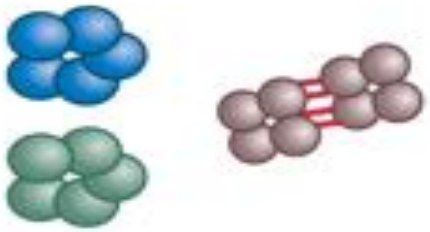
activated by

PENTRAXINS:

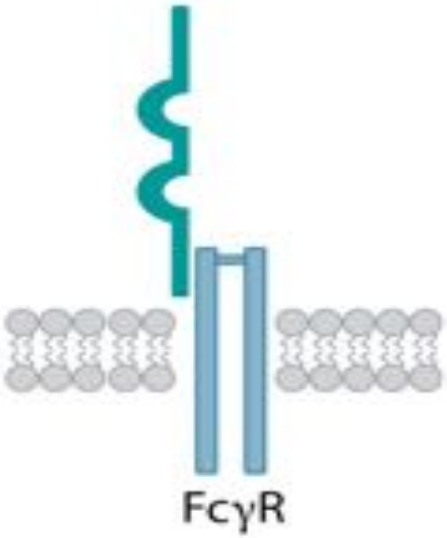
CLASSICAL COMPLEMENT ACTIVATION IN THE NATURAL IMMUNITY AND INFLAMMATION!

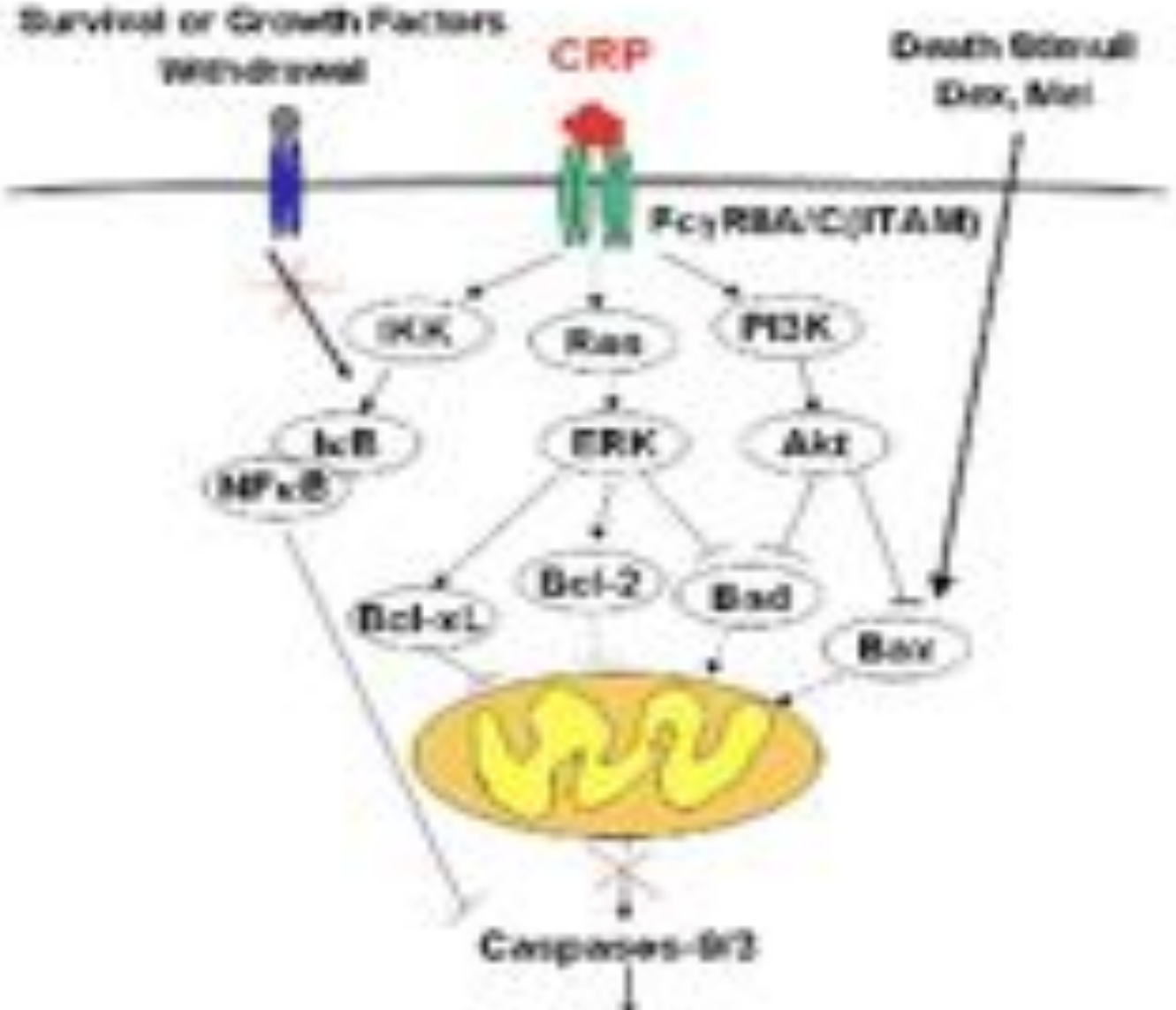


antibodies and activate phagocytosis!!



CRP/SAP PTX3



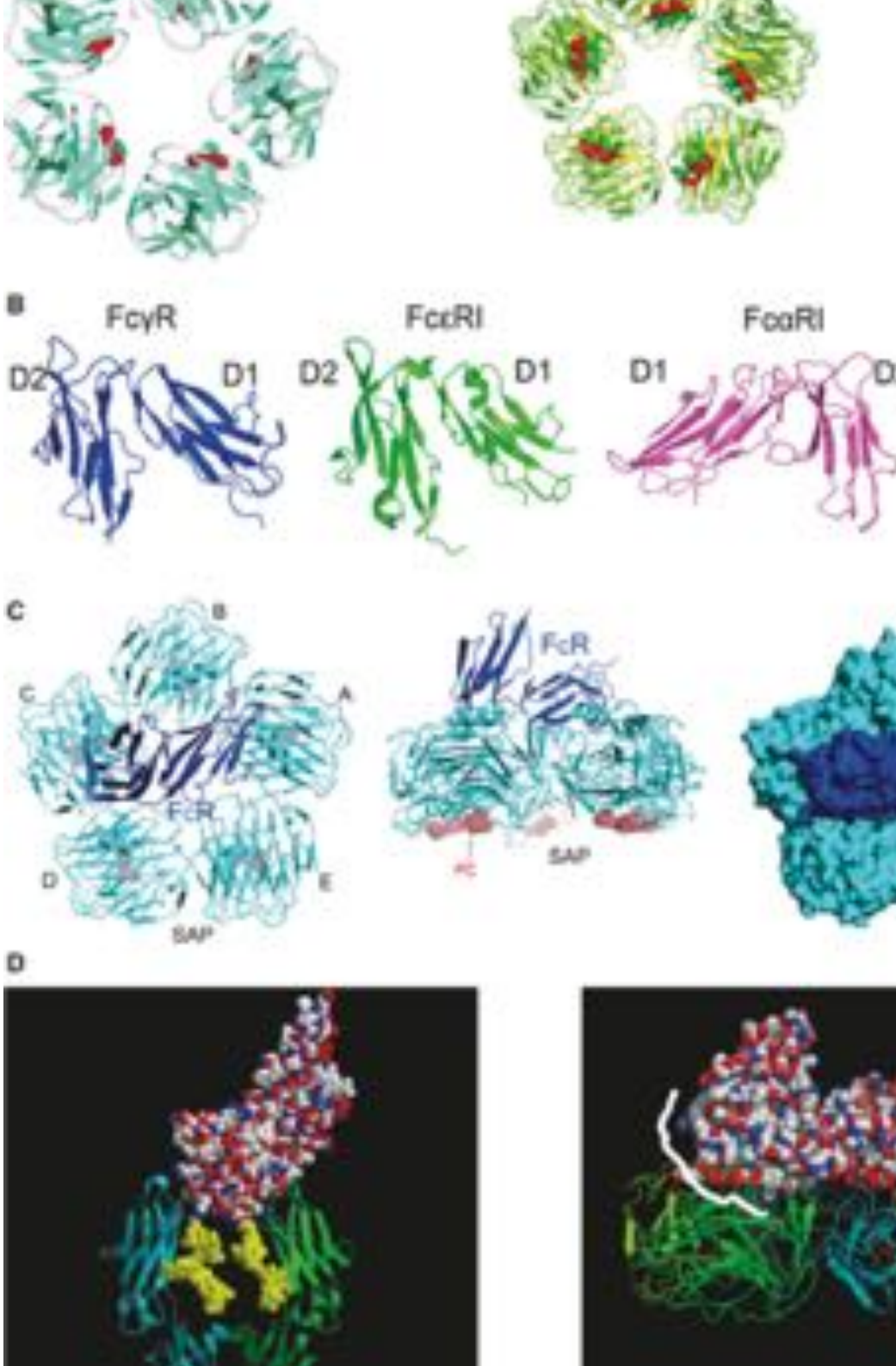


Crystal structure of phosphocholine
nd CRP (PDB entry 1B09, left) and
structural superposition between CRP
SAP (right).

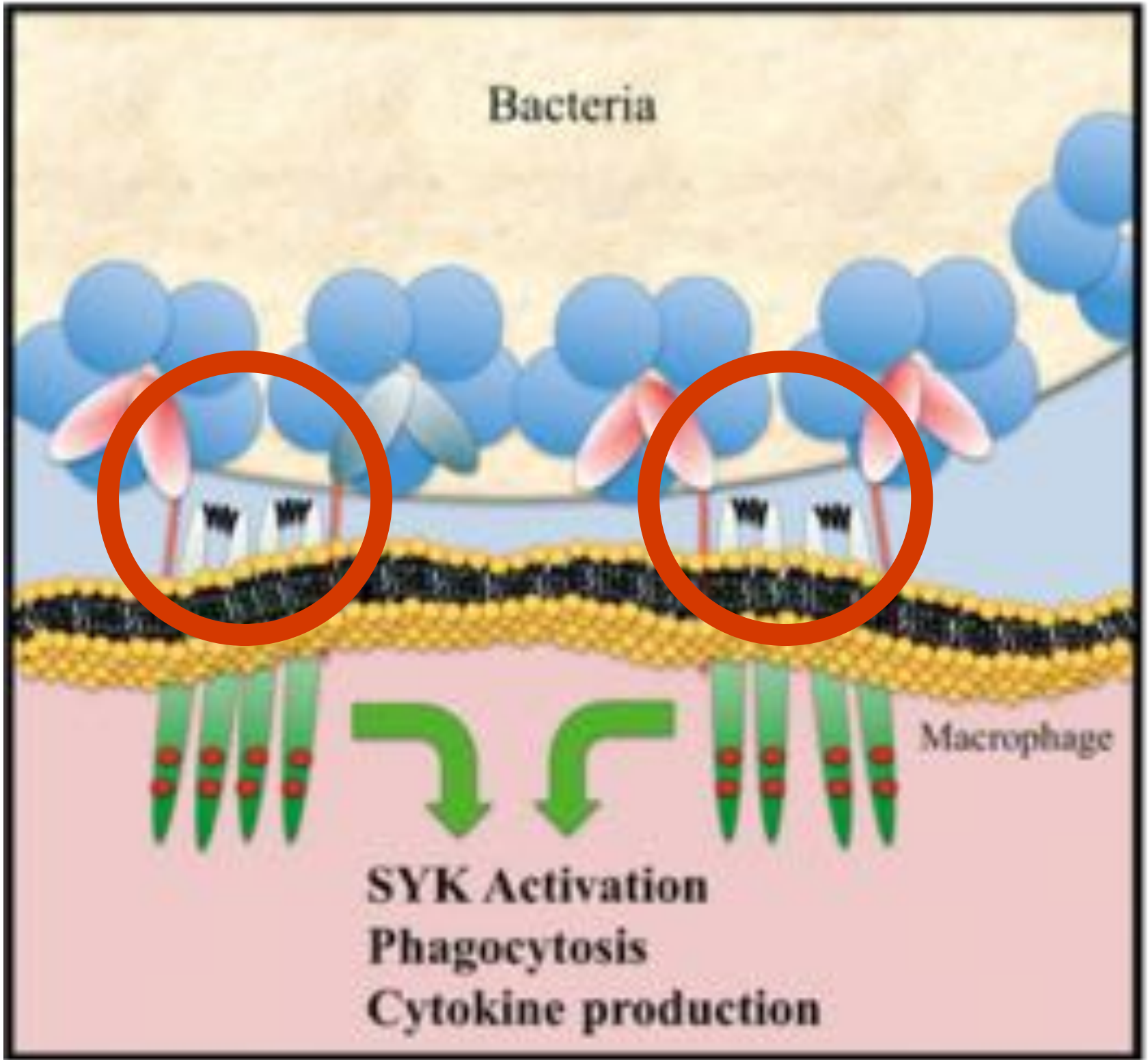
Structures of FcγRIIA, FcεRI (PDB
y 1F2Q), and FcαRI (PDB entry
/Z).

Structural complex between human
P (cyan) and FcγRIIA (blue) in two
hogonal views (left and middle panels)
in space filling model (right panel).

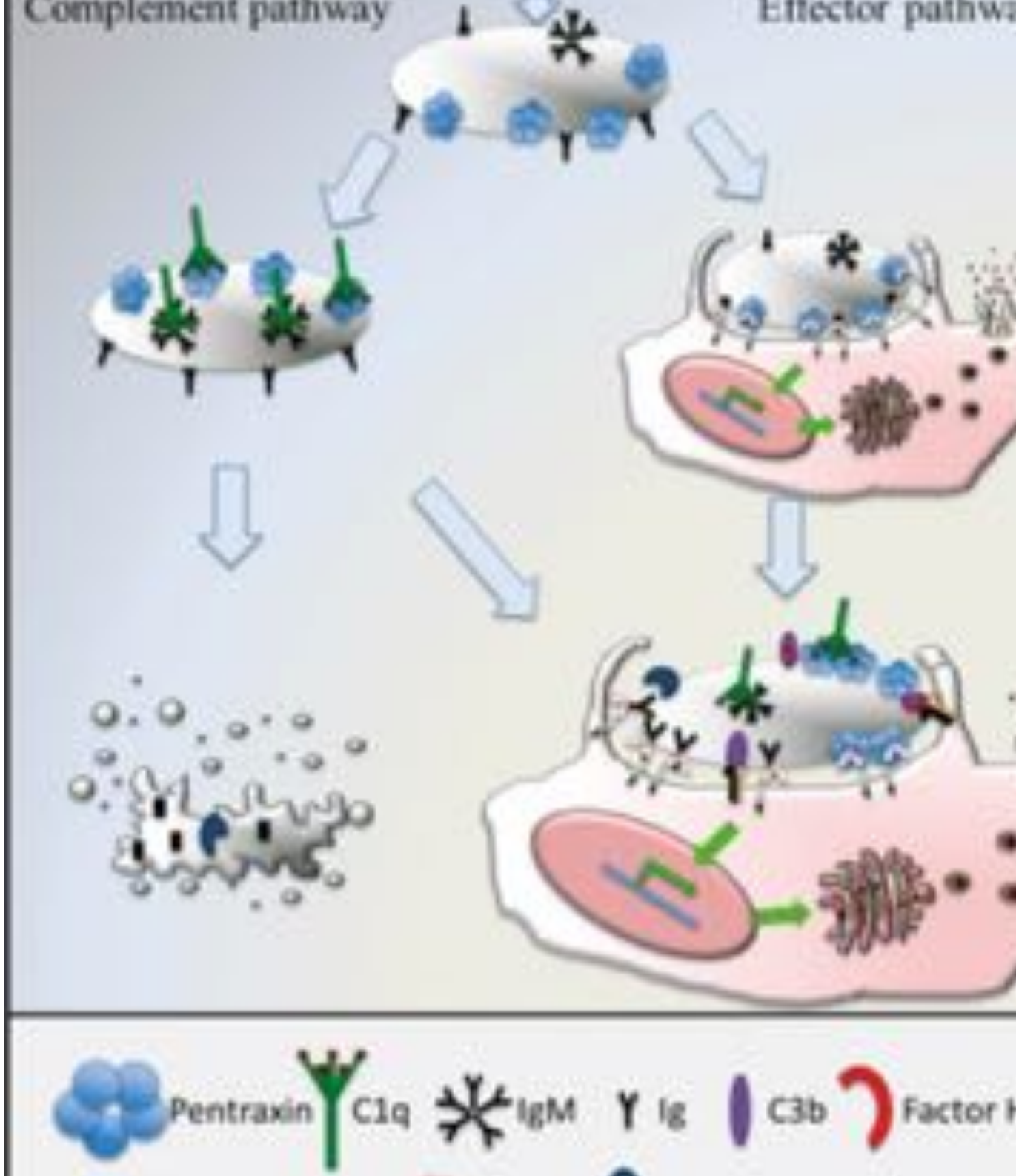
Binding mode of IgG−Fc on Fc
eptor (left panel) partially overlap with
of SAP (right panel). The IgG−Fc
rface region is highlighted in white
on the SAP complex structure.



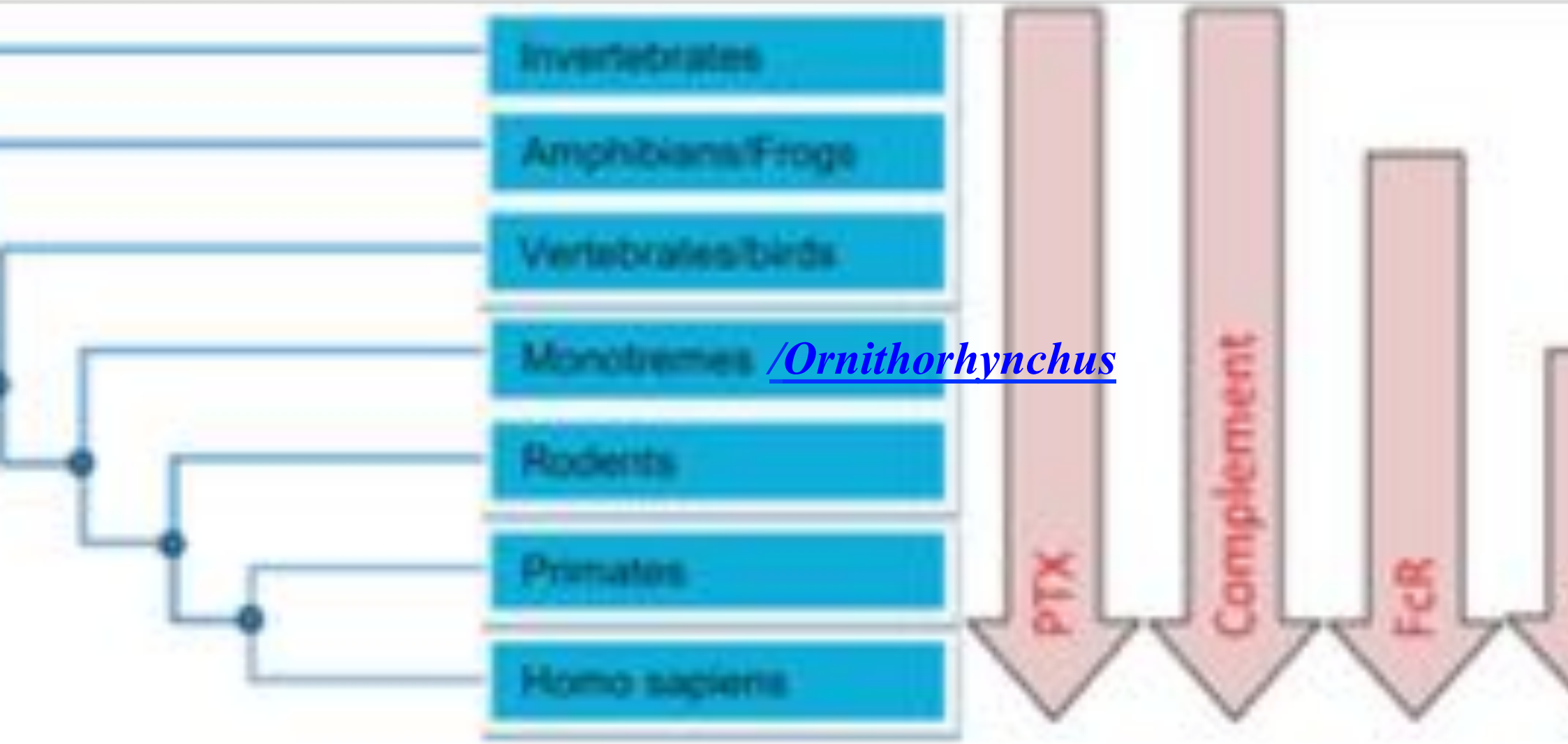
for IgG antibodies.



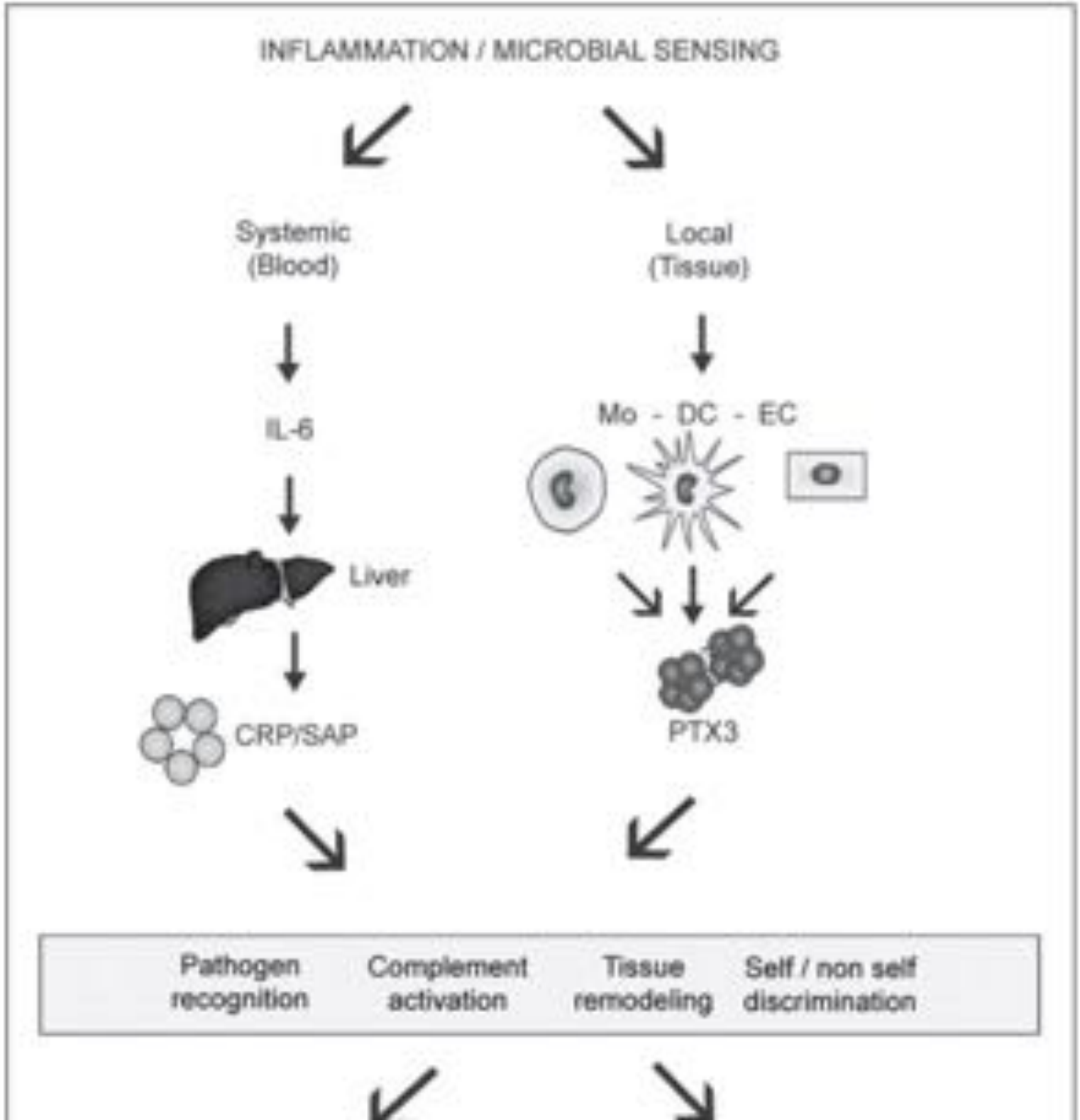
omparison of ntraxins and ntibodies in nplement and Fc receptor activation!



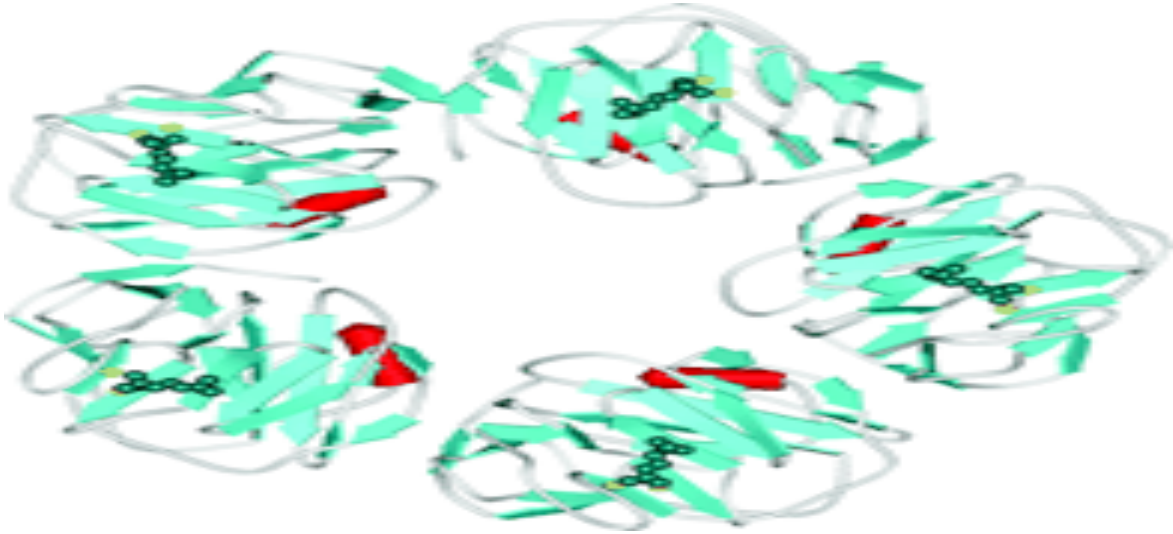
Pentraxins and Fc receptors



nunological Reviews


INFLAMMATION / MICROBIAL SENSING
Systemic
(Blood)
Local
(Tissue)
IL-6
Mo - DC - EC
Liver
CRP/SAP
PTX3
Pathogen recognition
Complement activation
Tissue remodeling
Self / non self discrimination

MAJOR POSITIVE APP!



physiological concentration is less than 1µg/ml (
ml at birth, 170 ng/mL in children and from 47(
0 ng/mL in adults), but increases by 100-1000 ti
ng inflammation.

ugh for a long time CRP levels have been used as a quick test for the presum
osis of bacterial infection (high CPR) distinct from viral infection (low CPR), today a
ase of the PCR can be observed in viral hepatitis, in bacterial acute flu-like syndrom
e TB, gout, in burns, in peritonitis, in rheumatic fever, rheumatoid arthritis, and a
icant increase occurs in scarlet fever and Guillon-Barré syndrome. CPR is often use

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

UNDS:

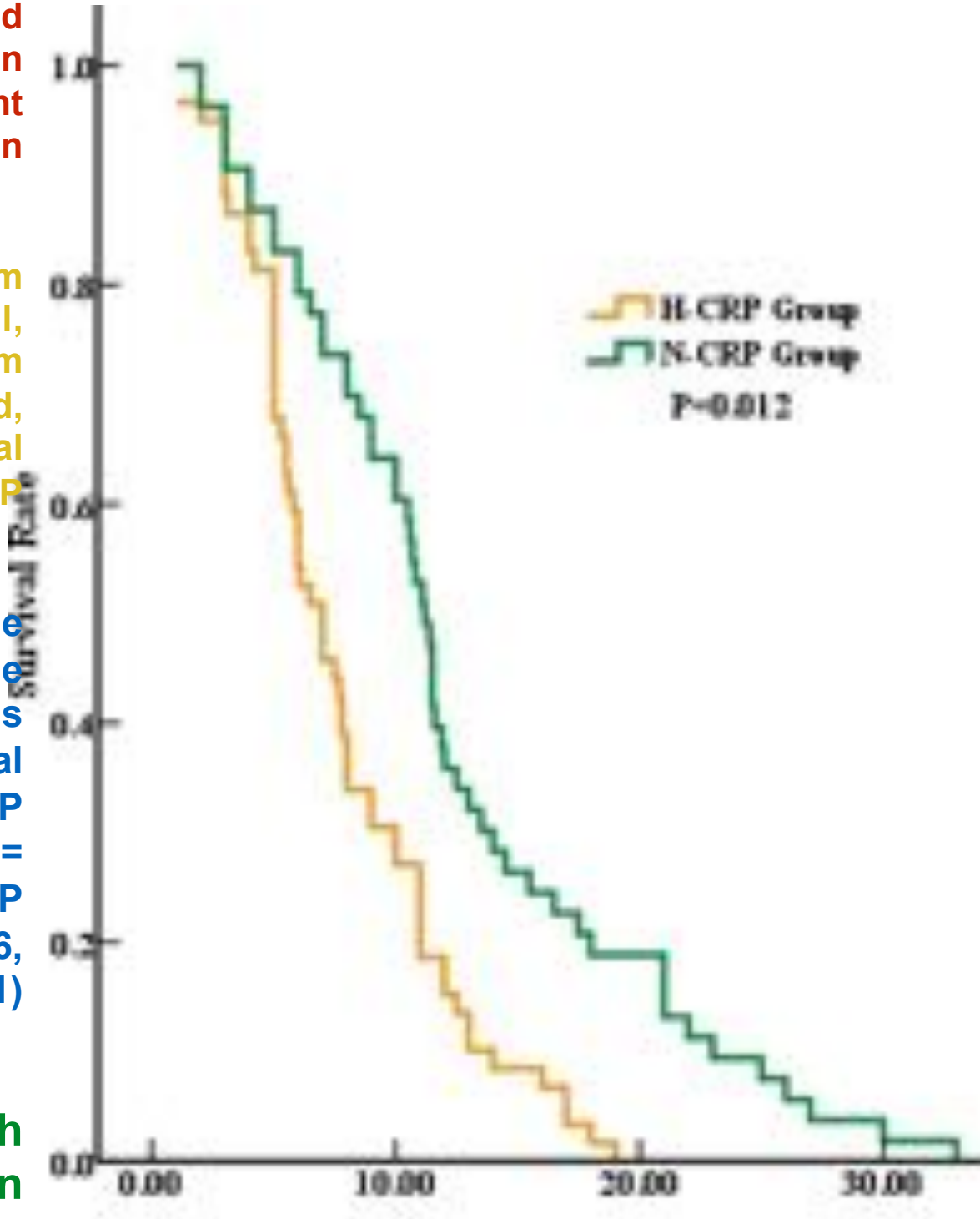
n C-reactive protein (CRP) was found to be associated rognosis in kinds of solid tumors, however, its role in ent gastric cancer (RGC) is unknown. The present d to explore the prognostic value of serum CRP in ts.

RGC patients who underwent radical surgery from 005 to May 2008 were enrolled. The clinical, al and survival information were collected. The serum was measured when the recurrence was confirmed, sociation between serum CRP and clinicopathological was analyzed. The prognostic value of serum CRP s investigated.

n CRP was elevated in 39 patients (H-CRP), while ts were within the normal range (N-CRP).The RP was associated with Lymph node metastasis) and tumor size (p = 0.004). The median survival recurrence was significantly worse in the H-CRP n N-CRP group (6.5 months vs. 11.5 months, p = ltivariate analyses identified that elevated CRP 2.325, p < 0.001), time to recurrence (HR = 0.466, and the follow-up treatment (HR = 2.650, p=0.001) pendent prognostic factors.

ONS:

rum CRP level was associated with sive pathological features, was an



POSITIVE APP:

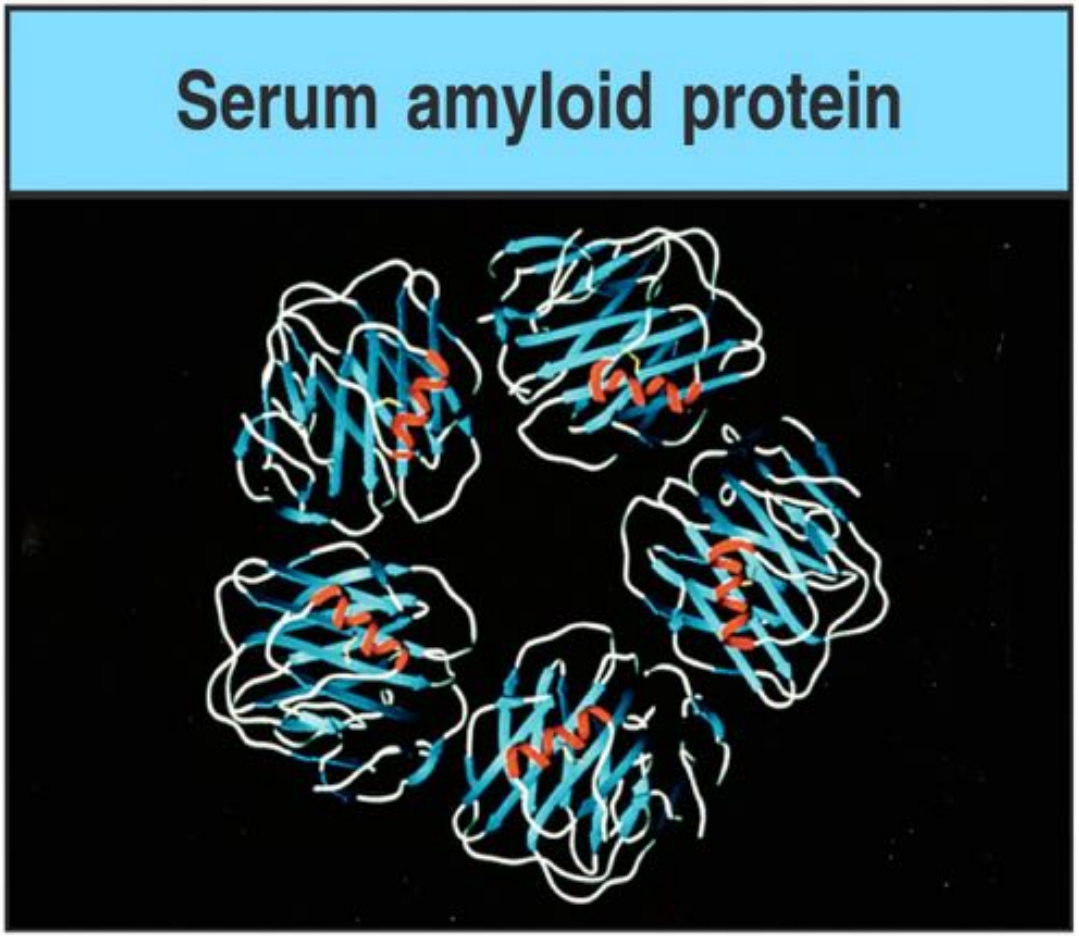


Figure 2-47 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

uthors have reported the existence of various types of SAA that can be classifie
constitutive SAA) and ASAA (Acute phase SAA).

atter have:
nological functions, such as promoting the lysis of apoptotic cells; pro
ytosis and adhesion and chemotaxis of leukocytes; inducing ECM-degrading e
nase, stromalisin, MMP2 and 3) and inflammatory cytokines (IL-6 , TNF-α);

linical importance of determination of serum amyloid A

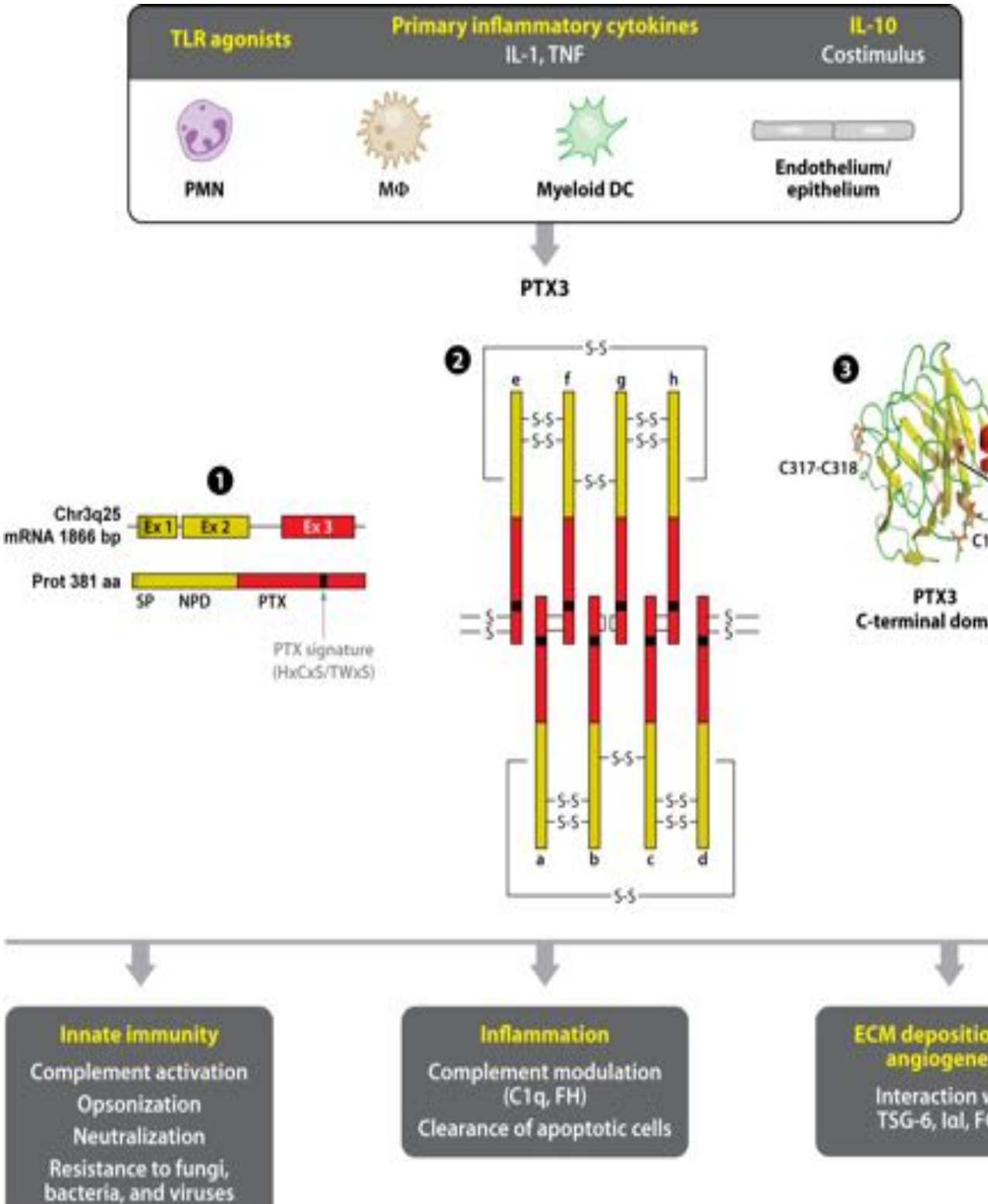
rum amyloid A (SAA) is an acute phase first class protein discovered a quarter of
ntury ago. Its concentration depends on clinical findings of the patient, illn
ivity and the therapy applied.

A increases moderately to markedly (100-1000 mg/l) in bacterial and fun
ections, invasive malignant diseases, tissue injuries in the acute myocard
arction and autoimmune diseases such as rheumatoid arthritis and vasculitis.

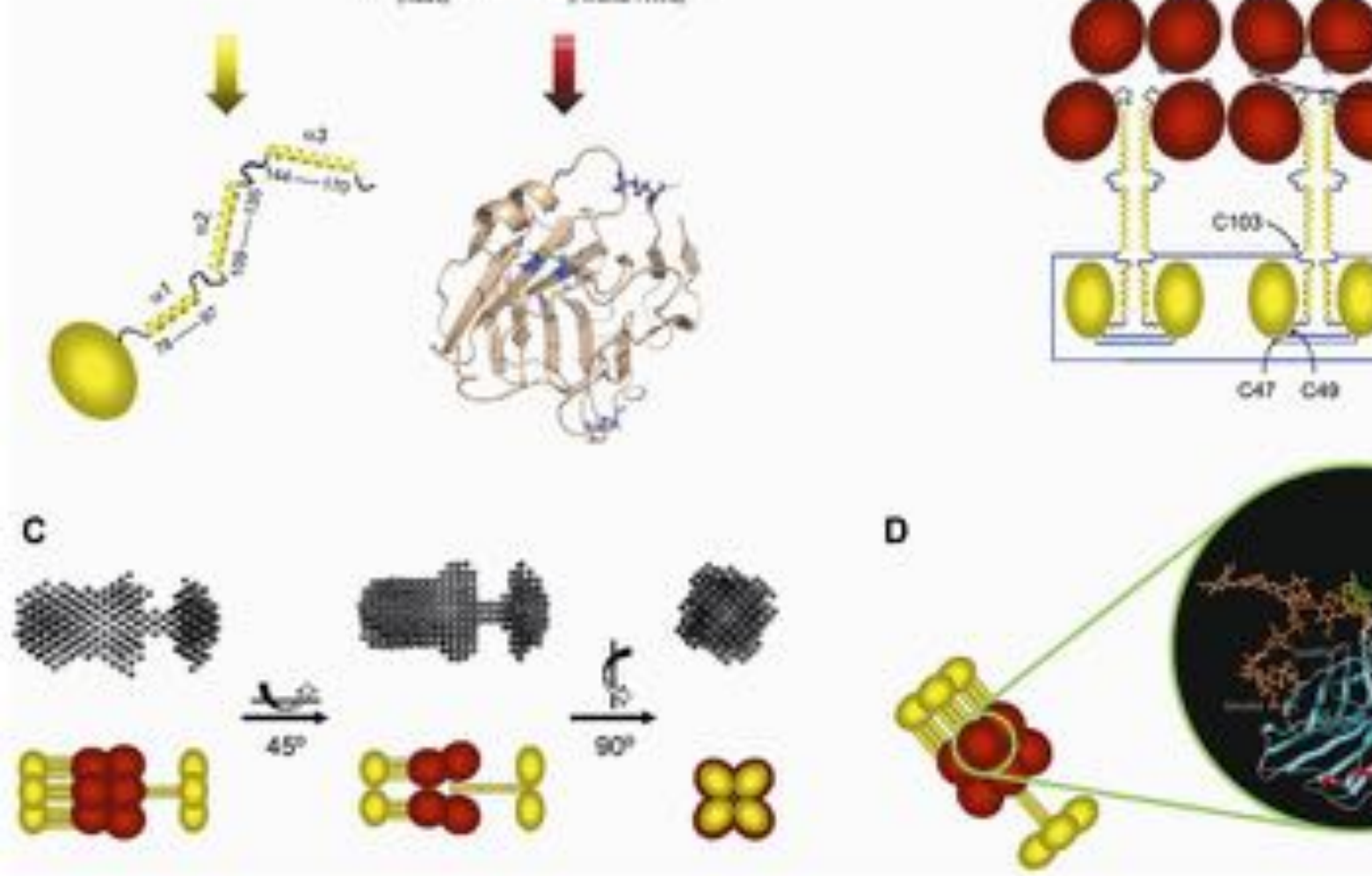
ld elevation (10-100 mg/l) is often seen in viral infections, systemic lup
ythematosus and localized inflammation or tissue injuries in cystitis and cereb
arction.

A as sensitive, non-invasive parameter is used in organ transplantation where ea
d correct diagnosis is needed as well as where prompt therapy is requir
nultaneous determination of C-reactive protein (CRP) and SAA may point to ac
ney allograft rejection.

Cellular sources and inducers of the long pentraxin PTX3!



The ntraxins NG PTX3/ TX4, after their rahepatic oduction can be cosylated!



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

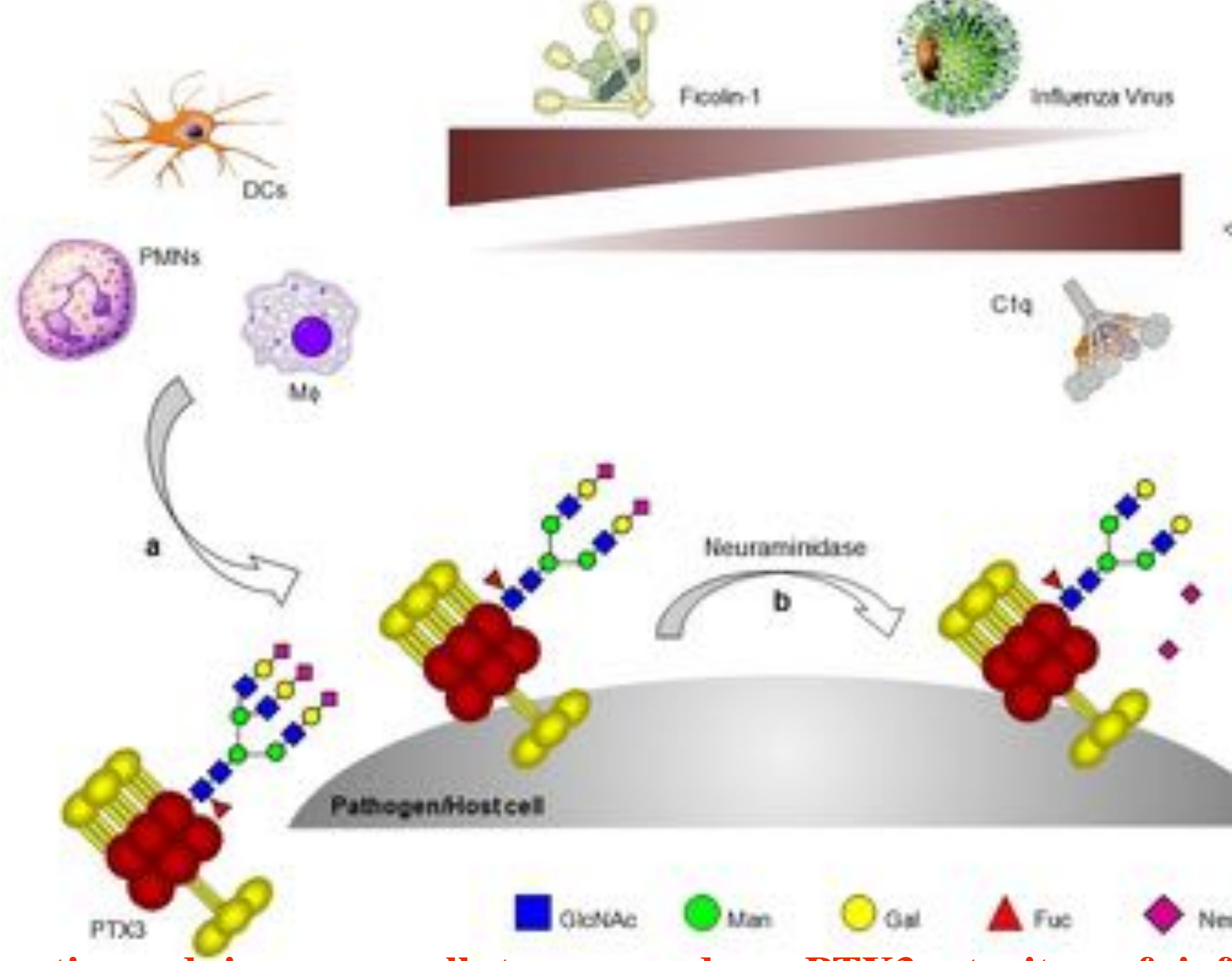
(B)Disulfide bond organization of the PTX3 octamer.

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

nt

nunol.

2; 3: 407. 7



mber of both somatic and immune cell types produce PTX3 at sites of inf
mation. The glycosylation status of PTX3 (e.g., branching and sialylation) might c
ding on cellular source and inducing stimuli (a). In addition, the protein oligosacch

PTX3 is related to the CPR, whose levels in case of infection rise dramatically i
r of hours. Compared to PCR , PTX3 is the fact of being produced from any tiss
ct to inflammation. In this way its concentration increases much more quick
ing an early diagnosis. For example, in the case of infarction was found that wh
atient arrives in the ER, PTX3 levels are already very high, while those of C
ge after a few hours. It can also give important information about prognosis.

ndition in which the evaluation of the levels of PTX3 could be very useful, it
lampsia. This is a very serious complication that may occur during pregnancy, w
crease of blood pressure. In these cases the raising of PTX3 concentration occu
while the clinical manifestations due to altered vascularization of the placenta a
much later. Even in this case PTX3 could become important for an early diagnos

bsence of PTX3 corresponds to a condition of infertility, since this protein is a k
onent of the structure of cells (the cumulus oophorus) surrounding the ooc
ovulation occurs. Without it, the egg cell remains virtually ' naked ' , deprived
nal elements that have the function of guiding the sperm in the right direction a
fertilization can not occur.

OF THE NEW YORK ACADEMY OF SCIENCES
r in Immunology

ng pentraxin PTX3: a paradigm for humoral pattern ition molecules

ntovani,[1,2] Sonia Valentino,[1] Stefania Gentile,[1] Antonio Inforzato,[1]
ttazzi,[1] and Cecilia Garlanda[1]

inical and Research Center, Rozzano, Milan, Italy. [2]Department of Medical Biotechnology and Translational ersity of Milan, Milan, Italy

rrespondence: Alberto Mantovani, MD, Scientific Director, Istituto Clinico Humanitas, Via Manzoni 113, 20089 no, Italy. alberto.mantovani@humanitasresearch.it

gnition molecules (PRMs) are components of the humoral arm of innate immunity; they recogni
ociated molecular patterns (PAMP) and are functional ancestors of antibodies, promoting complement sonization, and agglutination. In addition, several PRMs have a regulatory function on inflammation. e a family of evolutionarily conserved PRMs characterized by a cyclic multimeric structure. On the basis pentraxins have been operationally divided into short and long families. C-reactive protein (CRP) and id P component are prototypes of the short pentraxin family, while pentraxin 3 (PTX3) is a prototype ntraxins. PTX3 is produced by somatic and immune cells in response to proinflammatory stimuli and ptor engagement, and it interacts with several ligands and exerts multifunctional properties. Unlike ene organization and regulation have been conserved in evolution, thus allowing its pathophysiological aluated in genetically modified animals. Here we will briefly review the general properties of CRP and otypes of short and long pentraxins, respectively, emphasizing in particular the functional role of PTX3 ic PRM with antibody-like properties.

nate immunity; pentraxins; PTX3; pattern recognition molecules

Bacteria	
Pseudomonas aeruginosa	NT[a]
Klebsiella pneumoniae	NT
Salmonella typhimurium	–
Fungi and yeasts	
Aspergillus fumigatus	+
Saccharomyces cerevisiae (zymosan)	+
Paracoccidioides brasiliensis	NT
Viruses	
Influenza virus	–
Human cytomegalovirus (HCMV)	NT
Membrane moieties	
Phosphocholine (PC)	+
Phosphoethanolamine (PE)	–
LPS	–
Outer membrane protein A from *Klebsiella pneumoniae* (KpOmpA)	NT
Complement components	
C1q	+
Factor H	+
C4BP	+
M-, L-ficolin	+
MBL	–
Extracellular matrix proteins	
TNF-stimulated gene-6 (TSG-6)	NT
Inter-α-trypsin-inhibitor (Iαi)	–
Hyaluronan	NT
Laminin	+
Collagen IV	NT
Fibronectin	+
Growth factors	

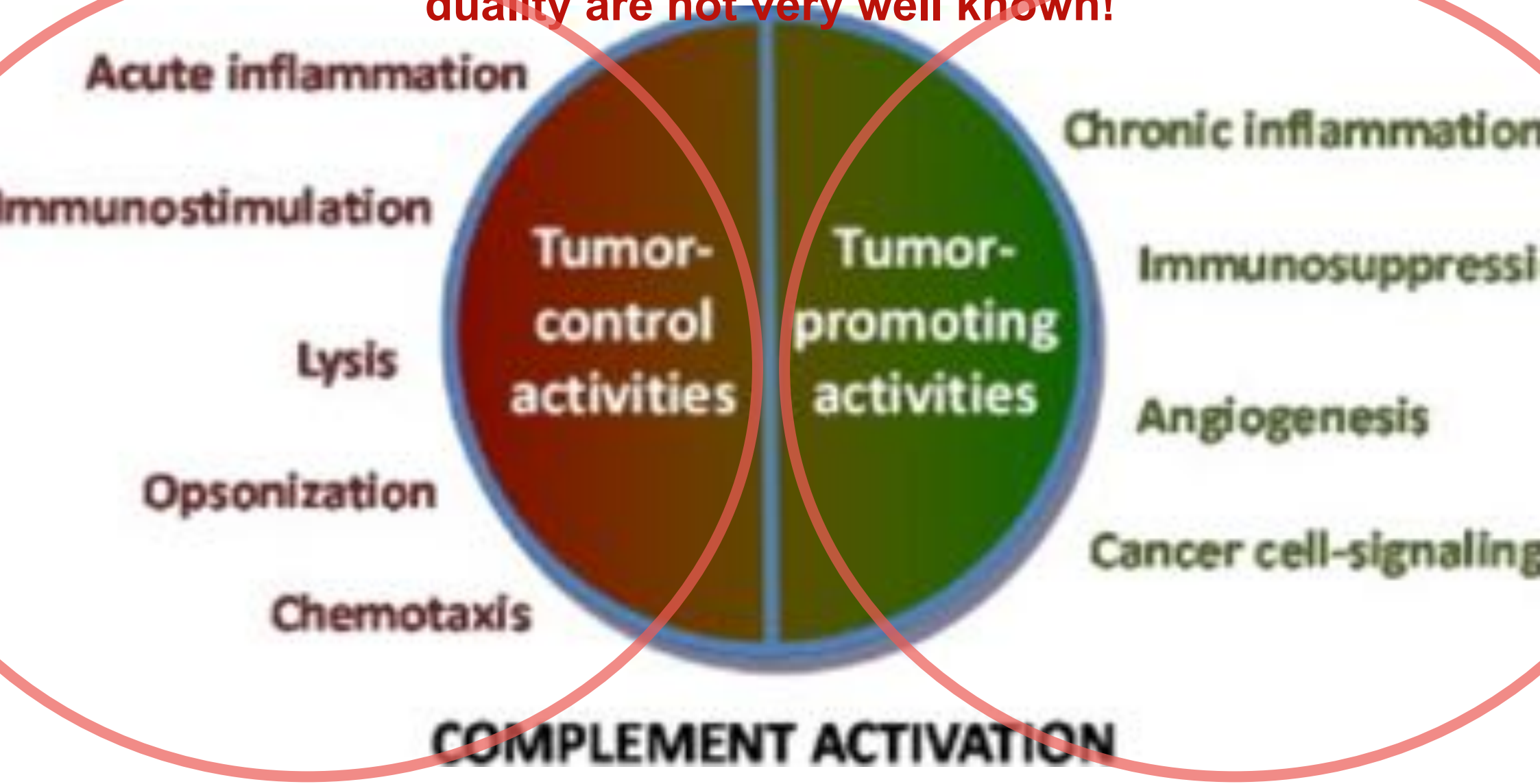
...s is an Extrinsic Oncosuppressor Regulating Complement-Dependent Inflammation in Cancer.

...a E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, Greco C, Feru...a M, Laface I, Tartari S, Doni A, Pasqualini F, Barbati E, Basso G, Galdie...hi M, Roncalli M, Colombo P, Laghi L, Lambris JD, Jaillon S, Garlanda C, Mantovan...

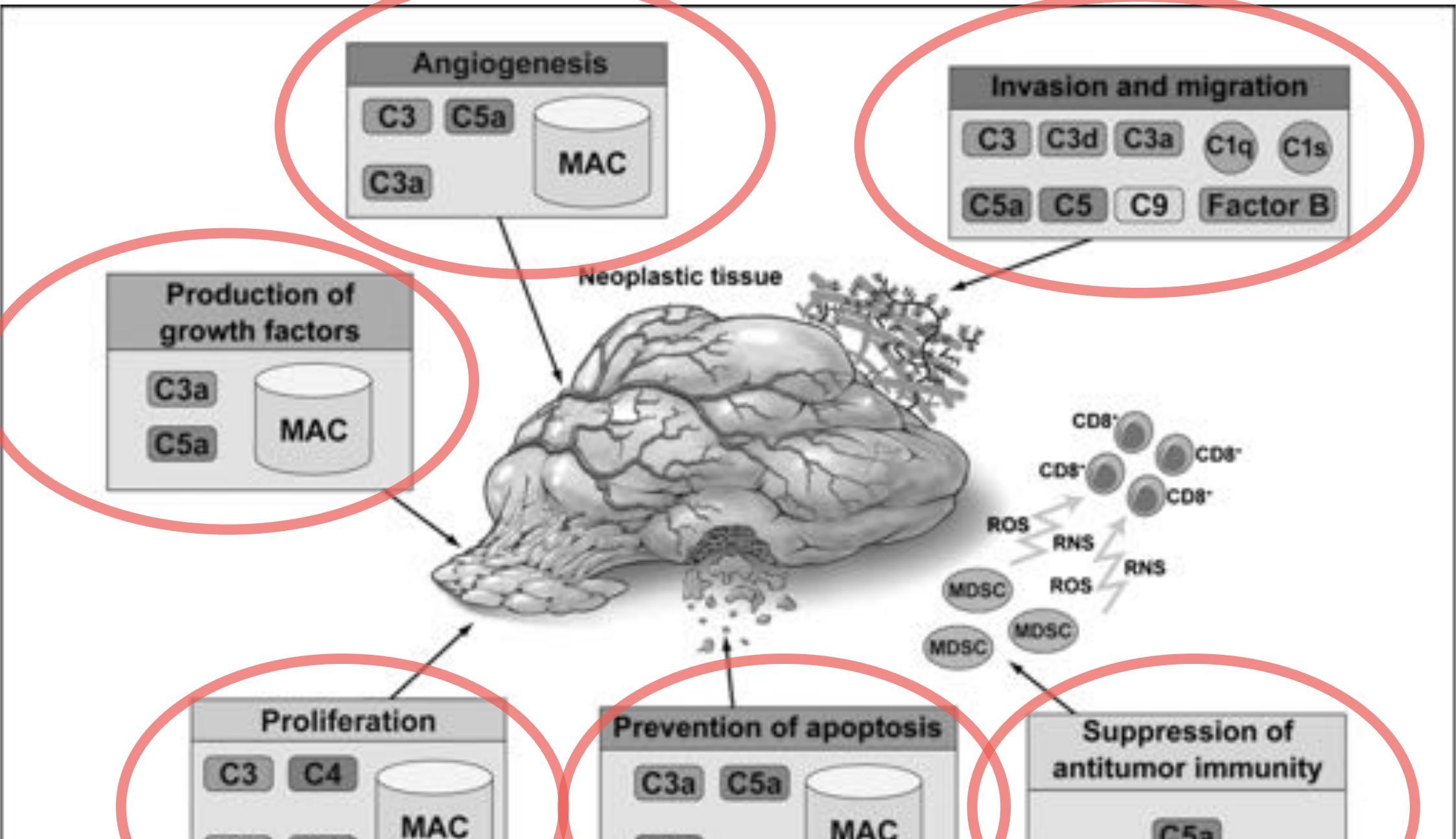
...ct

...s an essential component of the humoral arm of innate immunity, pla...undant role in resistance against selected microbes and in the regula...mation. PTX3 activates and regulates the Complement cascade by interacti...nd with Factor H. PTX3 deficiency was associated with increased susceptib...chymal and epithelial carcinogenesis. Increased susceptibility of Ptx3(-/-) mi...ated with enhanced macrophage infiltration, cytokine production, angiogenes...mutations. Correlative evidence, gene-targeted mice, and pharmacological bl...ments indicated that PTX3 deficiency resulted in amplification of Comp...ion, CCL2 production, and tumor-promoting macrophage recruitment...sion was epigenetically regulated in selected human tumors (e.g., leiomyosar...lorectal cancer) by methylation of the promoter region and of a putative enh...PTX3, an effector molecule belonging to the humoral arm of innate immunity,...rinsic oncosuppressor gene in mouse and man by regulating Compl...

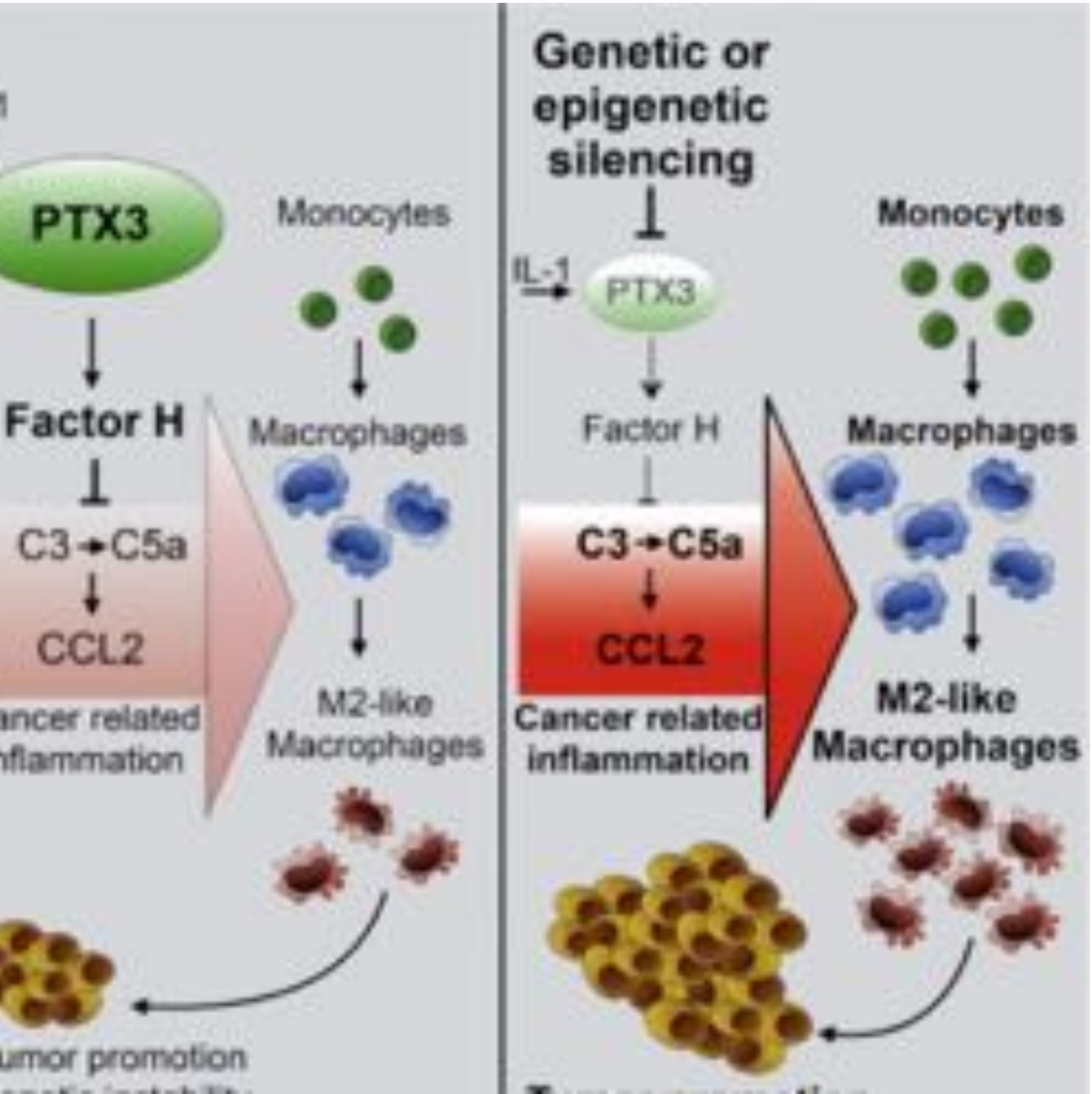
perimental data support the idea that complement is activated by tumor
ver, some studies also suggest that malignant cells evade the harmful e
mplement and make use of some complement effector molecules to pro
cer growth. Unfortunately, the exact mechanisms and consequences of
duality are not very well known!



r production, angiogenesis, protection from antigr
ls and apoptosis, cellular invasion and migration thr
xtracellular matrix, and suppression of antitumor immun

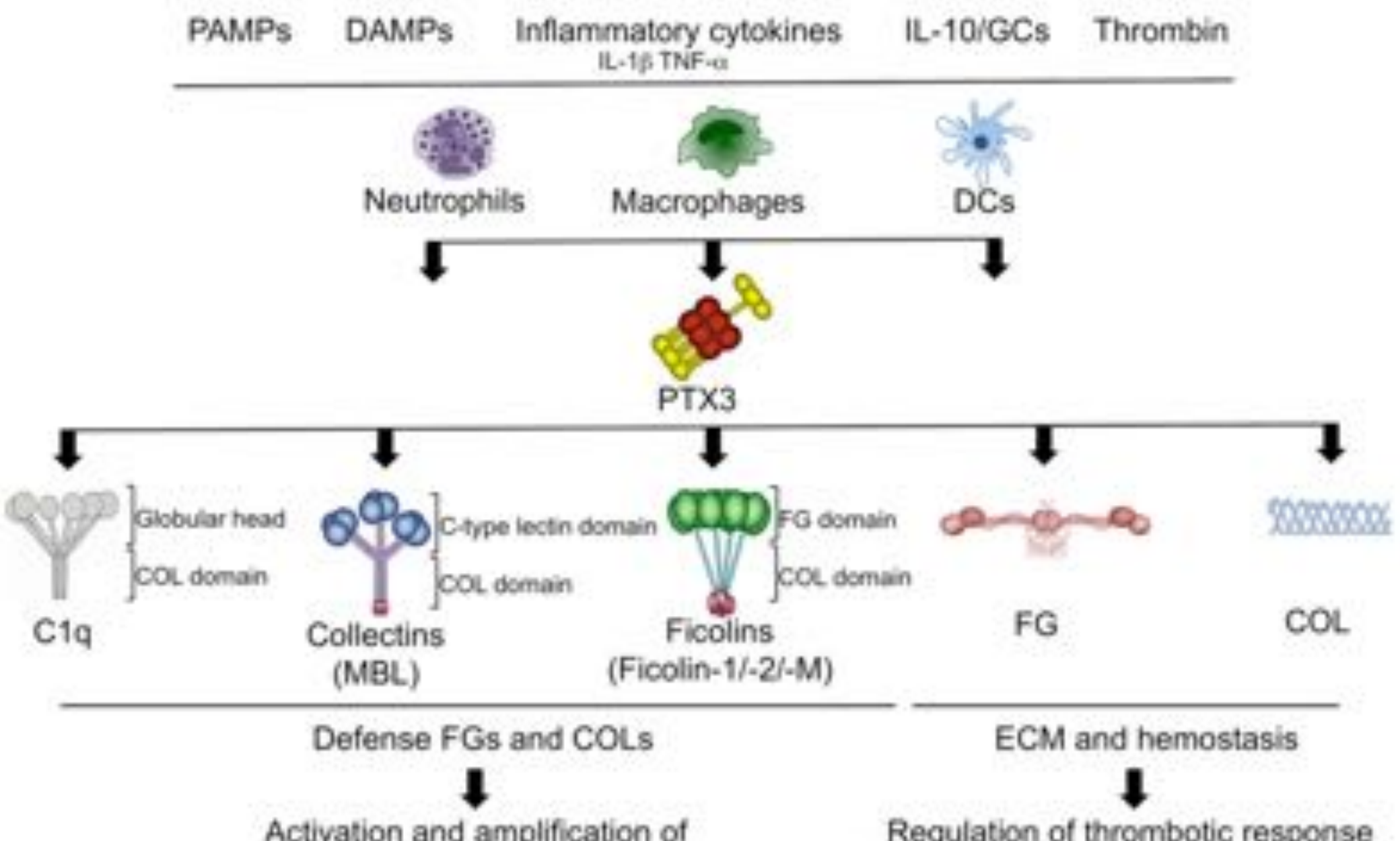


man!

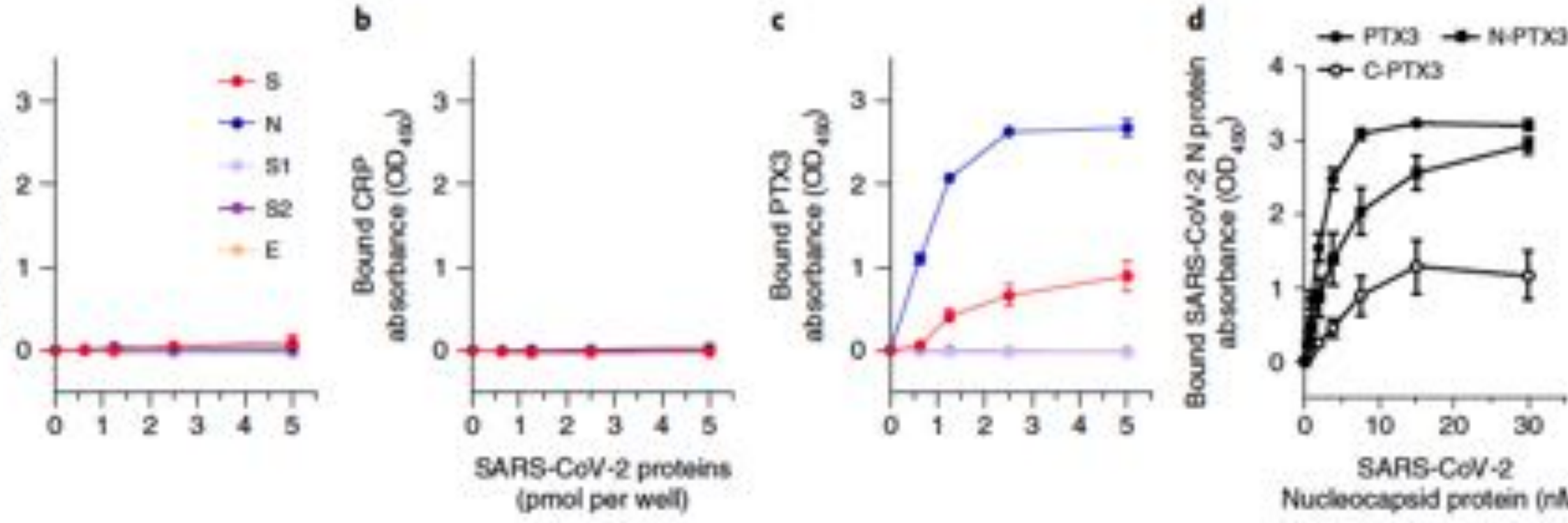


PTX3 gene i
silenced by
hypermethylat
in selected
human tumo
including
colorectal can
(CRC) and th
event occur
early in
progressior
already at th
level of

ogens amplifies other effector functions of the innate immune system. At v
PTX3 regulates the injury-induced thrombotic response and promotes w
g by favoring timely fibrinolysis. Therefore, PTX3 interacts with and
ns conserved in innate immunity, hemostasis and extracellular matrix and
ons related to both antimicrobial resistance and tissue repair.

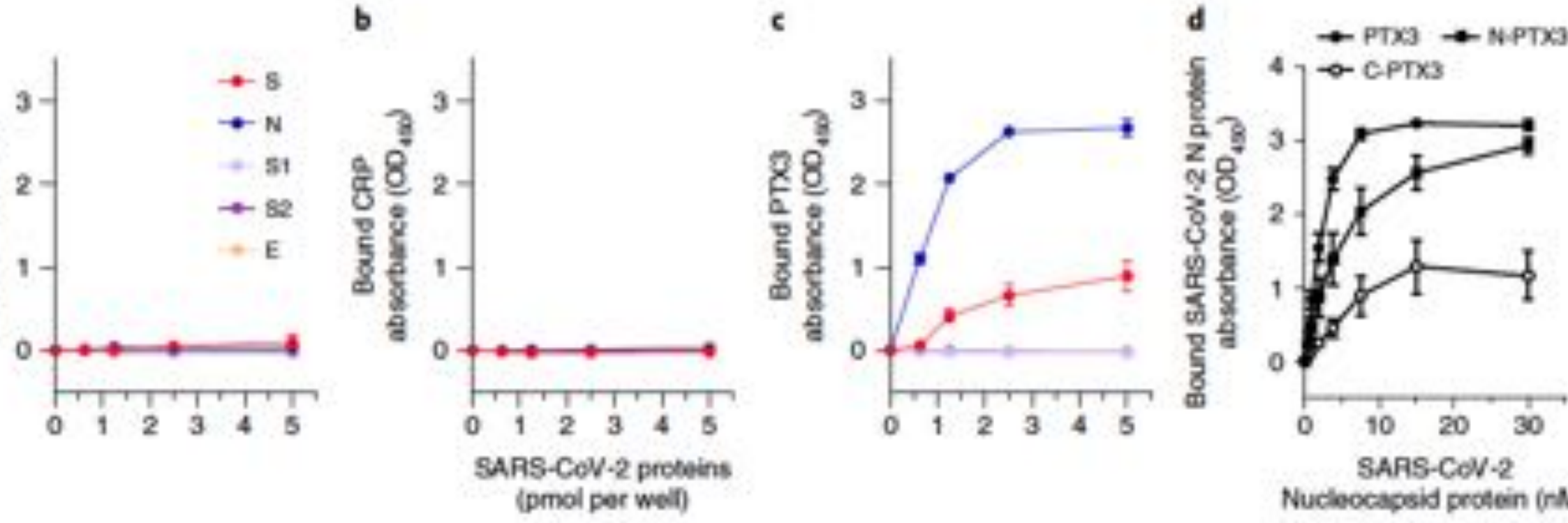


er to the nucleocapsid protein, one of the most abundant proteins of SARS-C



ction between pentraxins and SARS-CoV-2 proteins. a–c, Recombinant His tag SARS-CoV-2 proteins (spike active trimer (S), S1, S2, r
lope (E); the legend refers to **a–c**) were immobilized on 96-well nickel-coated plates at different concentrations. Fixed concentrations
PTX3 (**c**) were incubated over the captured viral proteins. Bound pentraxins were detected by enzyme-linked immunosorbent assay (E
ary antibodies. **d**, Full-length PTX3 or the N- or C-terminal domains were captured on 96-well plates. Biotinylated SARS-CoV-2 nucleo
ncubated at different concentrations. Bound nucleocapsid was detected by ELISA using horseradish peroxidase (HRP)-conjugated stre
resented as mean ± s.e.m.; $n = 3$ independent experiments performed in duplicate; OD_{450}, optical density at 450 nm.

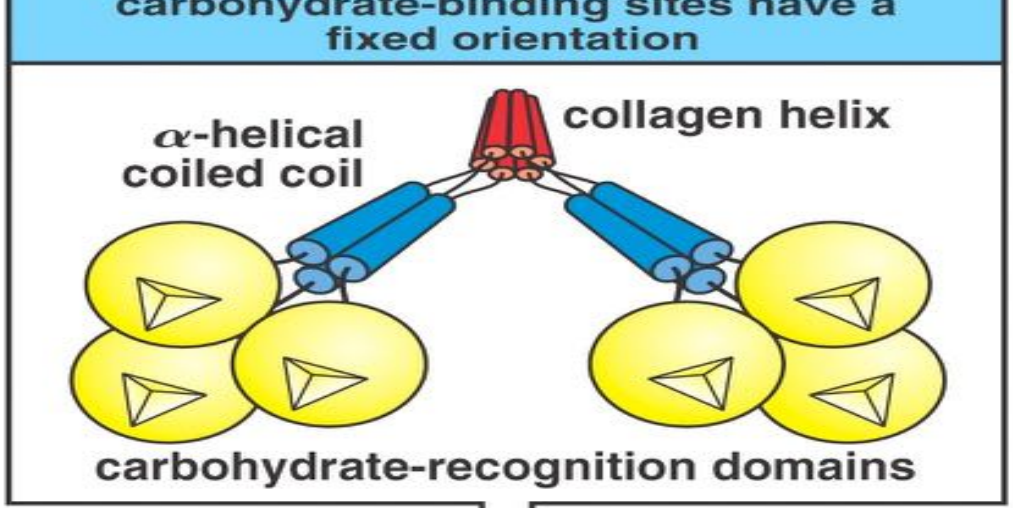
er to the nucleocapsid protein, one of the most abundant proteins of SARS-C



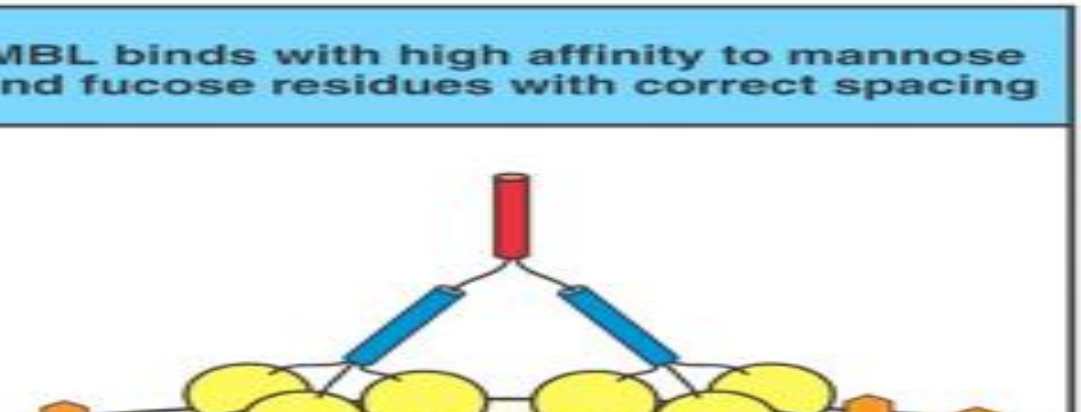
ction between pentraxins and SARS-CoV-2 proteins. a–c, Recombinant His tag SARS-CoV-2 proteins (spike active trimer (S), S1, S2, r
lope (E); the legend refers to **a–c**) were immobilized on 96-well nickel-coated plates at different concentrations. Fixed concentrations
PTX3 (**c**) were incubated over the captured viral proteins. Bound pentraxins were detected by enzyme-linked immunosorbent assay (E
ary antibodies. **d**, Full-length PTX3 or the N- or C-terminal domains were captured on 96-well plates. Biotinylated SARS-CoV-2 nucleo
ncubated at different concentrations. Bound nucleocapsid was detected by ELISA using horseradish peroxidase (HRP)-conjugated stre
resented as mean ± s.e.m.; $n = 3$ independent experiments performed in duplicate; OD_{450}, optical density at 450 nm.

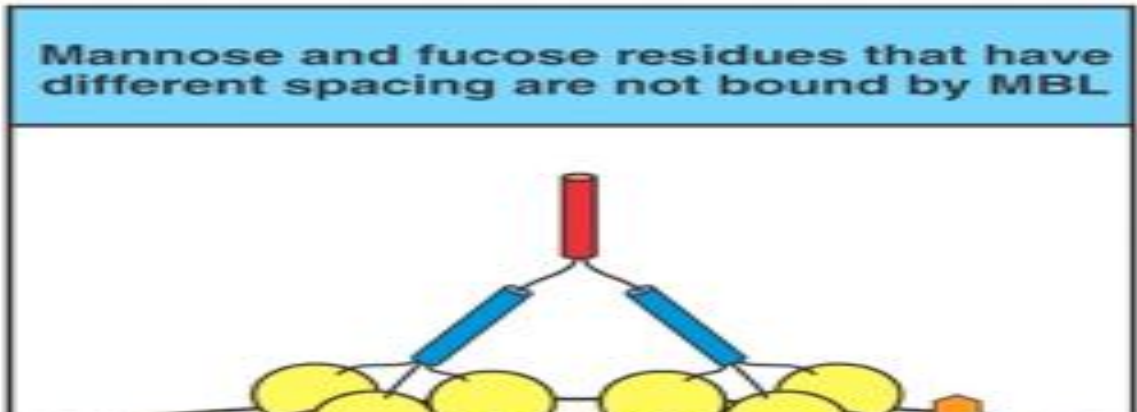
- MBL
- SPA
- SPD
- FICOLINs

is structurally related to the C1q and is part of the fam
otein collectins, together with proteins A and D of
onary surfactant.
as recently discovered that a second group of prote
d ficolins, which includes the L-ficolin, the M-and
n, possesses lectin activity.

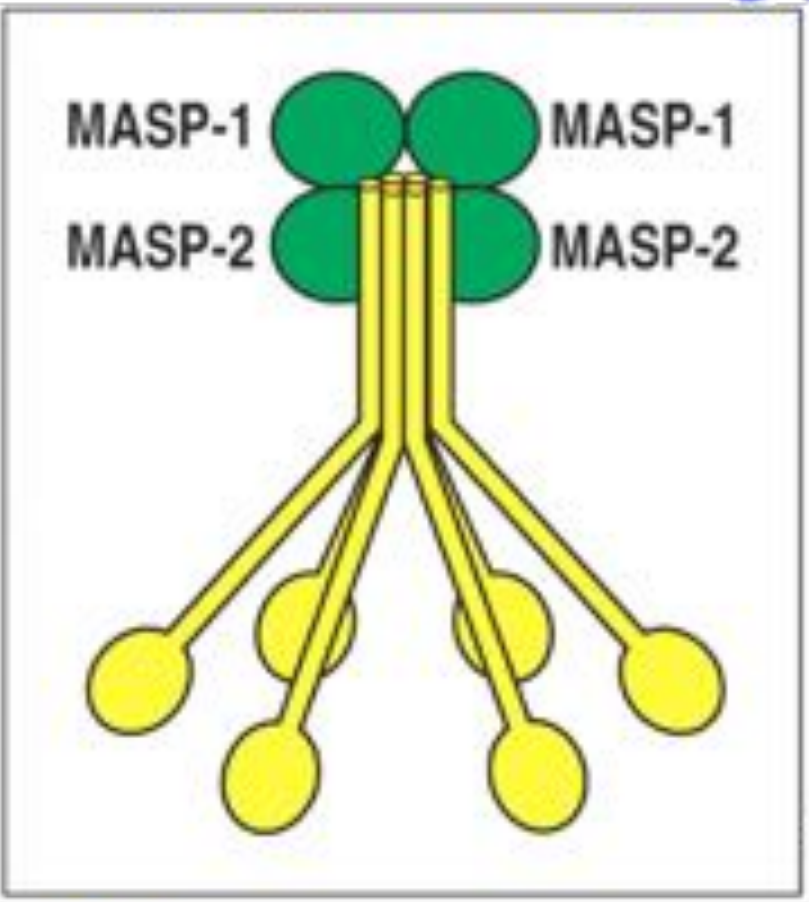


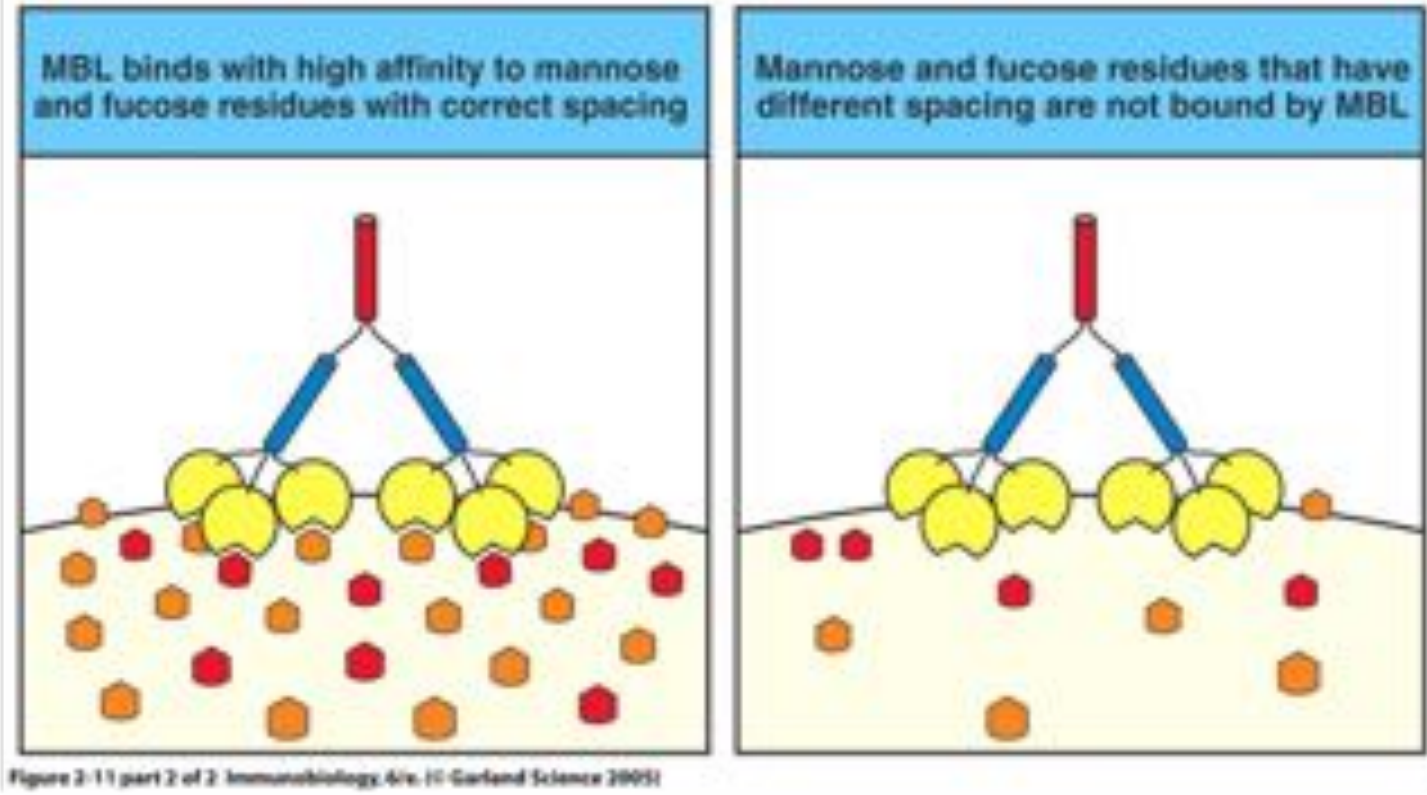
annose-binding lectin (MBL), or mannose-binding protein, recognizes different carbohy
t on the surface of many microorganisms, including bacteria, viruses, protozoa and fung
as an oligomeric structure (400-700 kDa), formed by subunits in their turn consist of
al peptide chains, each of 32 kDa and containing from two to six clusters "of carboh
ition sites" that can bind mannose, maltose, N-acetylglucosamine, N-acetylgalactosa
and glucose.
been shown that the mere presence of these sugar residues is not sufficient for the bind
BL, but their orientation is critical, as they are related only the residues that have a c
arrangement. The bond has a low affinity (Kd 10-3) and, in order to be effective, it is ess
ore "carbohydrate recognition sites" bind simultaneously.





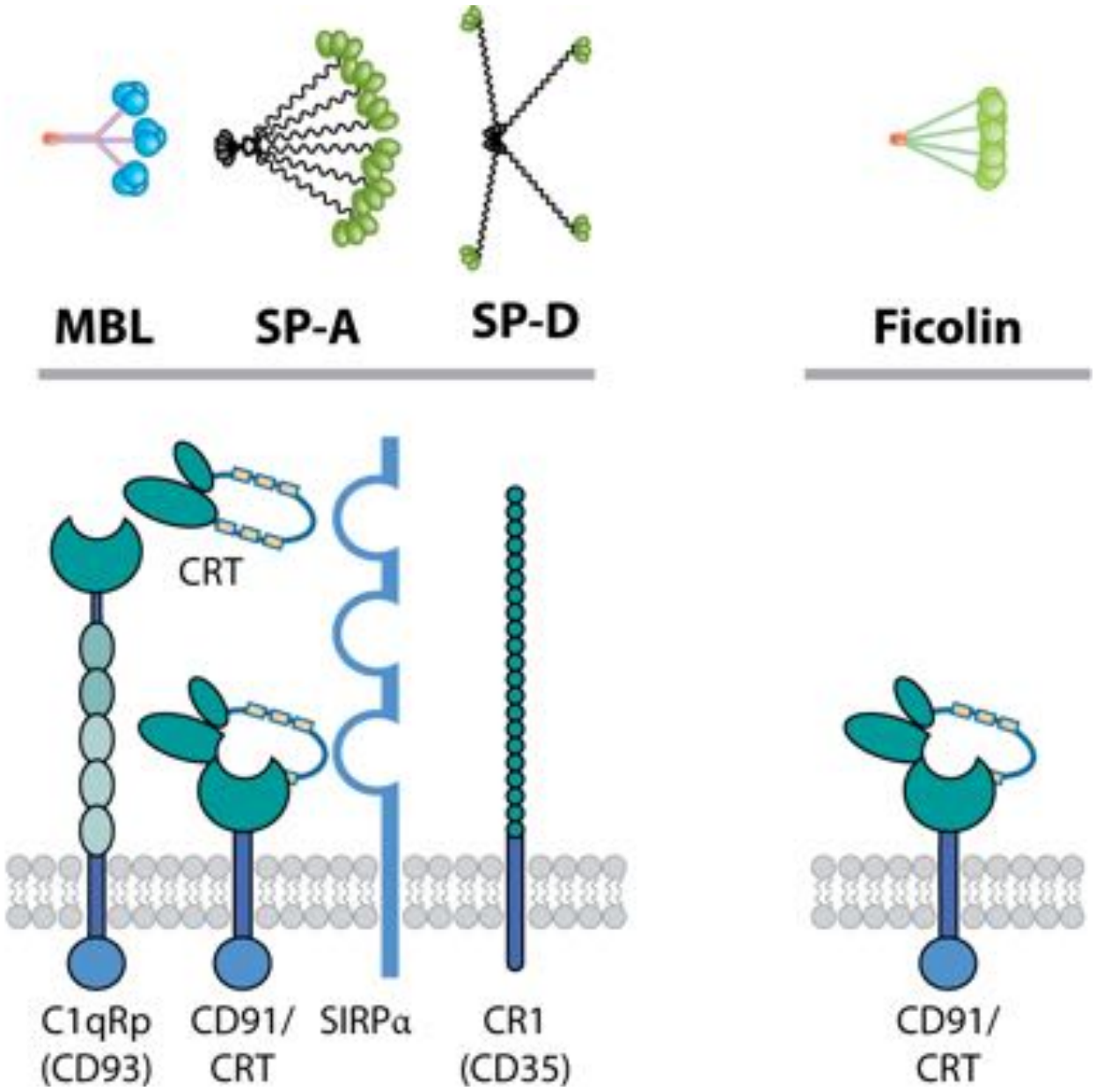
The lectin-binding pathway of complement activation





MBL
SPA
SPD
FICOLINs

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



The humoral collectins activate

The clinical MBL!

plasma concentration of MBL immediately after birth from 1000
o a maximum of 2500 ng/mL within a few weeks, after which it f
ually up to 1700 ng/mL in adults where it can increase up to 20 tir
g infections and inflammatory processes.

anges in the plasma concentration reveal the physiological importance of the MBL th
t at every age during the early stages of contact with the pathogens prior to the incr
concentration of IgM but it is crucial in the period following childbirth, when
ntration of antibodies decreases maternal and starts the production of those of
orn.

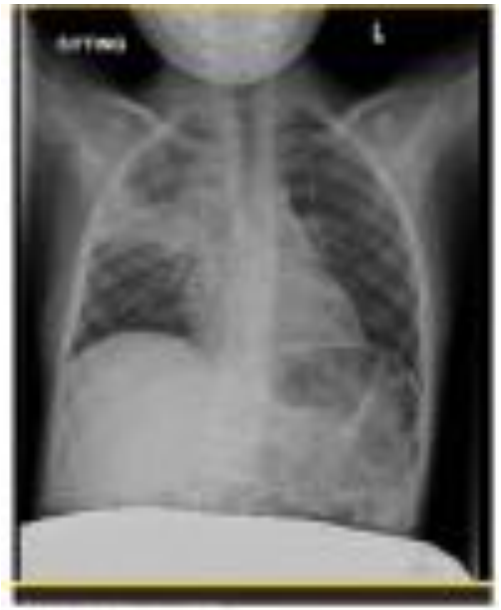
um concentrations of MBL are genetically controlled polymorphisms and/or mutati
moter and coding region of the MBL2 gene and MBL deficiency, the incidence of
to approximately 20-25% of the world population, are associated with incr
bility to infections such as ear infections, pneumonia, gastroenteritis, meningiti
velitis.
dren, a heterozygous mutation of the MBL gene doubles the risk of hospitalization
ous diseases than children with normal MBL levels; in case of a homozygous mutat
ne, the risk of infection is also increased and the disease is worse.
ts with cystic fibrosis with a mutation in the MBL gene are more susceptible to infect
omonas aeruginosa and have a lower life expectancy than patients with cystic fi
ut this mutation.
r patients undergoing chemotherapy and consequently to that, develop neutropen
susceptible to long periods of fever if they have a low concentration of MBL.
dren under one year suffering from Kawasaki disease, whether MBL deficiency is
for coronary aneurysms.
-positive patients the progression of AIDS is faster in case of deficiency of MBL.
deficiency is associated with the occurrence of recurrent spontaneous abortions
bly, to intrauterine infections.
MBL deficiency is also associated with autoimmune diseases such as systemic
matosus (SLE) and rheumatoid arthritis (RA)

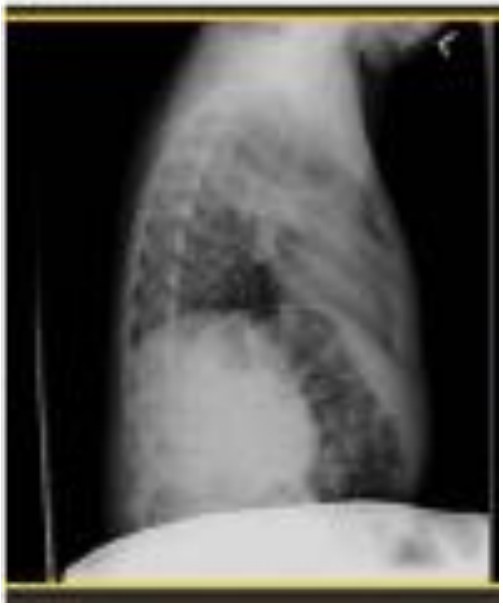
d Insights Pediatr. 2012; 6: 89–94.

se Binding Lectin Deficiency: lore than Meets the Eye

nelle Halbrich, Moshe Ben-an, and Christine McCusker

report describes a 5-year-old boy who to the emergency department with ymptoms and chest X-ray findings of pneumonia. Further history revealed other infections, and workup for eficiency revealed a deficiency of binding lectin (MBL), a pattern receptor involved in activation of the nt system. Innate immunodeficiency may ommon than currently appreciated, with of MBL affecting up to 50% of individuals populations. While pneumonia is a resentation in the Pediatric Emergency t, clinical presentations of children with innate immunity can be unpredictable. nay initially appear well with sudden tion. These cases pose particular to physicians, and the level of suspicion defects must remain high. It is crucial to tients with such impairments to better d prevent future complications.





X-ray from the emergency department demonstrating a

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 DEFIC

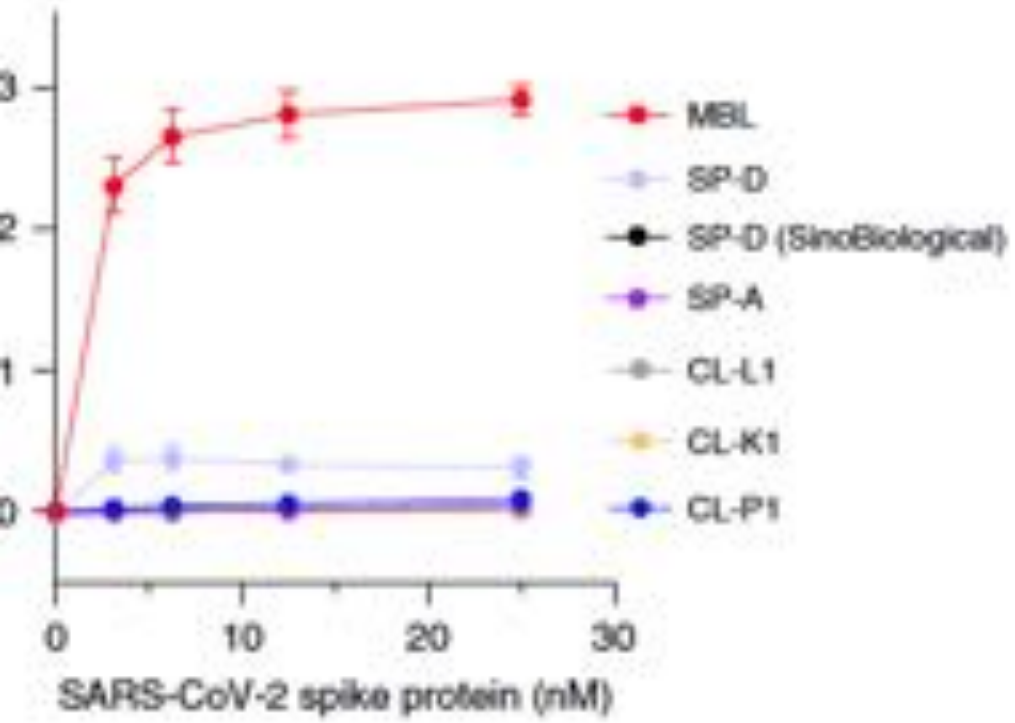
MBL	1470	(>100 ng/ml V.N.)

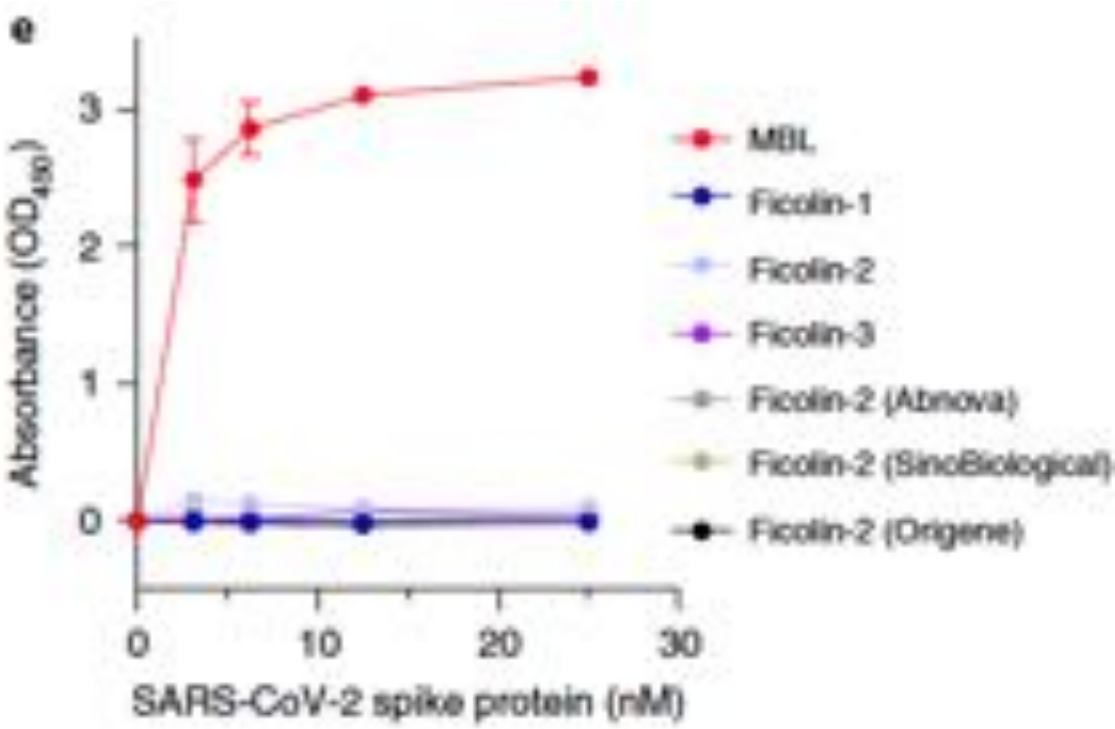
Il test è stato eseguito mediante MBL Oligomer ELISA kit (BioPorto Diagnostics).

Il Responsabile

Fabrizio Mainiero

l specifically to the spike protein of SARS-CoV-2!





action of C1q, MBL, ficolins and surfactant proteins with SARS-CoV-2 proteins. a–c, Recombinant His tag SARS-CoV-2 proteins (s
, S2, nucleocapsid (N) and envelope (E)) were immobilized on 96-well nickel-coated plates at different concentrations. Fixed conc
MBL (**b**) were incubated over the captured viral proteins. Recombinant SARS-CoV-2 spike proteins tested were expressed in differ
. MBL (2 μg ml^{-1}; 6.7 nM) was incubated over the captured viral proteins. In **a**–**c**, bound proteins were detected by ELISA with speci
odies. Data in **a** and **b** are presented as mean ± s.e.m.; $n = 3$ independent experiments performed in duplicate. Data in **c** are presen
.; $n = 2$ independent experiments, one performed in duplicate and one in triplicate. **d**,**e**, MBL-, CL-L1, CL-K1, CL-P1-, SP-D- and SP-A
ficolin-1-, ficolin-2- and ficolin-3-coated plates (**e**) were incubated with various concentrations of biotinylated SARS-CoV-2 spike p
protein was detected by ELISA with HRP-conjugated streptavidin (mean ± s.e.m.; $n = 3$ independent experiments in duplicate). **f**, S
combinant full spike protein trimer to immobilized MBL (dissociation constant (K_d) = 34 nM, left). No binding was detected in the a
; RU, resonance units.

The SPA and SPD!

Proteins A and D of the pulmonary surfactant or SPA and SPD are also defined as pulmonary collectins found mainly in bronchial fluids where they are synthesized by alveolar macrophages and Clara cells.

Their serum concentrations are around 100 ng/ml but may change in relation to the lung pathology in progress.

Their concentration may increase in pulmonary disorders such as pulmonary alveolar proteinosis (PAP) and decrease in pulmonary fibrosis and acute respiratory distress syndrome (ARDS).

humans have been identified three ficolins: the H-ficolin (Ficolin-2), also known as th
ata antigen, the L-ficolin/P35 (Ficolin-3) both serum proteins, and a third ficolin call M
lin/P35-related (Ficolin-1), not present in the serum.

st recently these ficolins has been associated with a new membrane protein calle
CD1, which binds groups of acetylated sugar residues expressed by pathogens ar
naged cells.

ilar to the MBL, the three human ficolins bind carbohydrates on the surface
nogens and activates the lectin pathway of complement through the MASPs.

e H-Ficolin is synthesized in both the liver in the lung and
esent in serum at a concentration average of 15 µγ/ml.

H-ficolin may be absent in patients with SLE, probably due to the presence
pantibodies anti-H-Ficolins, and in liver diseases where the serum levels decrease wi
easing severity of cirrhosis.

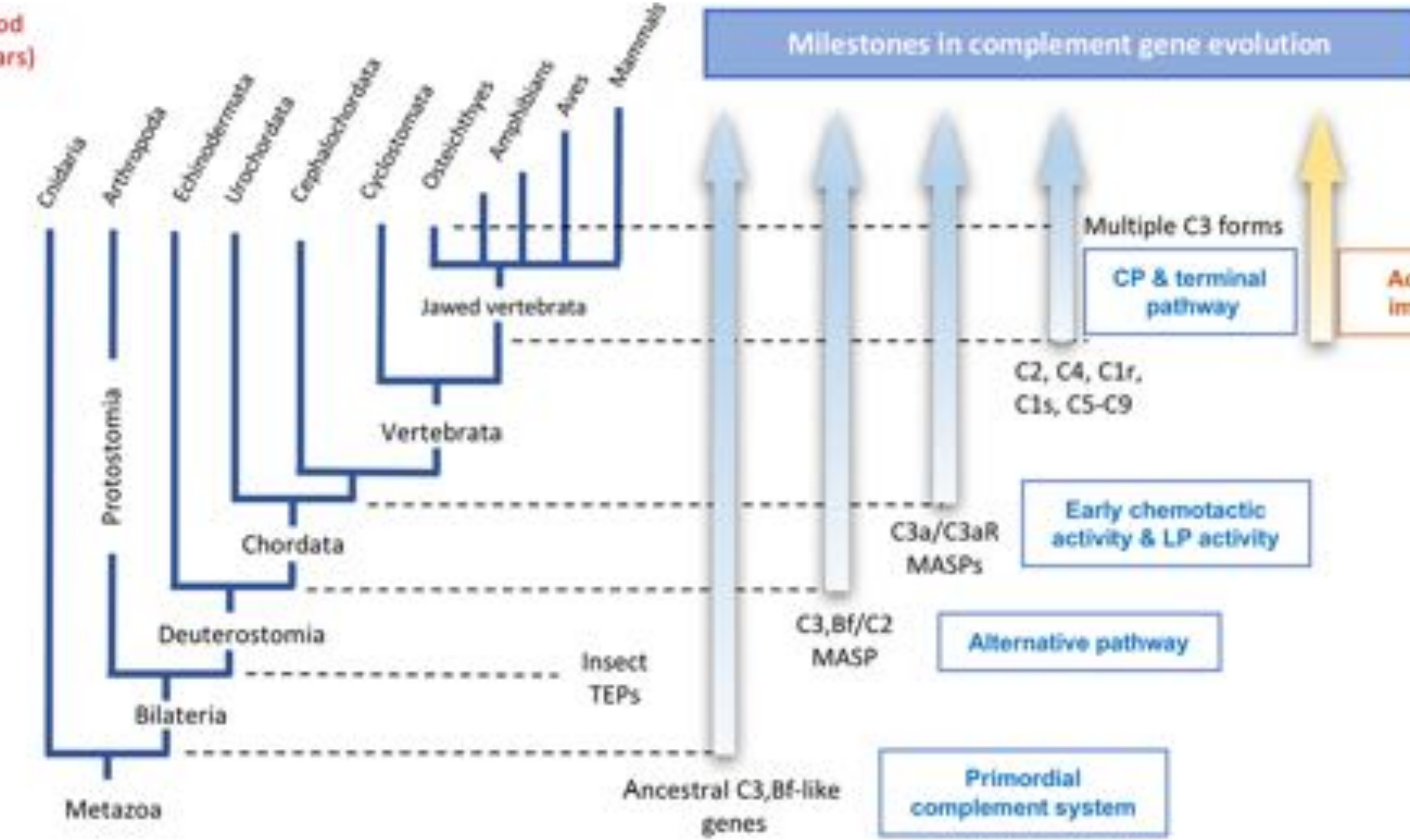
y recently has been described a mutation of the gene FCN3, coding for the H - ficol
cause defects of complement activation, and gene polymorphisms FCNI whi

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	C5a C3a

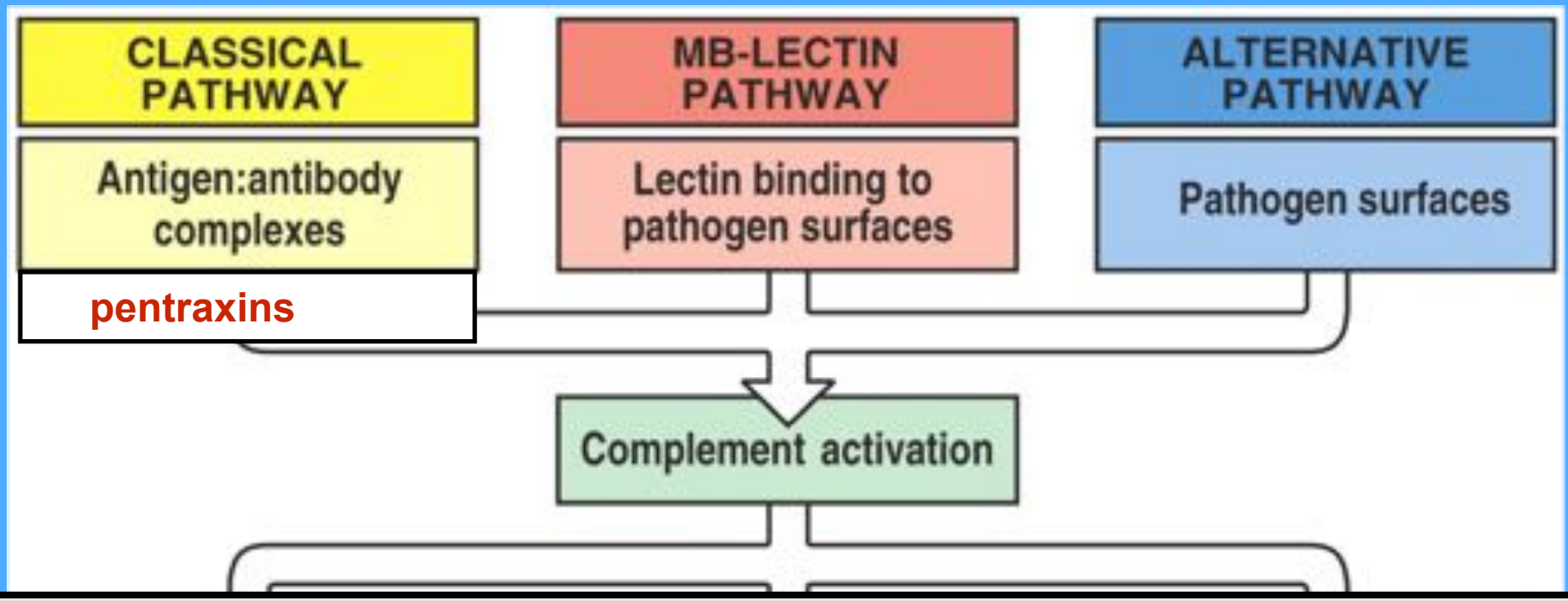
Functional protein classes in the complement system	
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

The COMPLEMENT is the oldest defense system!



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

E THREE MECHANISMS OF COMPLEMENT ACTIVATI

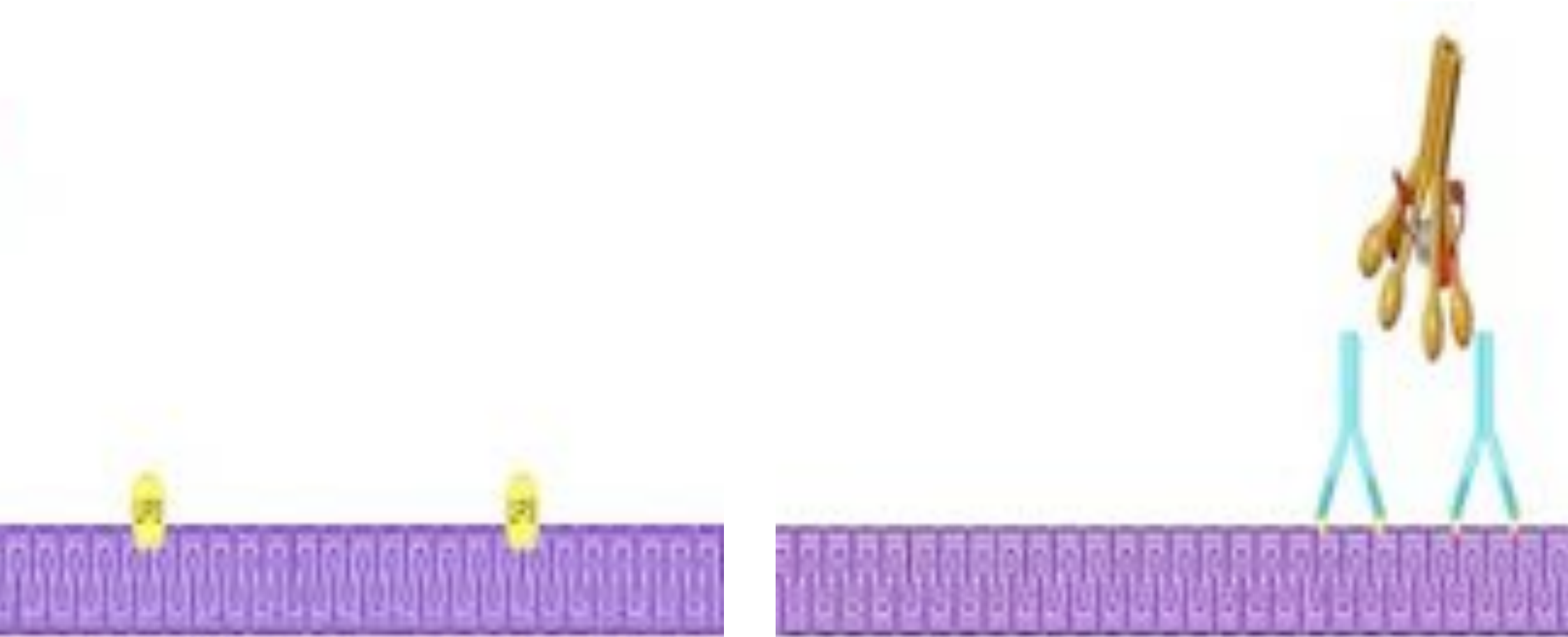


ALL THREE MECHANISM HAVE C3 AS CENTRAL PROTEIN AND CONVERGE IN THE ACTIVATION OF C5!!!

OF COMPLEMENT ACTIVATION!

TERNATIVE PATHWAY

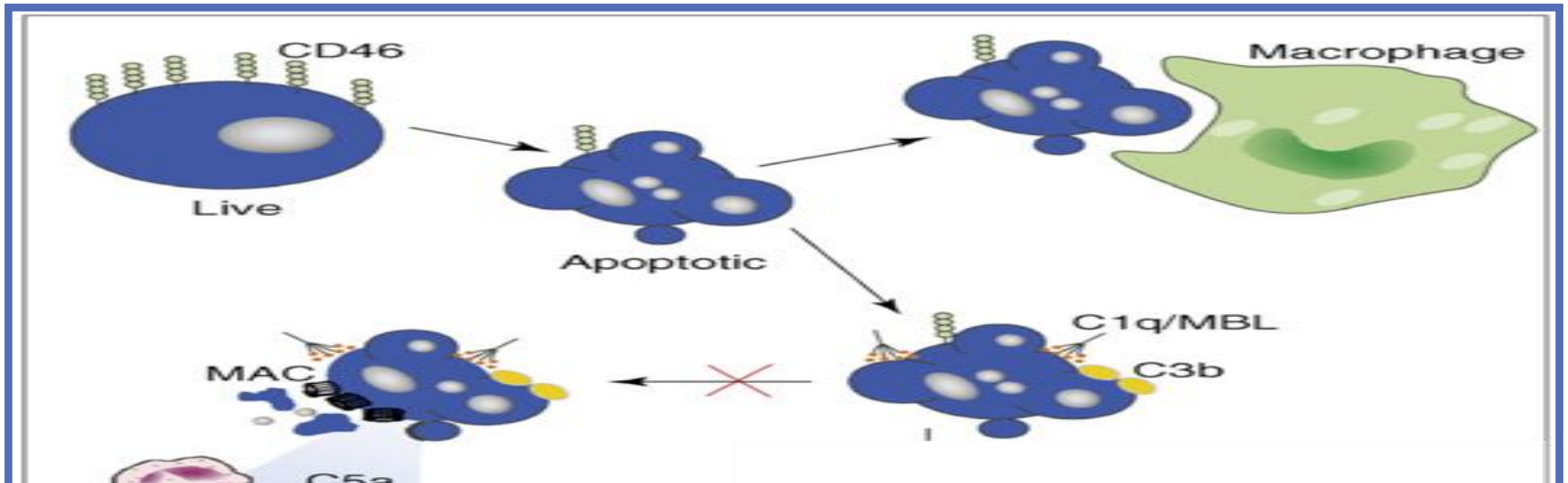
CLASSIC PATHWA



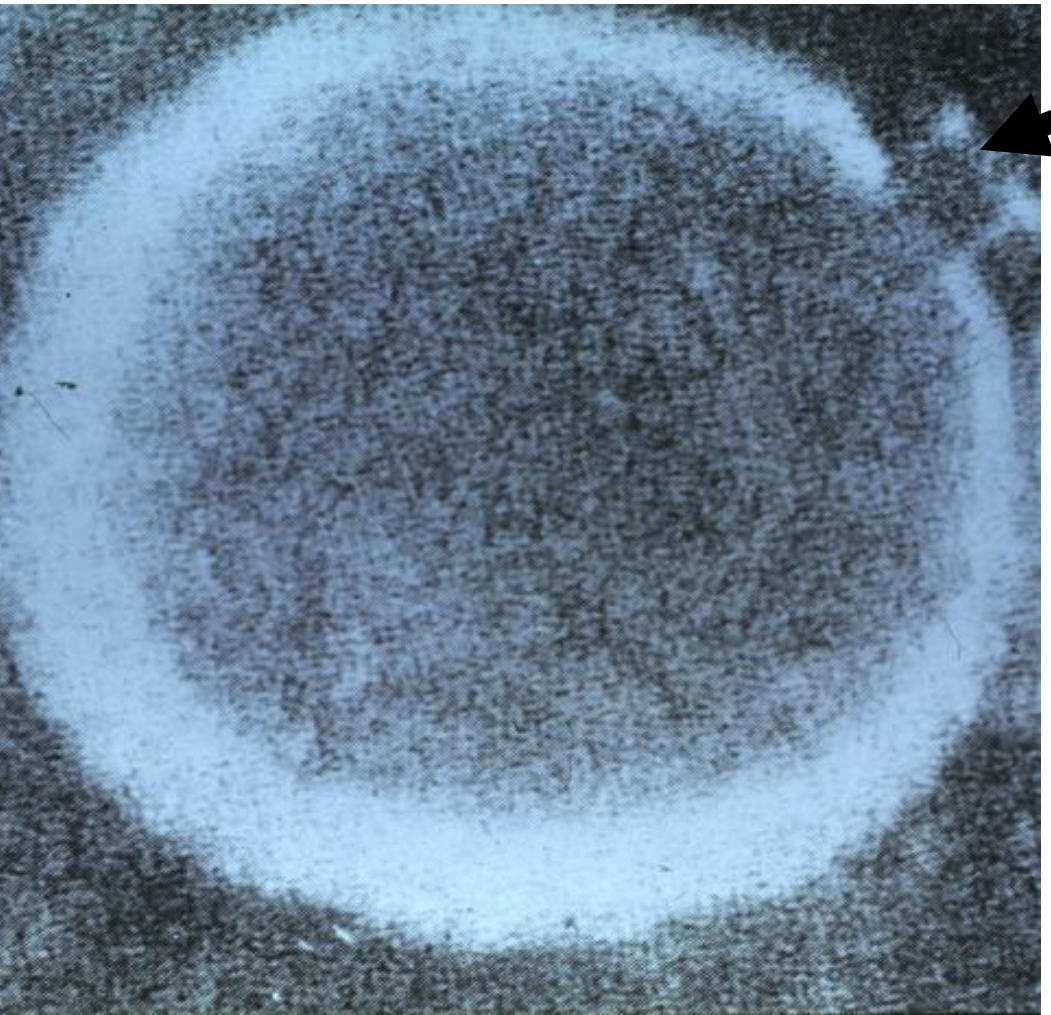
activated by pentraxins !

ASSICAL COMPLEMENT ACTIVATION IN THE NATUR IMMUNITY AND INFLAMMATION!

ddition to recognizing the Fc portion of antibodies, C1q binds to pentraxins (CRP, S
X3) through its gC1q domain. gC1q also binds directly to many gram-negative bact
ugh Omp, LPS, or lipid A and to viruses (e.g., gp41 of HIV-1 or gp21 of HTLV-1). C
o interacts with misfolded proteins, such as amyloid Aβ peptide and prion proteins fo
neurodegenerative diseases and with several ECM proteins (such as fibromodu
eoadherin, fibronectin, and laminin). Finally, C1q binds via the globular head domai
face blebs on apoptotic cells and to necrotic cells directly or through **pentraxins**!



LITHIC COMPLEX IN CELL MEMBRANE or MAC, WHICH DESTROYS PATHOGENS .....



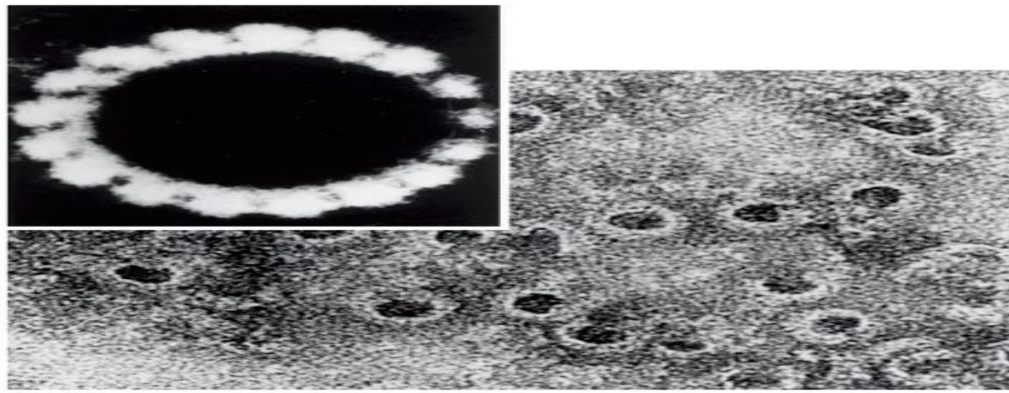
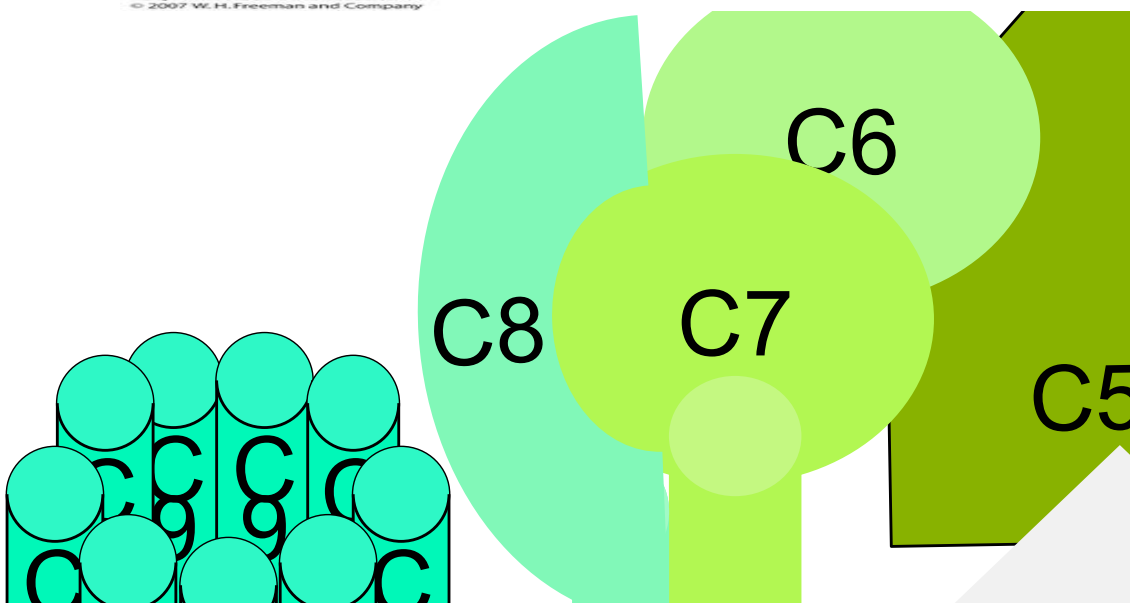
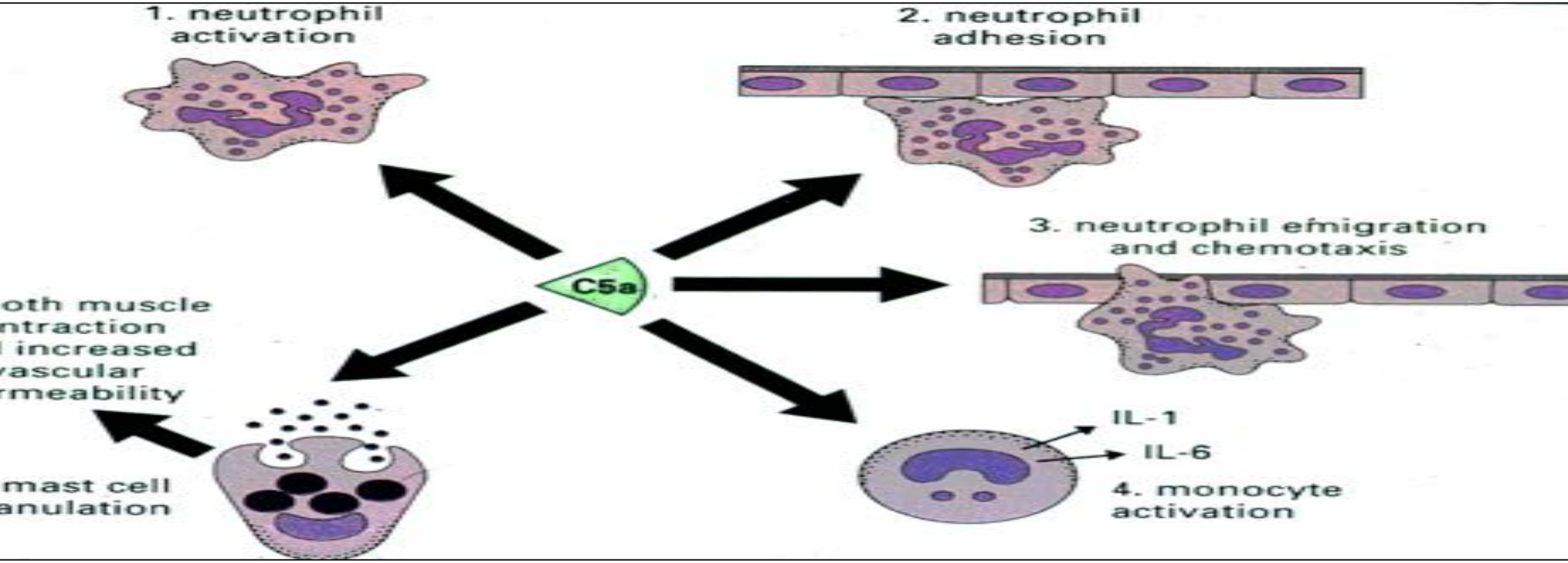


Figure 7-8
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W. H. Freeman and Company



AND
OF THE PHAGOCYTOSIS OSPONINS!

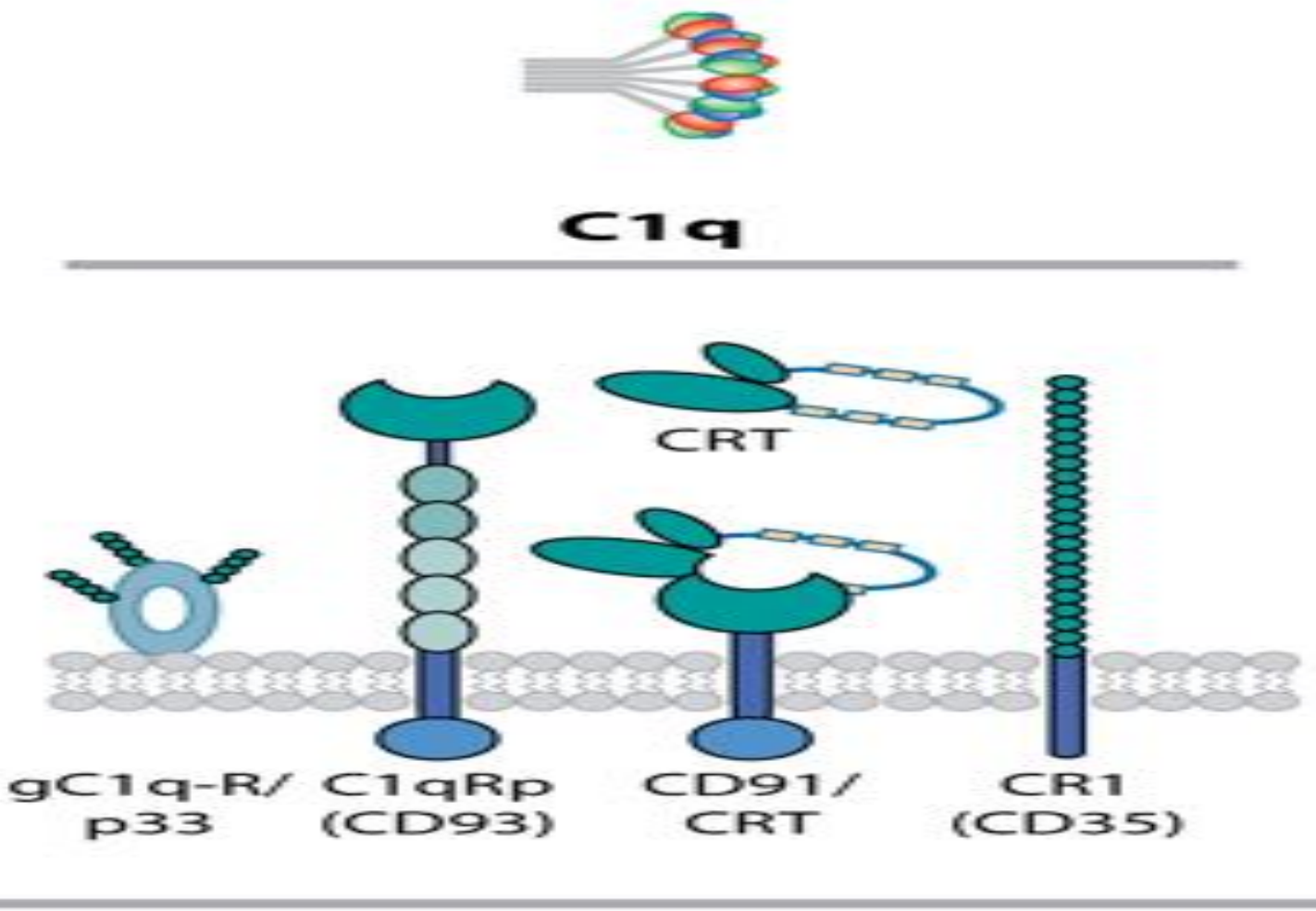
APHYLATOXINS (C5a-C3a)



ONINS (C4b-C3b)-PHAGOCYTOSIS

binds to a wide range of cell types (PMN, monocytes, lymphocytes, DCs, ECs
ets), resulting in the induction of cell-specific biological responses, which in
ocytosis, chemotaxis, the generation of procoagulant activity, activation of
nhancement of FcγR- and CR1-mediated phagocytosis and superoxide produc

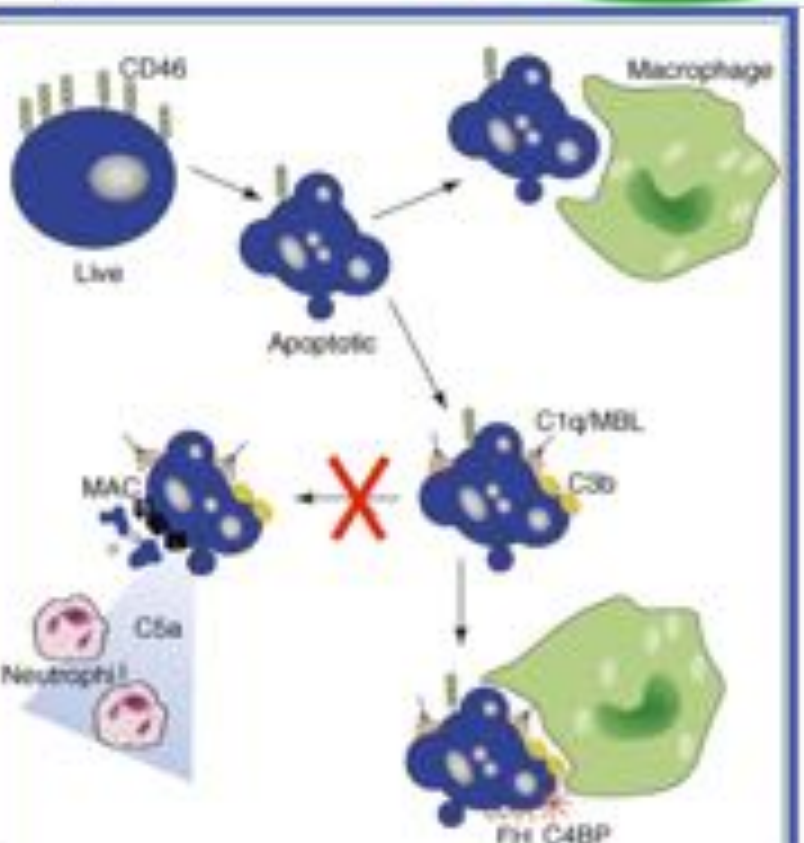
ceptors for 1q humoral omplement factor !



ate, investigators have described four types of C1q-binding proteins/receptors exp
e cell surface. These include cC1q-R/calreticulin (CRT), a 60-kDa protein ; gC1q-R/
Da homotrimeric protein; C1q-Rp (CD93), a 120-kDa O-sialoglycoprotein; and
5), the receptor for C3b. In addition to C1q, CRT reportedly serves as a recep

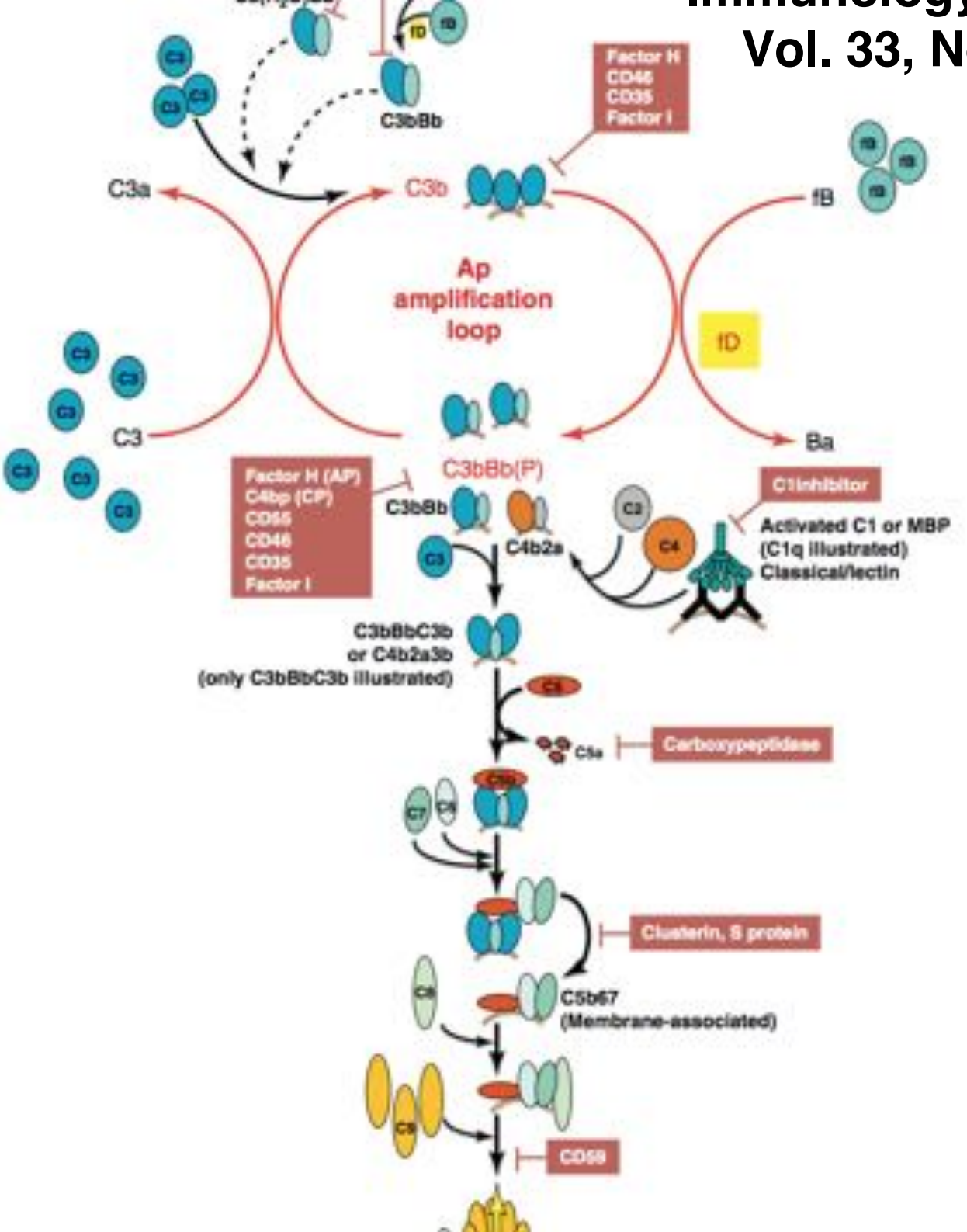
Table 1 | **Complement interactions with pathogens and self**

	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

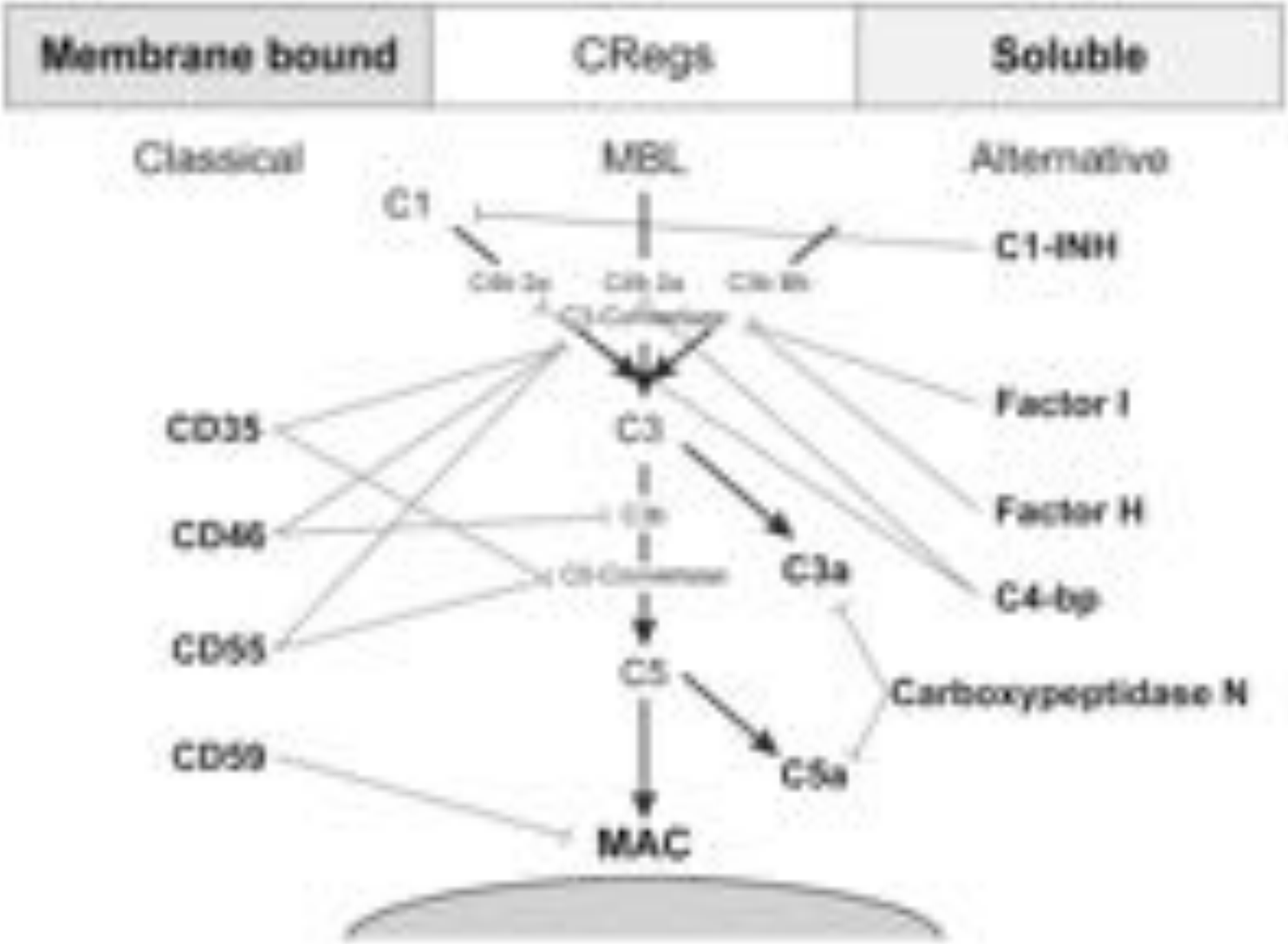


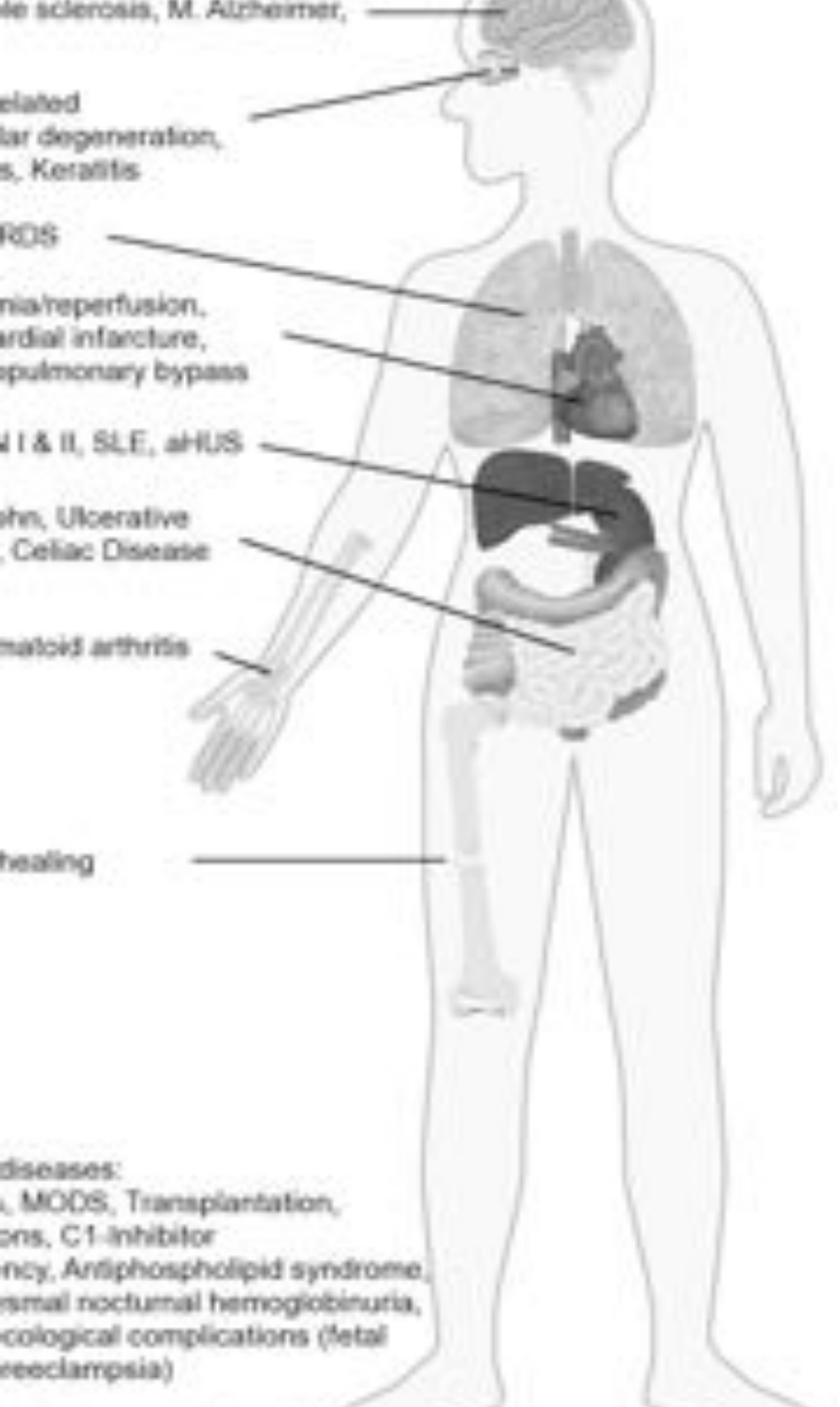
Complement activation on apoptotic cells depends on recognition by C1q and C3b/iC3b; the binding of Factor H and C4BP allows phagocytosis, without substantial activation of the terminal complement pathway and inflammation

lysis of the C3 thioester, or cleavage to C3b by plasma proteases. Fluid production of either molecule results rmation of the AP C3 convertase, b, and production of further C3b either binds a surface or remains phase. Each newly produced C3b can n form a convertase, which cleaves esulting in exponential production of This self- propagation, referred to as mplification loop' and indicated here , is responsible for amplifying a small er to yield large responses. C3b ed through any activation pathway into the amplification loop. Binding of to C3 convertase creates C5 rtase; cleavage of C5 and generation 5b marks the start of the terminal way. C6 and C7 bind C5b to form 7, which is released from convertase, membrane and incorporates C8 and ple C9 molecules to form the MAC. issues are protected from accidental lement damage by regulatory proteins nt in plasma and on membranes,

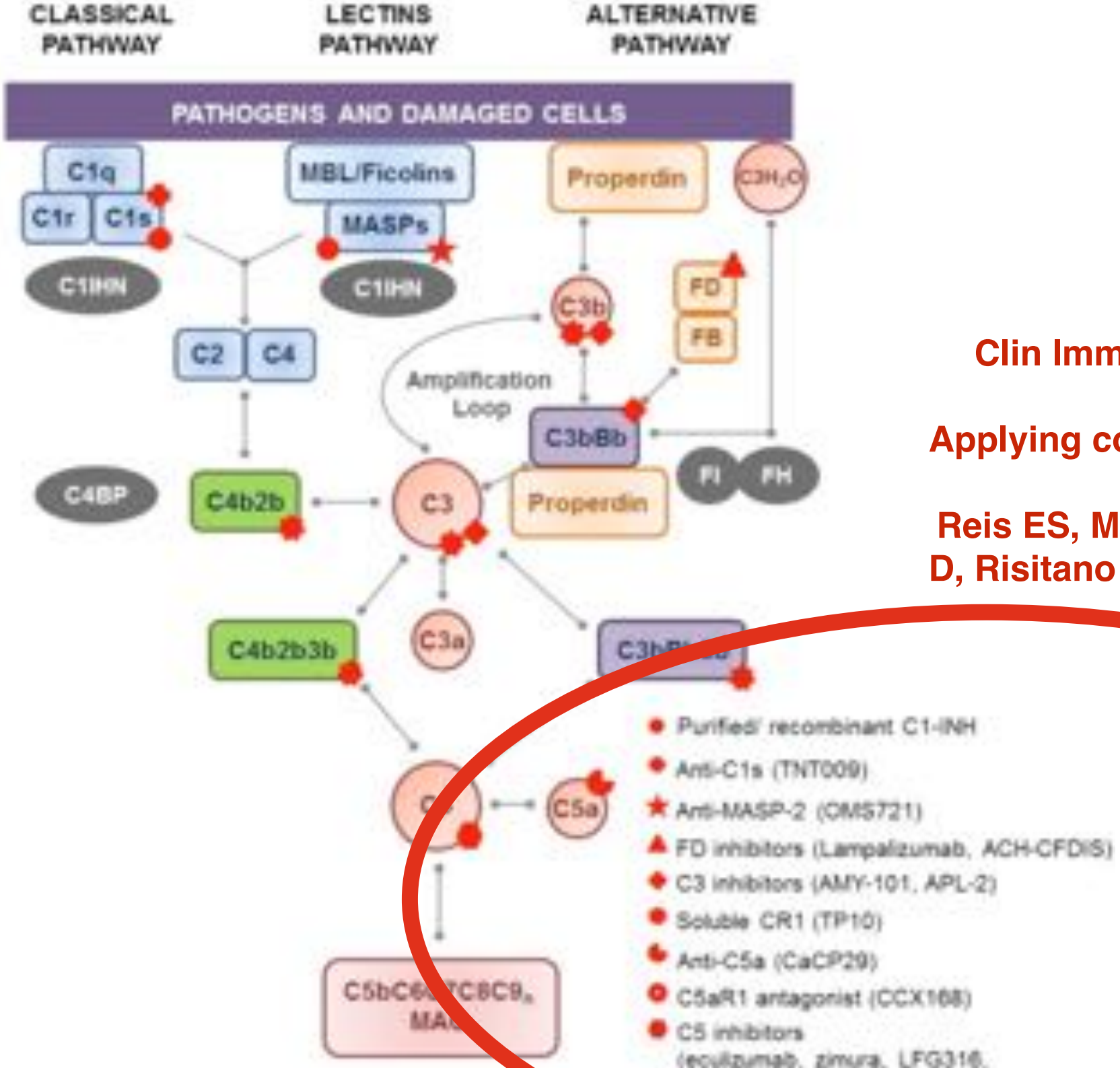


complement cascade!



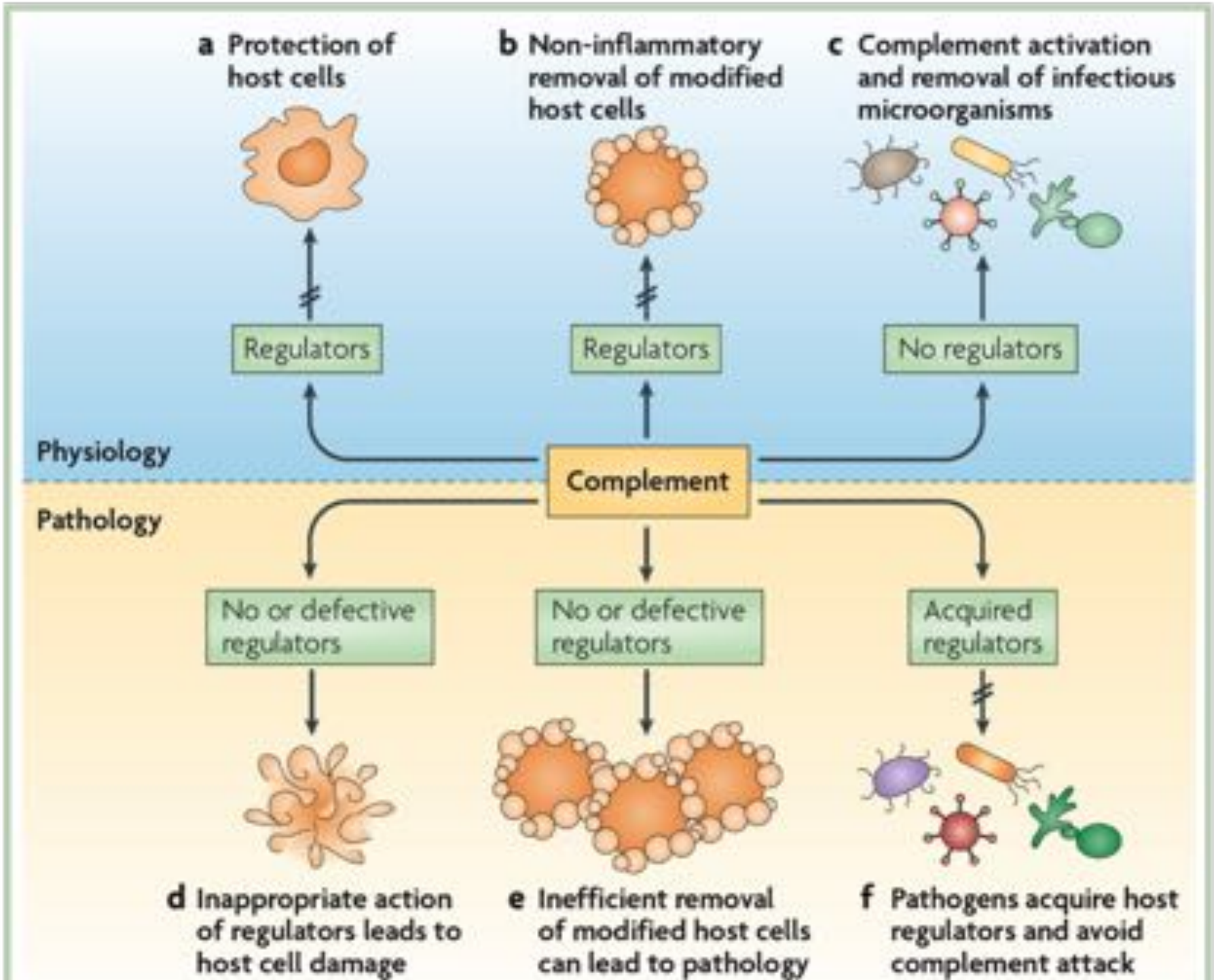


ALS, amyotrophi lateral sclerosis; AL acute lung injury ARDS, adul respiratory distres syndrome; MPGN membranoproliferativ glomerulonephritis SLE, systemic lupu erythematosus; aHUS atypical hemolyti uremic syndrome MODS, multiple orga dysfunctio syndrome.



Clin Immunol. 2015 Sep 1;161(
225-240.
Applying complement therapeuti
rare diseases.
Reis ES, Mastellos DC, Yancopou
D, Risitano AM, Ricklin D, Lambri

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



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.

ectrophotometric measurement of the hemoglobin releas

Correlation between hemoglobin released.

CH50 reduction correlated with the reduction of the levels

observed reduction of complement by:

onsumption of C for the formation of immune-comple

ecreased synthesis of C;

creased catabolism of C.

n of tion	CH50	C4	C3	Factor B	Conditions wi Activation Pa
	Decreased	Decreased	Decreased	No change	SLE, SS, RA, an cryoglobuline
tive	Decreased	No change	Decreased	Decreased	Endotoxemia; II MPGN
al and tive	Decreased	Decreased	Decreased	Decreased	SLE, shock, an immune comp diseases
hase on— l	Decreased	Decreased	No change	No change	Hereditary angioedema; malarial infecti vivax)
hase	Significantly increased	Significantly increased	Significantly increased	Significantly increase	Acute and chr inflammation; pregnancy

nic lupus erythematosus; SS, Sjogren syndrome; RA, rheumatoid arthritis; MPGN, membranoproliferative

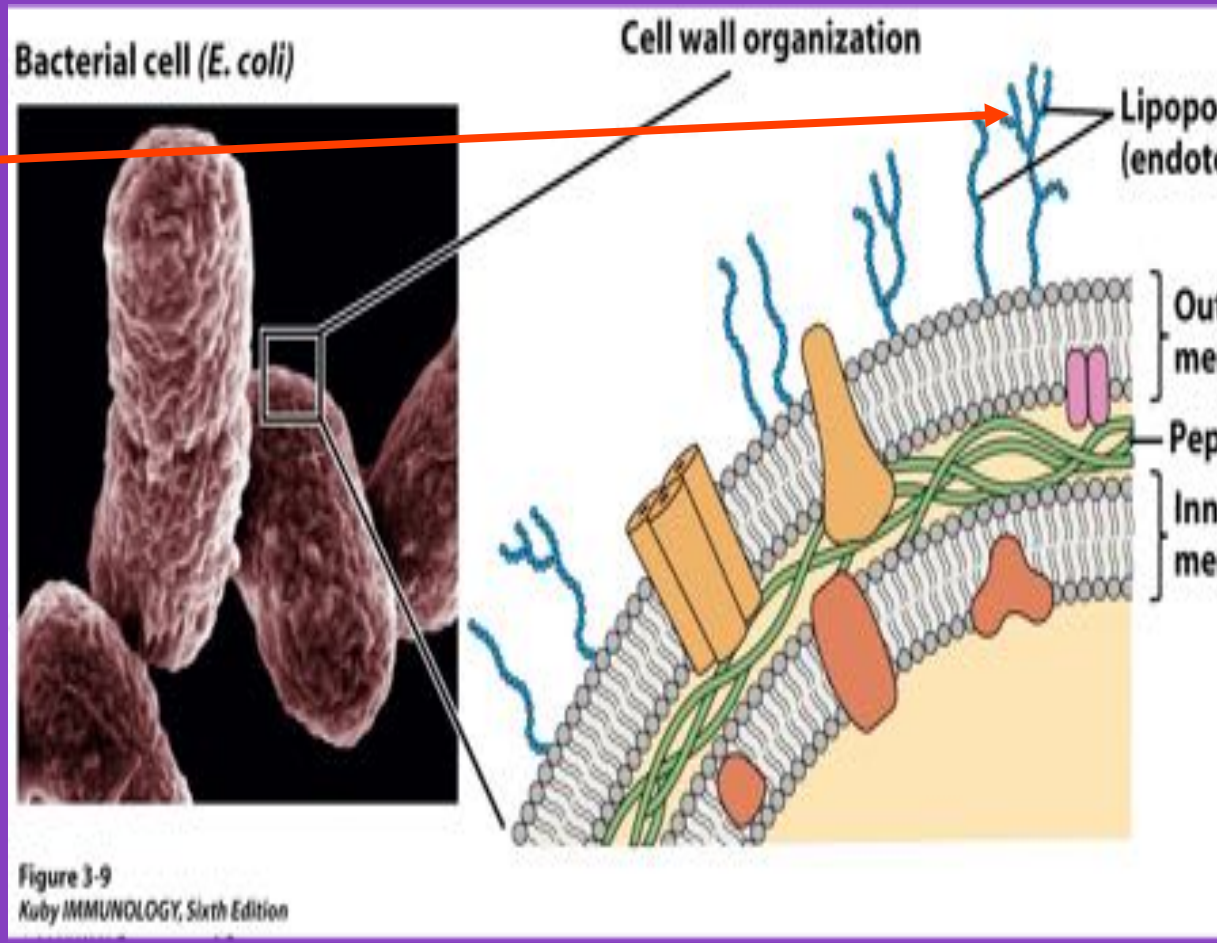
he LPS binding protein or LBP was identified in 1990 ar
present **in serum at a concentration of less than 0.5 µ**
l but which reaches 50 µγ/ml at 24 hours during an AP

is a 60 kDa protein synthesized by hepatocytes, ha
inding sites for the lipid A of LPS, which binds with hig
ffinity to CD14 and transports of phagocytes to th
ubsequent binding with TLR4 and activation of th
roduction of inflammatory cytokines.

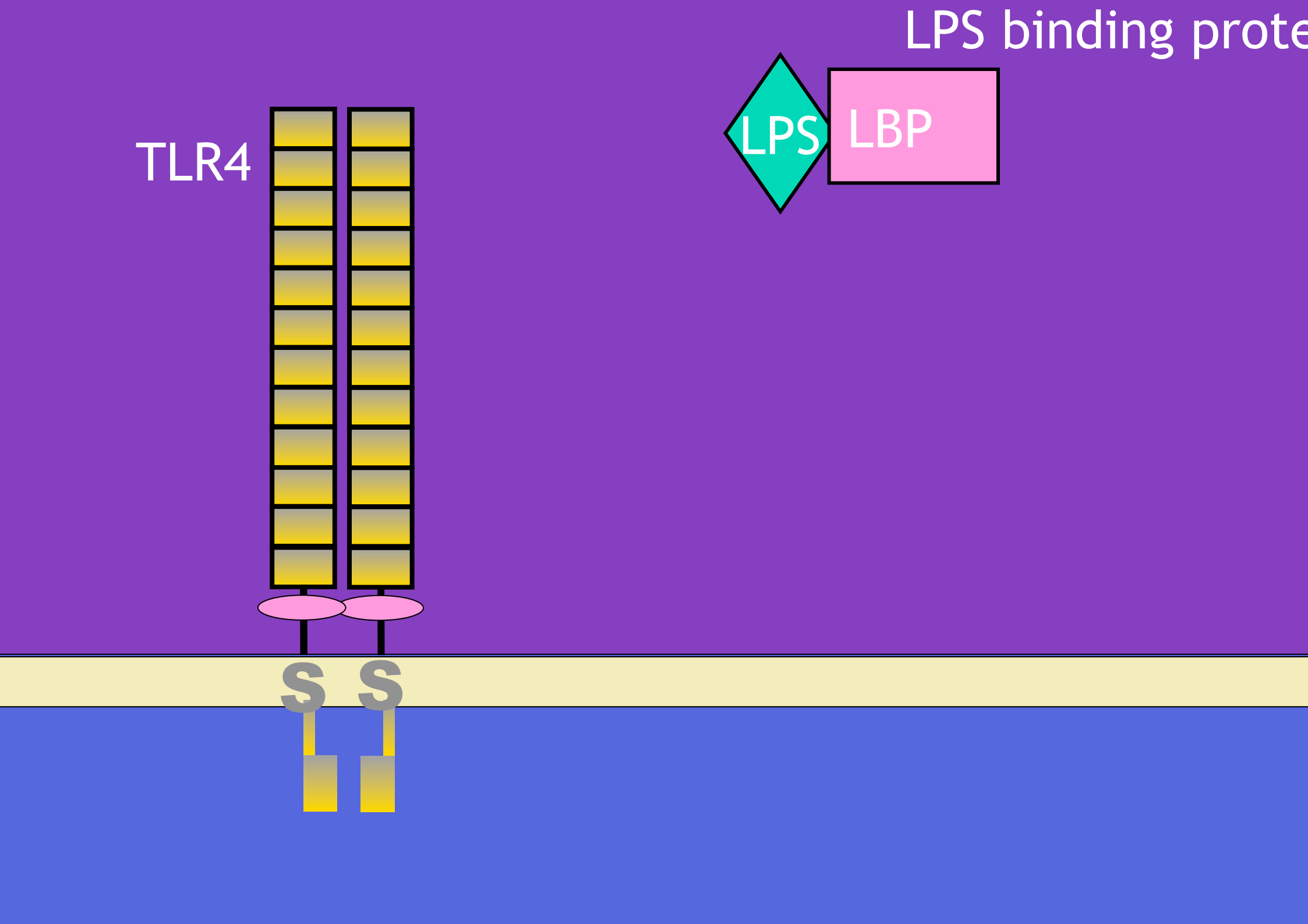
lthough deficits have not been found in humans, th
nportance of LBP is underscored by the fact that knocko
nice (KO) to LPB are much more susceptible to Salmonel

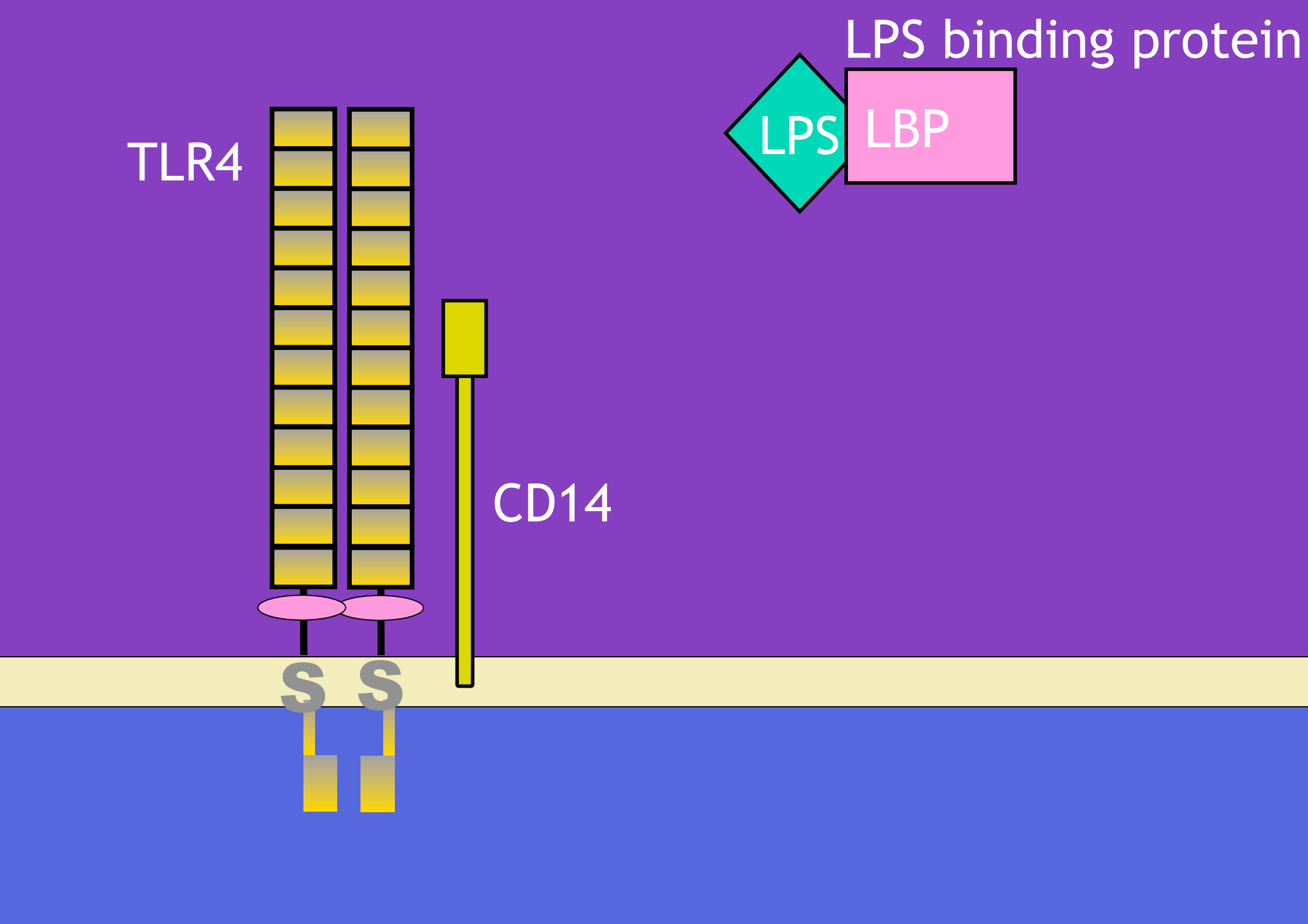
TLR4

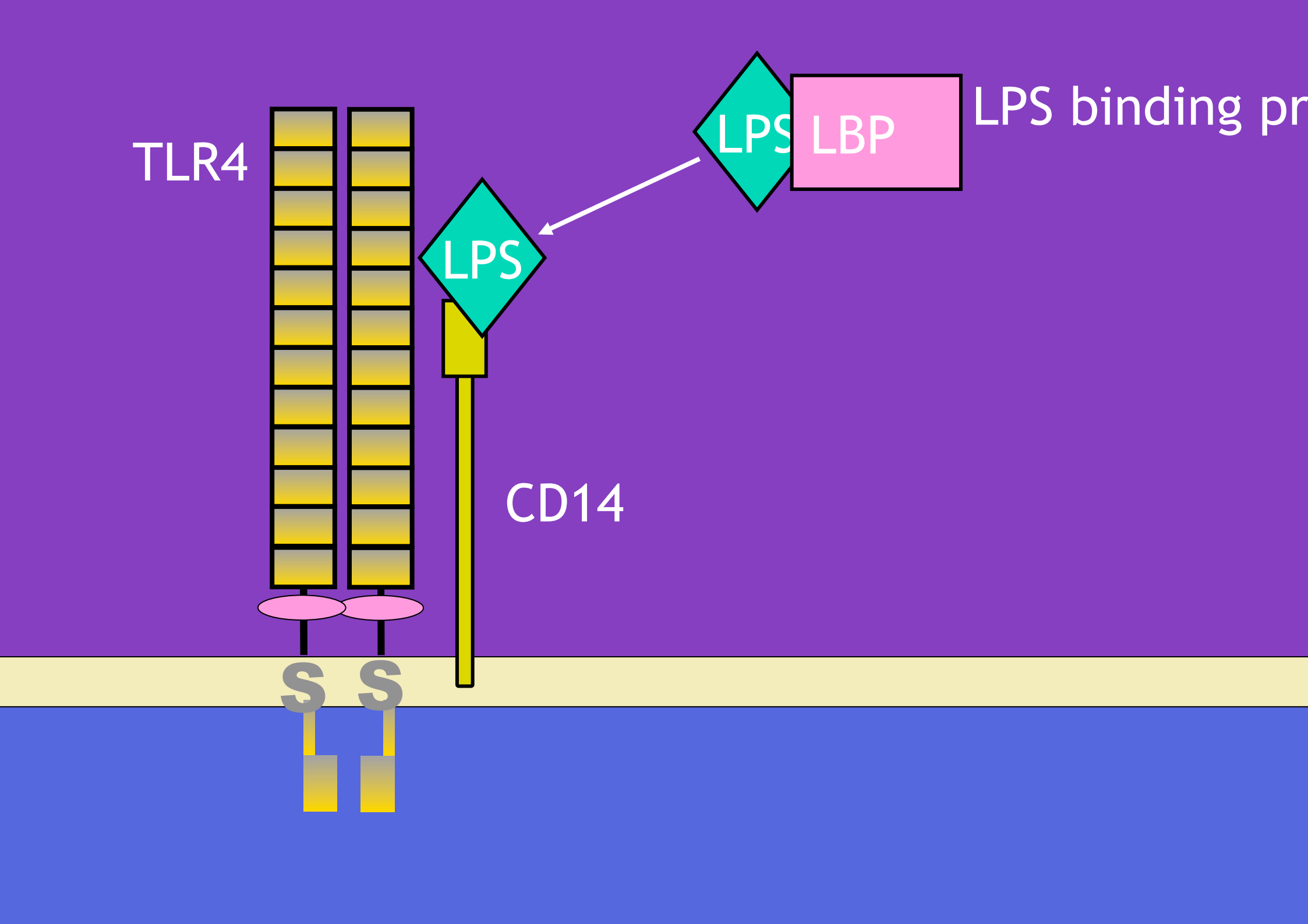
LPS

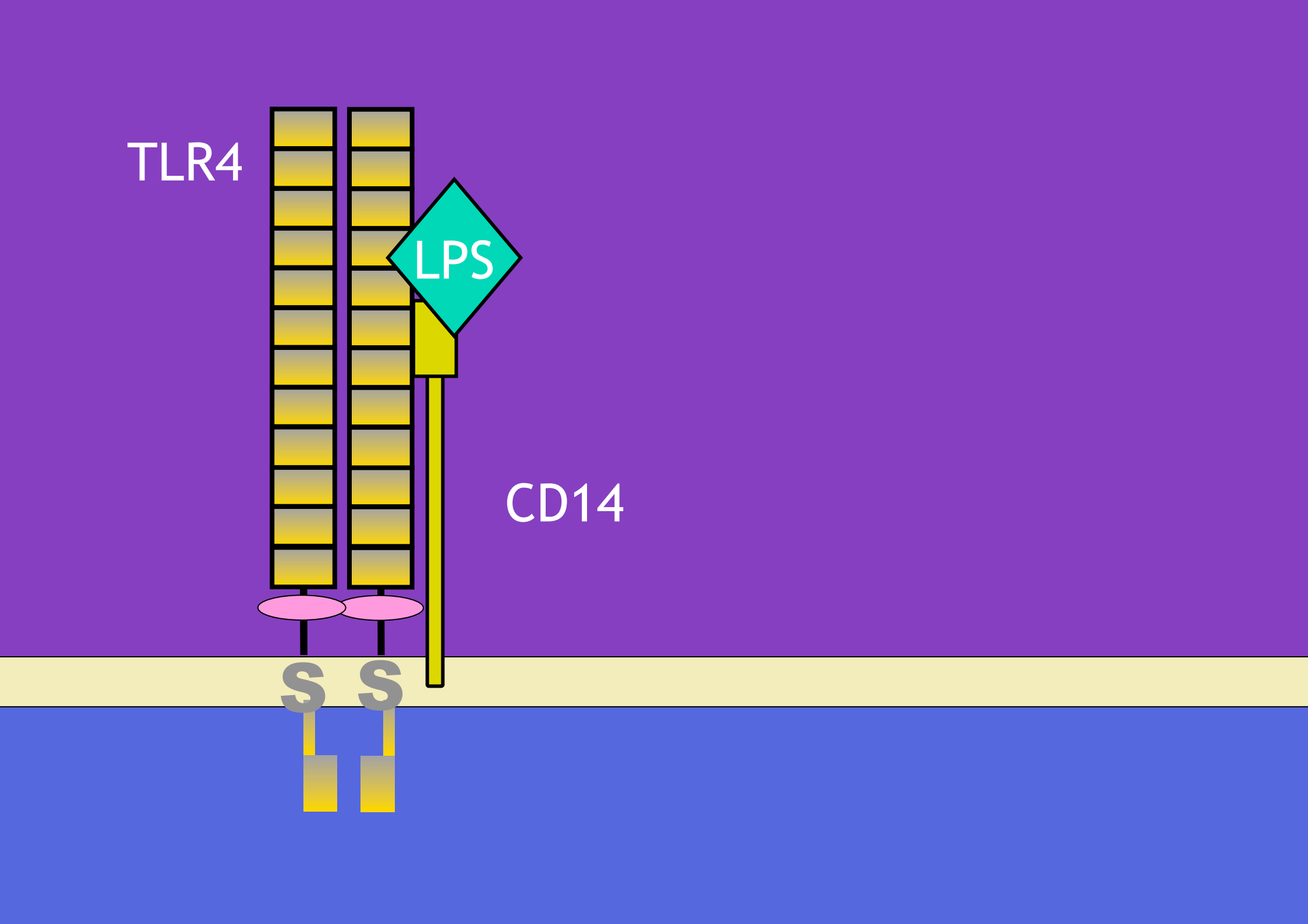

Bacterial cell (E. coli)
Cell wall organization
Lipopo
(endot
Ou
me
Pep
Inn
me
Figure 3-9
Kuby IMMUNOLOGY, Sixth Edition

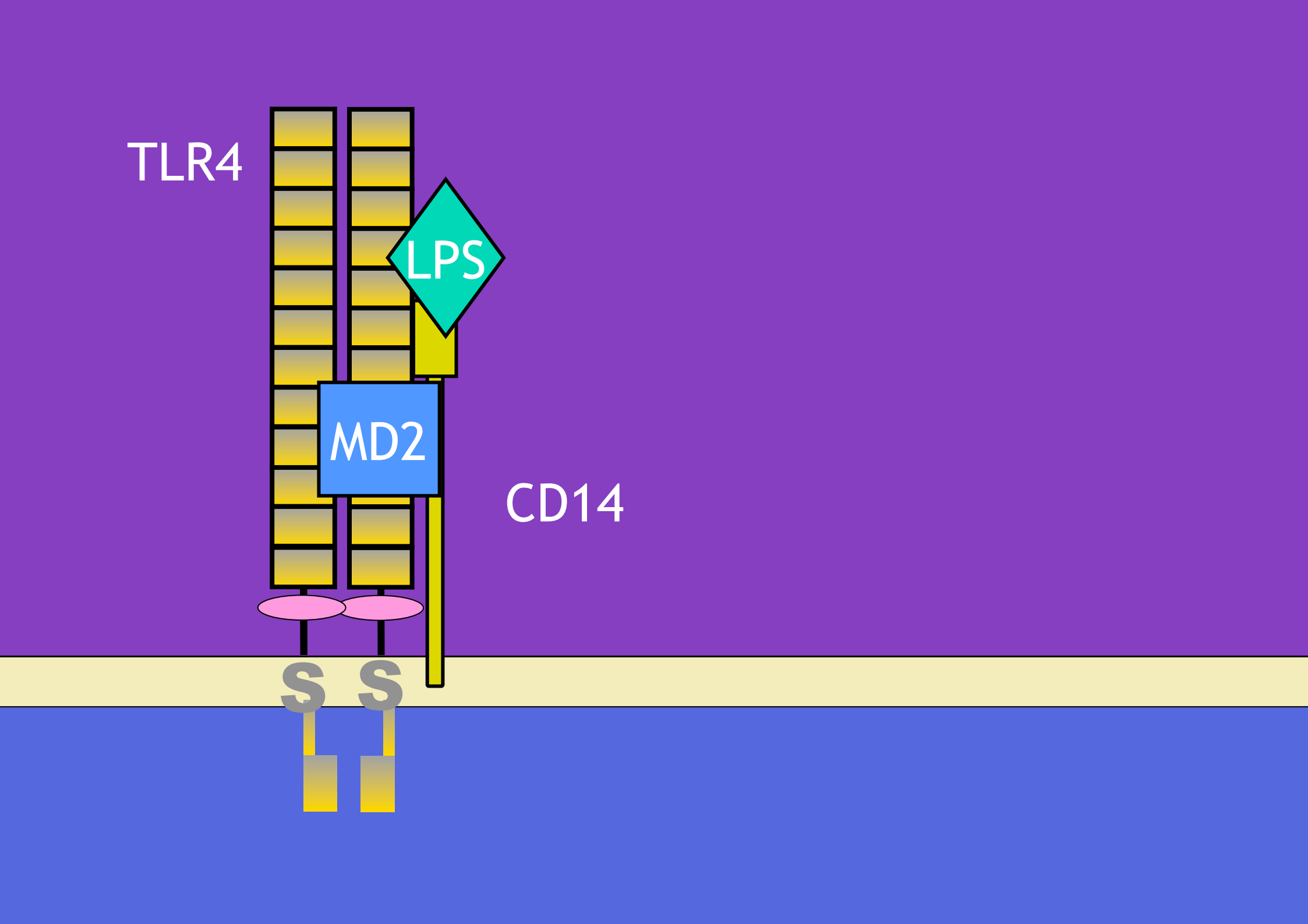
S S

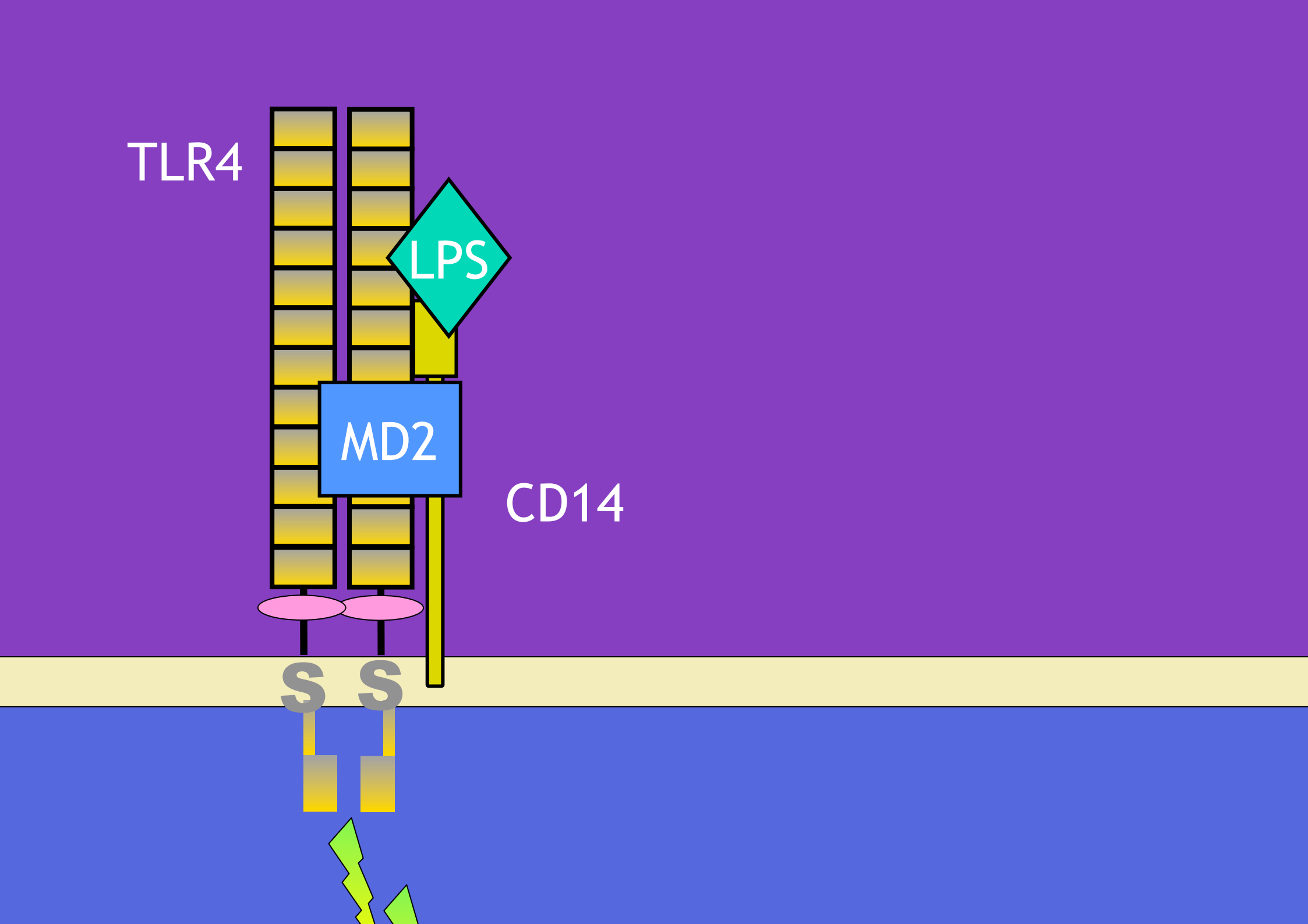

LPS binding prote
LPS
LBP
TLR4
S
S


LPS binding protein
LPS
LBP
TLR4
CD14
S
S


TLR4
LPS
LBP
LPS binding pr
LPS
CD14
S
S


TLR4
LPS
CD14
S
S


TLR4
LPS
MD2
CD14
S
S


TLR4
LPS
MD2
CD14
S
S

SIGNAL TRANSDUCTION PATHWAYS:

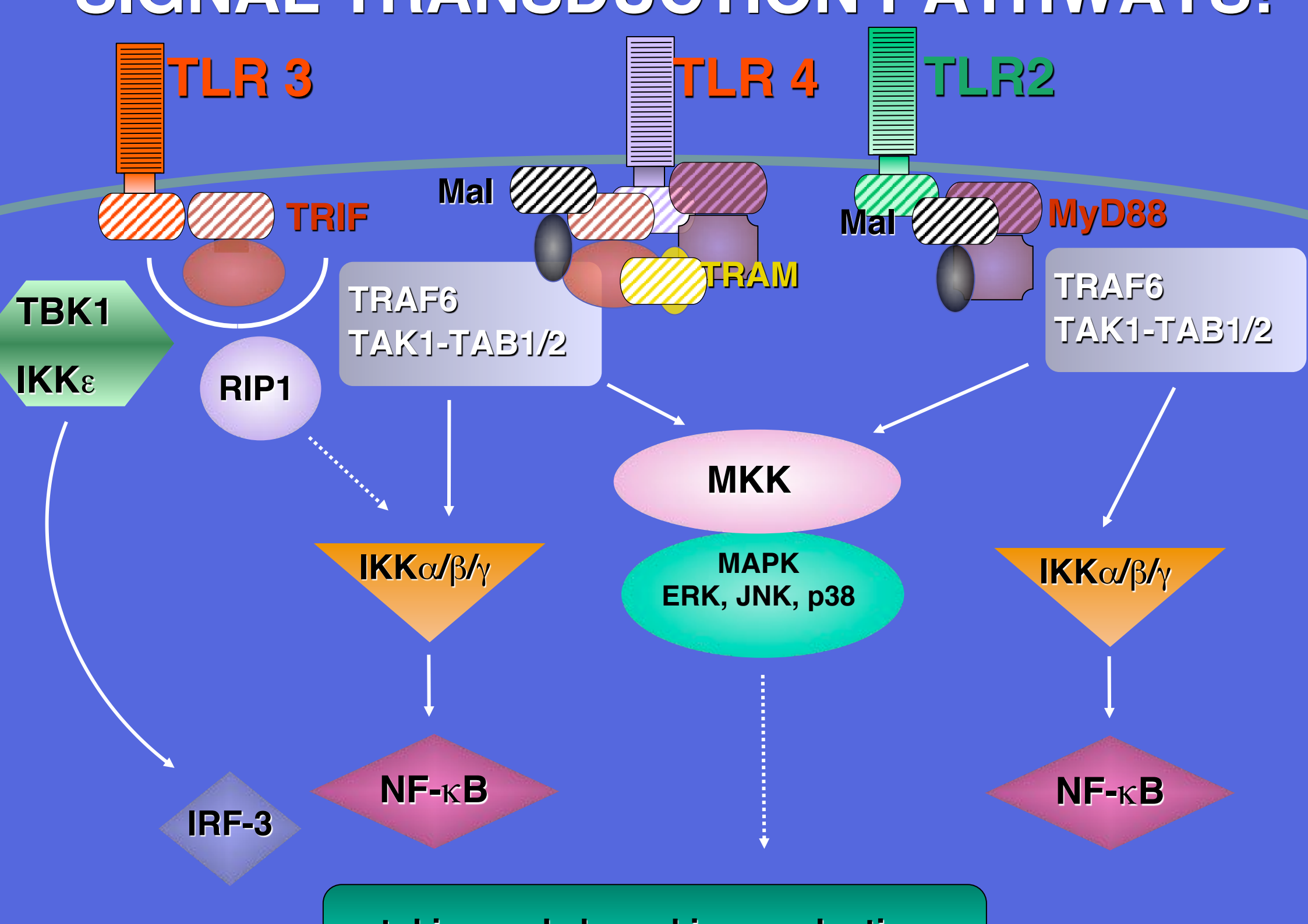


Table 1 Ligands recognized by pattern-recognition molecules

gnition	Producers[a]	Ligands
ns	Liver (hepatocytes)	•Complement components (C1q, Factor H, L-ficolin, M-ficolin)
		• Microorganisms (bacteria, viruses, fungi, parasites)
		• Phosphorylcoline, carbohydrates
		• Modified LDLs
		• ECM protein (fibronectin, collagen IV, laminin, proteoglycans)
		• Amyloid fibrils
		• DNA
rs	Monocytes, MΦ, PMN, EC, DC, fibroblasts, epithelial cells	• Complement components (C1q, Factor H, L-ficolin)• Microorganisms (bacteria, viruses, fungi) and microbialmoieties (OmpA)• ECM protein (tsl, TSG-6)• Apoptotic cells• FGF2
	MΦ, DC, EC	• Fc portion of immunoglobulin
		• Pentraxins (CRP, SAP, PTX3)
		• Microorganisms and microbial moieties (LPS, lipid A, Omps)
		• Aβ peptide of prions
		• ECM protein (fibronectin, laminin, fibromodulin, osteoadherin)
		• Apoptotic cells
L, SP•	Liver (hepatocytes), lung (type II alveolar cells), MΦ	• Carbohydrates• Microorganisms and microbial moieties (LPS, LOS, LTA, PDG)
	Liver (hepatocytes), lung (type II alveolar cells), PMN, monocytes	• Carbohydrates• Microorganisms and microbial moieties (LTA, PDG,1,3-β-D-glucan)
	Monocytes, MΦ, PMN, mast cells	• Complement components (C3b)
		• Microorganisms, zymosan
	Liver (hepatocytes, monocytes, MΦ)	• Microorganisms and microbial moieties (OmpA)

roteins of the coagulation system an fibrinolysis:

fibrinogen, plasminogen, tissue plasminogen activator, Protein S.

gen is the most abundant plasma contains from 100 to 400 mg/dl. With an
lar weight of 340 kDa, fibrinogen is a dimer composed of three pairs of peptide
and gamma-B) linked by disulfide bridges, multiple proximate to the N-terminal.
ains extend outside in two other identical domains (D) at the C-terminal in w
hains are intertwined. Thrombin detaches the fibrinopeptides of A and B from
al ends, forming a fibrin monomer, which polymerizes into fibrils, ar
dinally, which in turn form the clot macroscopic.

Fibrinogen levels become elevated in acute phase!

ogen levels increase during pregnancy and the use of contraceptives.

levels generally indicate an extensive activation of coagulation with consump
ogen.

are several variants of hereditary fibrinogen pathologies, some with relative alt
gulation and bleeding diathesis, others with an increased tendency to thrombosi

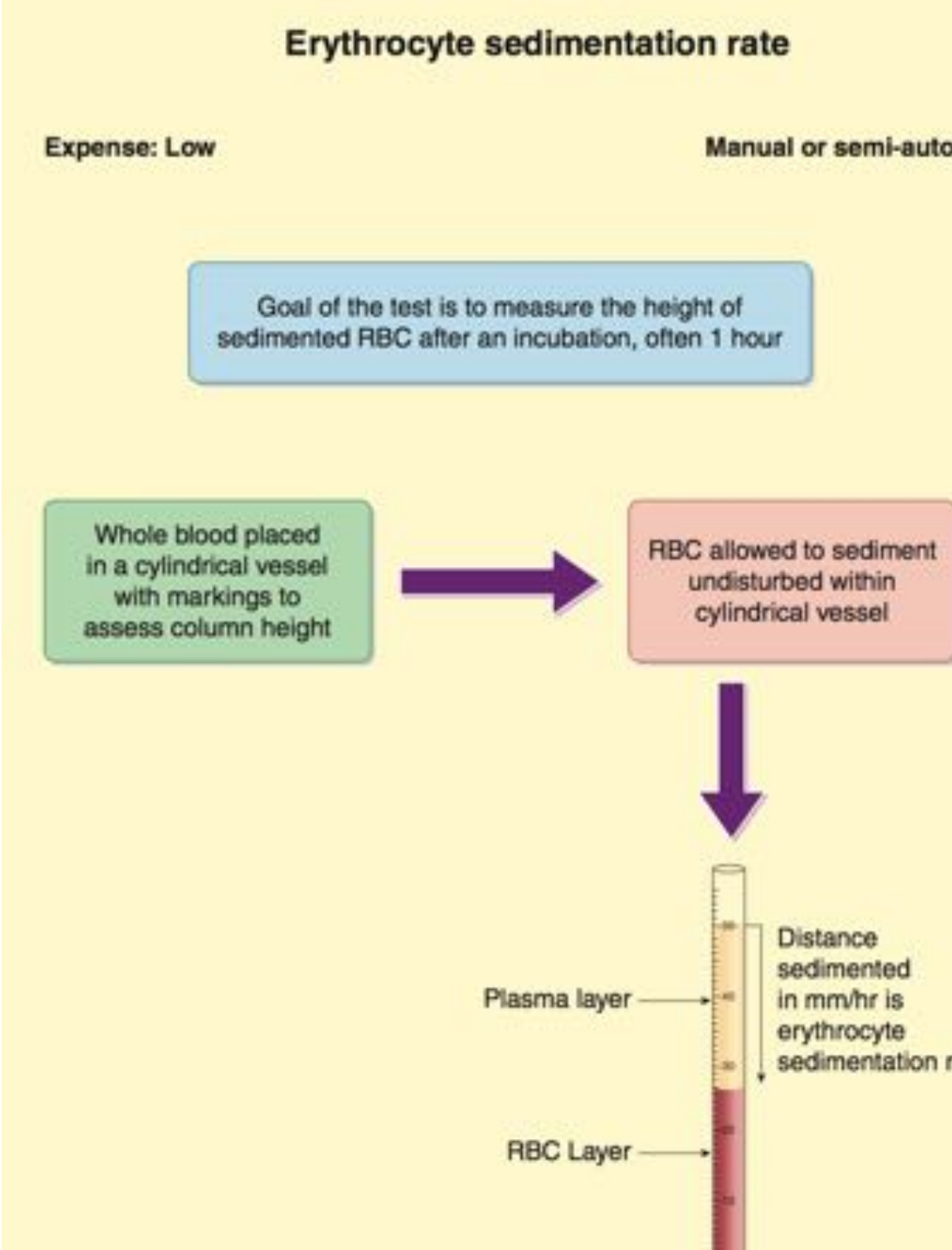
Erythrocyte Sedimentation Rate

R measures the rate at which erythrocytes fall or settle in the plasma of a randomly c
gulated blood specimen over a specified period of time (usually 60 minutes) in millim
hour; however, newer methods involving centrifugation can generate resul
imately 5 minutes. This phenomenon was first observed by Edmund Faustyn Biernacki
hat the rate at which blood settled varied among individuals and that red blood cells (
more quickly in the presence of increased levels of fibrinogen.

3, Dr Robert Fahraeus noted that ESR differed in pregnant versus nonpregnant wome
e test as a possible indicator of pregnancy. In 1921, Dr Alf Vilhelm Albertsson Westergren
a laboratory indicator of the prognosis of patients with pulmonary tuberculosi
gren defined the measurement standards for the ESR test that still are used widely t
ng utilization of sodium citrate as an anticoagulant.

SR can be confounded by many factors, leaving this widely used test vulnerab
rpretation in clinical practice. Aggregation of erythrocytes promotes falling and increase
owever, RBCs are negatively charged and tend to repelone another. Thus, the preser
ely charged, large, asymmetric acute phase proteins such as fibrinogen and immunoglol
es the ESR. The rate of erythrocyte settlement can be influenced by a wide variety of im
nimmune factors, including alterations of the quality and quantity of the RBCs, as w
s in the normal patterns and amounts of various plasma proteins.

rinogen levels become
ted in acute phase up to
es of occasional over 1.0
In this case also becomes
kedly elevated the
hrocyte sedimentation
(ESR): it is believed that
0 % of the increase of ESR
ue to the fibrinogen
ralizing effect on the sialic
residues of red blood cells
are known to inhibit the
rocyte aggregation!



Antiproteases:

-antitrypsin (AAT) and the α1-anti-chymotryps

a 1-antitrypsin is also referred to as alpha-1 proteinase inhi
l) because it inhibits a wide variety of proteases.

a reference range in blood of 1.5 - 3.5 gram/liter.

ough AAT is a powerful APP, its increase in inflammatory proc
clinical specificity.

lly there are no appreciable amounts of trypsin in the circul
d, it and other similar proteases, such as collagenases, are prod
ominantly by leukocytes in response to inflammatory stimu
tive or damaged cells. The AAT is able to neutralize these prote
h may cause tissue damage, and from this derives its physiolo

function is very important and it was revealed by the discovery that the seru
ome young adults with pulmonary emphysema and cirrhosis of the children w
cient AAT.

majority of individuals are homozygous for M, the functional allele of the AA
has the MM phenotype. About l0% of the Caucasian population is heterozygou
M and other alleles of ATT, as the PiZ. More than 2% are carriers of the allele P
has the MZ phenotype. Although these individuals are asymptomatic, the
endants ZZ are susceptible to lung disease or liver disease.

serum protein electrophoresis can be used for screening for AAT deficiency, b
necessary to perform confirmatory testing complex, such as trypsin inhibito
city (TIC), so the phenotype seeking to cross electrophoresis or isoelectr
sing in order to exclude the presence of some other allele as PiS or PiF th
ates differently. The ZZ phenotype ICT has a very low which corresponds to ve
concentrations of AAT. It is essential that such persons should avoid cigaret
ke, as this activates alveolar macrophages to release proteases

α1-anti-chymotrypsin!

is not only highly specific for chymotrypsin, a protease aks the peptide bonds at the carboxyl site of tyrosine nylalanine, but it is the only APP.

, which has a molecular weight of 68 kDa with approxima % of the carbohydrate content and a normal se centration from 40 to 60 mg/dL, can rapidly increase up to es during and for the duration of inflammation.

Transport proteins:

ceruloplasmin, haptoglobin and hemopexin.

oplasmin consists of a single polypeptide chain, can bind six atoms of cop
n give a blue color to the protein and " in vitro " activity manifests oxid
ough at birth is lower, its serum level ranges from 20 to 40 mg/dL in yo
ts, increasing to twice in the treatment of contraception and pregnancy (
cute-phase reactive.

loplasmin is a glycoprotein essential for the body to transport the copper
he removal of iron from the tissues through the activity of the enz
xidase.

aceruloplasminemia is a genetic disease with an autosomal recessive
ed by a mutation of a gene located on chromosome 3. Unlike Wilson's dise
mitted in an autosomal recessive and caused by mutations in the ATP7B
ng for ATPase that controls the transport of copper into the bile an
poration in the enzyme ferroxidase, there are no apparent defects in

Hemopexin!

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

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

haptoglobin has two heavy chains and two light chains that, of di
ecular weight and joined by disulfide bridges, determine three hapto
notypes: (1-1), (2-1) and (2-2).

mean serum HTG concentration is (2.5 +/- 1.2 g/L).

nds hemoglobin released by lysis of erythrocytes in order to preserve th
protein reserves. The hemoglobin - haptoglobin complexes are remov
rophages in the liver and spleen of the reticuloendothelial system to ensu
very of the heme-iron. Therefore, the physiological function of haptogl
ly to allow the recovery of iron when red blood cells, at the end of their
irculation are destroyed (hemolysis saline).

ormal conditions the concentration of haptoglobin in the circulation is the
e balance between its synthesis in the liver and its elimination.

concentration of haptoglobin is therefore inversely proportional to the
emolysis. The serum haptoglobin also increases in response to stress, inf

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

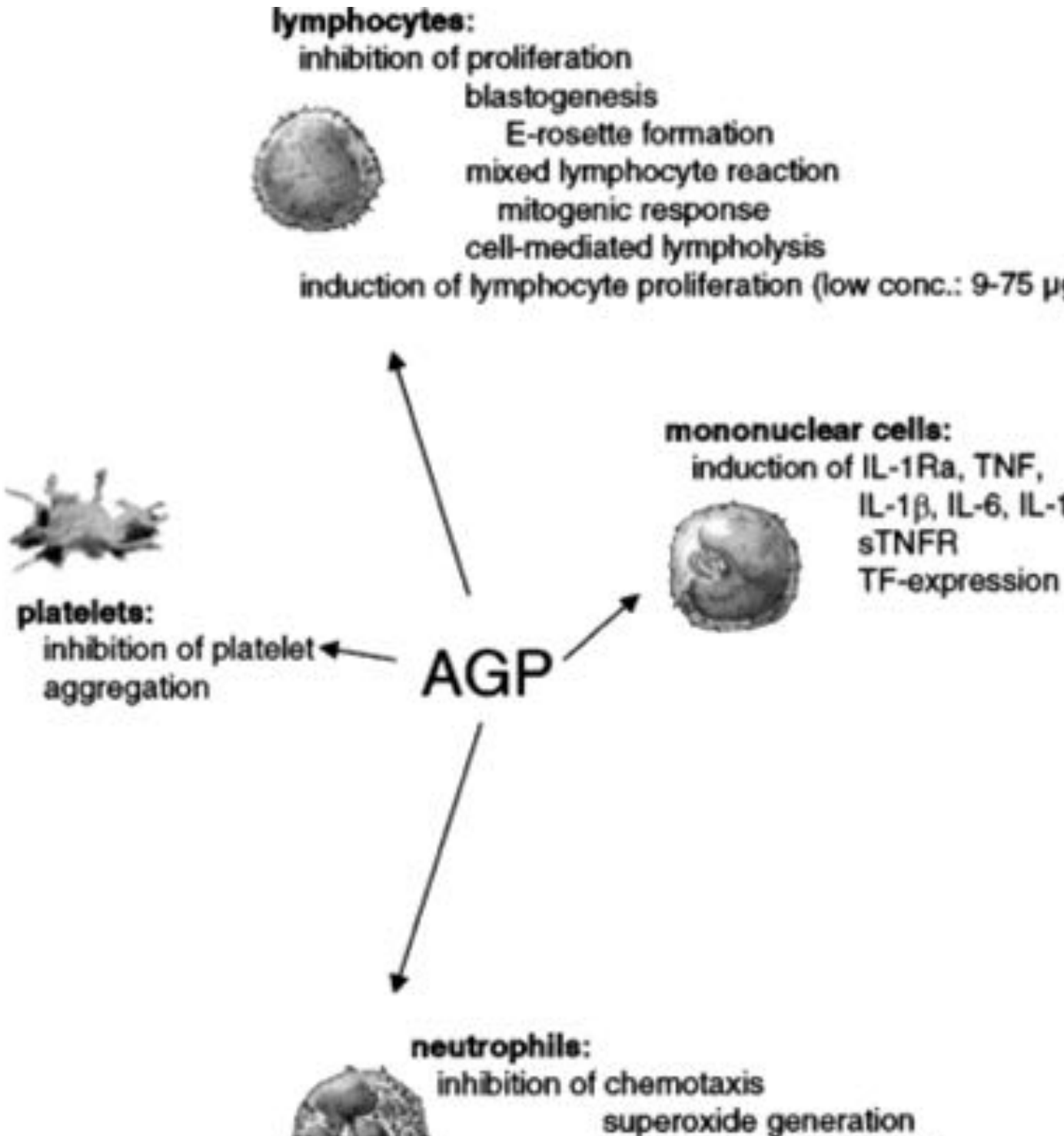
α1-acid glycoprotein, soluble CD14 or CD14S, gocyte-specific S100 calcium-binding proteins an procalcitonin!

e α-1 acid glycoprotein (AGP) or **Orosomucoid (ORM**
protein with a molecular weight of 41-43 kDa a
cosylated (45%).

P serum concentrations are between 0.6-1.2 mg/
d increase considerably in the case of acute ph
lammatory response.

s known as the primary carrier of basic drugs (wher
umin carries acidic drugs), steroids, and prote
ibitors.

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



The soluble CD14 (sCD14) is the soluble form of CD14, a prote of the membrane of monocytes-macrophages, anchored by glycosyl bond-fosfatidilico-inositol and that functions as coreceptor for the LPS.

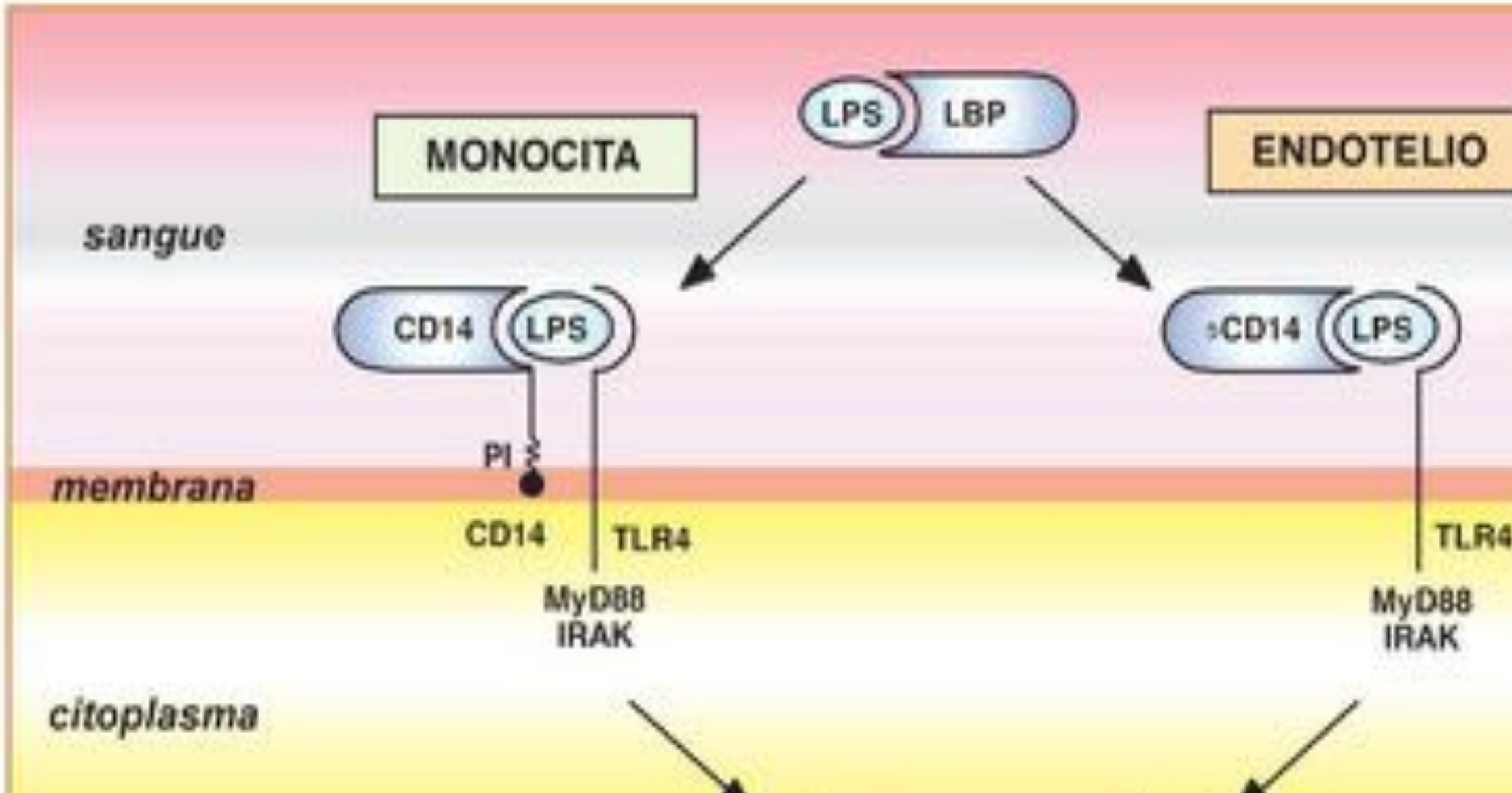
Its **serum levels are low (around 100 ng/ml)** but increase as consequence of the activation LPS-mediated monocyt macrophages that release large amounts of sCD14.

If it was initially postulated that the sCD14 was released desensitize monocytes-macrophages and to limit the production of inflammatory cytokines. Recently, it has bee detected its presence in breast milk, where it seems th enhances the differentiation of B lymphocytes. Plasma levels

CHEMOKINES

MONOCYTES

ENDOTHELIUM



sin as a potential marker for bacterial infection relapse in critical care pa
A preliminary study.

ini V, Ceccarelli G, D'Alessandro M, Collepardo D, Morelli A, D'Egidio A, Mariotti S, Nicole
Evangelista B, D'Ettorre G, Angeloni A, Venditti M, Bachetoni A.

bacterial infection carries a high risk of mortality in critical care patients. Improve
c procedures are required for effective management of sepsis. Recently, **the soluble CD14
psin, has been suggested as a reliable marker of sepsis,** and we set out to compare its c
nce with that of procalcitonin (PCT). We focused on a cohort of septic patients who, du
cation, relapsed after a period of clinical relief from symptoms.

: In total 21 adult patients were studied during their hospitalization in the Critical Car
o Umberto I hospital; 74 plasma samples were collected at multiple time points, and **p
ere measured using a PATHFAST® analyzer.**

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

ons: This study confirms the importance of monitoring a combination of several biomarker
a reliable diagnosis. **Maximal presepsin levels could alert clinicians not to suspend a
nts and to carefully monitor septic patients' state of health, even after clinical sympto**

ting presepsin (soluble CD14 subtype) as a marker of host response in patients
vere sepsis or septic shock: data from the multicenter, randomized ALBIOS tria

n S1, Caironi P, Fanizza C, Thomae R, Bernasconi R, Noto A, Oggioni R, Pasetti GS, Romero M, Tognoni G, Latini R, Gattino

s a soluble fragment of the cluster-of-differentiation marker protein 14 (CD14) involved in pathogen recog
nunity. We evaluated the relation between its circulating concentration, host response, appropriateness of
nd mortality in patients with severe sepsis.

esepsin was measured 1, 2, and 7 days after enrollment of 997 patients with severe sepsis or septic sho
r Albumin Italian Outcome Sepsis (ALBIOS) trial. They were randomized to albumin or crystalloids. We tes
and adjusted models the association of single measurements of presepsin or changes over time with clinica
unctions, appropriateness of antibiotic therapy, and ICU or 90-day mortality.

concentration at baseline (946 [492-1,887] ng/L) increased with the SOFA score, the number of prevale
ns or failures, and the incidence of new failures of the respiratory, coagulation, liver, and kidney syste
ion decreased in ICU over 7 days in patients with negative blood cultures, and in those with positive blood
priate antibiotic therapy; it increased with inappropriate antibiotic therapy ($p = 0.0009$). Baseline prese
ntly associated with, and correctly reclassified, the risk of ICU and 90-day mortality. Increasing concentr
from day 1 to day 2 predicted higher ICU and 90-day mortality (adjusted $p < 0.0001$ and 0.01, respectively).
ect on presepsin concentration.

USIONS:

in is an early predictor of host response and mortality in septic patients. Cha

Review Article

Presepsin as a novel sepsis biomarker

i Zou, Wei Wen, Xin-chao Zhang

nergency Medicine Department, Beijing Hospital, Beijing 100730, China

***orresponding 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 biom1arker 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, preseptin 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

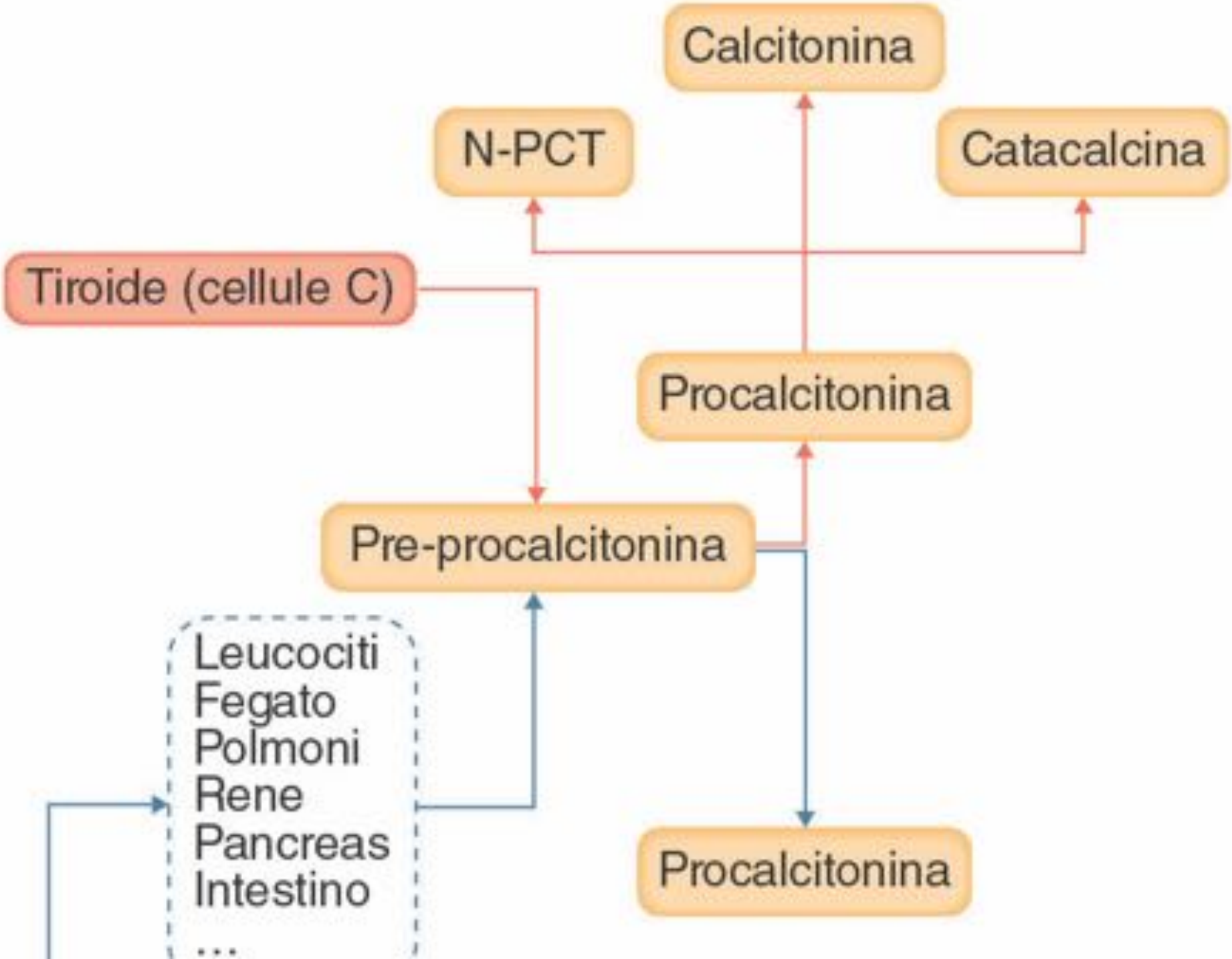
World J Emerg Med 2014;5(1):16–19

procalcitonin, or PCT, a precursor of calcitonin identified in 1975, is produ
he C cells of the thyroid gland and released into the circulation in nor
na levels of about 5-50pg/ml.

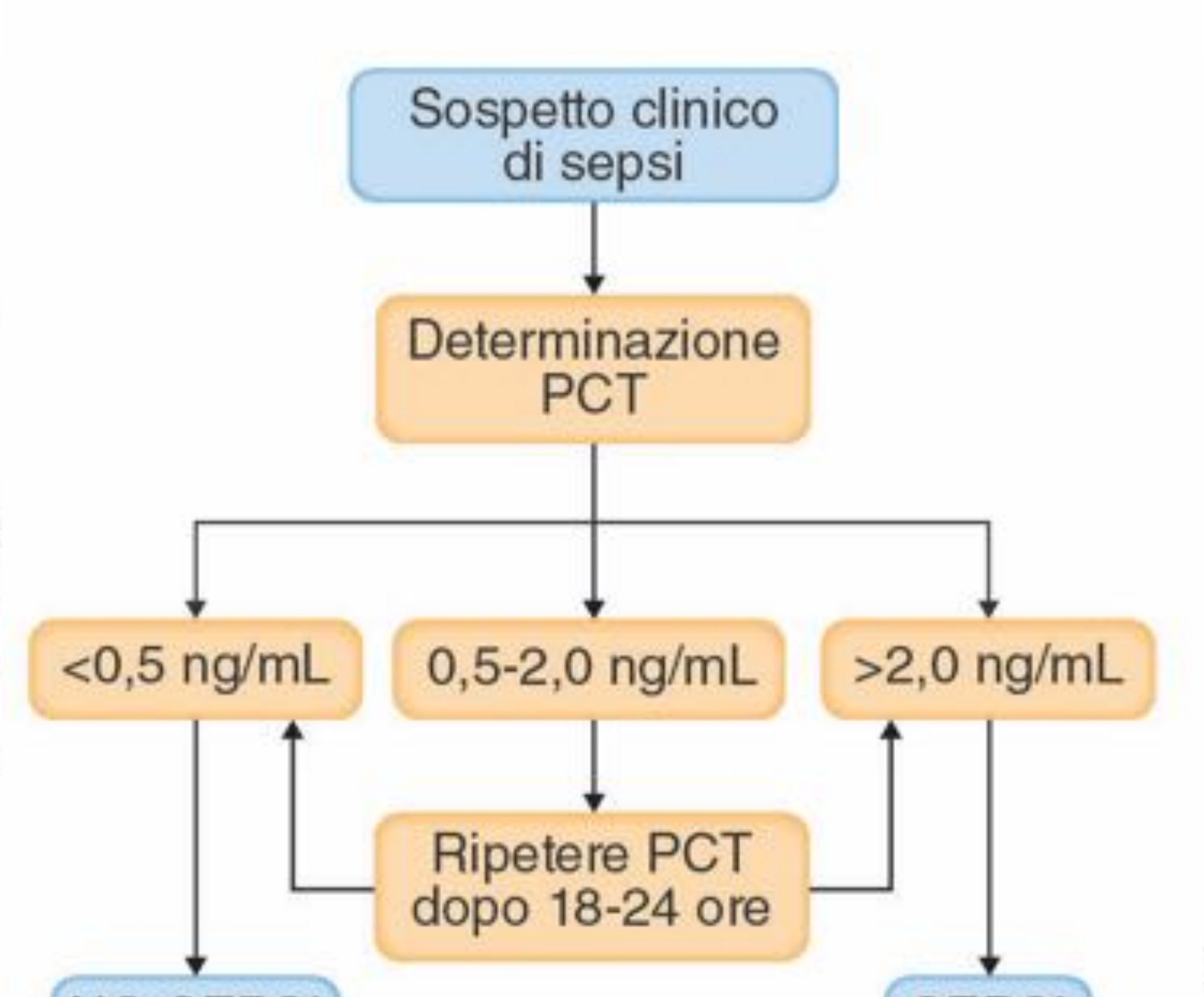
e course of inflammation is produced by the liver and PMNs and is a us
nostic marker of severe bacterial infections, but not viral infections and
surement may be useful to discriminate infectious SIRS from non-infect
because PCT levels increase in meningitis patients with bacterial infecti
are around 2.4 ng/ml in patients with pneumonia without sepsis and 31
patients with pneumonia and sepsis.

appears to be a good indicator of early onset and l
et sepsis.

can also be used to distinguish between septic ARDS and ARDS not se
use of these characteristics, the PCT is currently used in intensive care in


Calcitonina
N-PCT
Catacalcina
Tiroide (cellule C)
Procalcitonina
Pre-procalcitonina
Leucociti
Fegato
Polmoni
Rene
Pancreas
Intestino
...
Procalcitonina

TO CHECK IN TIME THE SEPSIS!!



SEPSIS HISTORY AND DEFINITIONS!

William Osler

American College of Chest Physicians; Society of Critical Care Medicine Consensus Conference

SCCM/ESICM/ACCP/ ATS/SIS International Sepsis Definitions Conference

The Third International Consensus Definitions for Sepsis and Shock Sepsis

00 a.c.

epsis:
fazione,
osizione o
scenza,
come una
ondizione
a ad alto
di morte,
elata a
fezione

1913

"con alcune eccezioni, sembra che il paziente muoia a causa della reazione del corpo all'infezione, piuttosto che a causa dell'infezione stessa"

1992

Introduzione del termine SIRS. La sepsi è definita come la presenza contemporanea di SIRS e infezione

2001

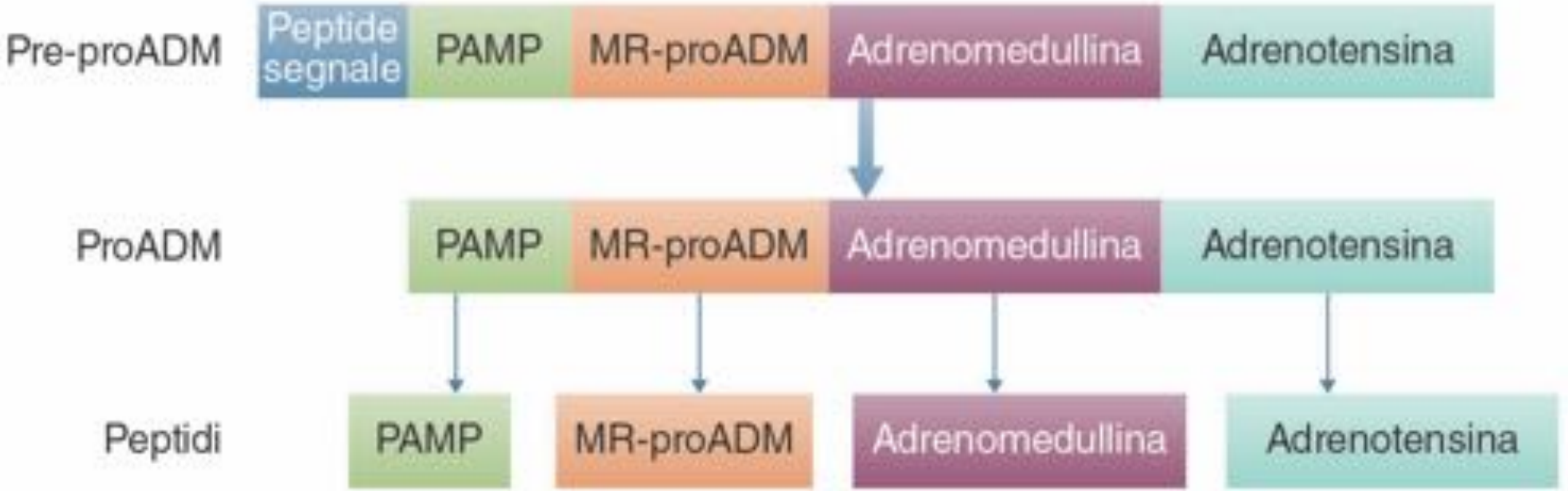
This document reflects a process whereby a group of experts and opinion leaders revisited the 1992 sepsis guidelines and found that apart from expanding the list of signs and symptoms of sepsis to reflect clinical bedside experience, no evidence exists to support a change to the definitions.

2016

Disfunzione d'organo pericolosa per la vita, causata da una disordinata risposta dell'ospite a un'infezione

(ADM).

BIOSINTESIS OF ADRENOMODULIN



H LEVELS OF ADM ARE PRESENT IN SEPSIS, BUT TH
ARE UNDERESTIMATED BECAUSE BIND VARIOUS
RECEPTORS OR ARE DEGRADED BY PROTEASE.
ERNATIVE, A FRAGMENT OF 48 aa, MR-proADM, H
EEN IDENTIFIED AND IS RELEASED BY EQUIMOLAR

Test di laboratorio	Scopo
Procalcitonina	Diagnosi di sepsi e guida alla terapia antibiotica
Presepsina	Diagnosi di sepsi
MR-proADM	Indicatore prognostico
Proteina C reattiva	Diagnosi di sepsi
Emocoltura	Diagnosi eziologica di sepsi
Esame emocromocitometrico	Diagnosi di anemia e piastrinopenia
Bilirubina	Compromissione epatica
Aminotransferasi	Compromissione epatica
Creatinina	Compromissione renale
Glicemia	Scompenso metabolico
Test di coagulazione	Diagnosi precoce di CID (coagulazione intravascolare disseminata)
Troponine cardiache	Compromissione cardiaca

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

yte-specific calcium-binding S100 proteins as clinical laboratory markers of inflam

Foell D1, Frosch M, Sorg C, Roth J.

rst member of the S100 family of proteins, which are part of the larger gr
m-binding proteins, was isolated in 1965.

100 proteins comprise the group of calgranulins, are pro-inflammatory mol
ssed and secreted by phagocytes. The three members of this group, S100A8, S
100A12 are over -expressed at the site of inflammation.

eterodimer of S100A8 and S100A9, known as " leukocyte protein L1", is now still
tectin by some research groups.

the complex S100A8/S100A9 that S100A12 are useful diagnostic facto
mation especially in the case of arthritis, chronic inflammation of the lung
nal diseases.

E, Van Assche G.
troenterol Belg. 2013 Sep;76(3):322-8.

ics and prognostics of inflammatory bowel disease with fecal neutrophil-derived biomarkers
tin and lactoferrin.
n T. Dig Dis. 2013;31(3-4):336-44.

ecal calprotectin in gastrointestinal disorders.
M, Gallo A, Santoro L, D'Onofrio F, Landolfi R, Gasbarrini A.
Med Pharmacol Sci. 2013 Jun;17(12):1569-82.

nostic Accuracy of Fecal Calprotectin During the Investigation of Suspected Pediatric Inflamm
sease: A Systematic Review and Meta-Analysis.
on P, Anderson NH, Wilson DC.
stroenterol. 2013 May 14.

disease: small bowel motility impairment correlates with inflammatory-related markers C-react
nd calprotectin.
upt S, Pazahr S, Chuck N, Blume I, Froehlich JM, Cattin R, Raible S, Bouquet H, Bill U, Rogler G
A, Patak MA.
stroenterol Motil. 2013 Jun;25(6):467-73.

tility of calprotectin and lactoferrin in patients with inflammatory bowel disease: is there some
the literature?
R, D'Incà R, Pathak S, Sturniolo GC.
ev Clin Immunol. 2012 Aug;8(6):579-85.

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

Role of fecal calprotectin in gastrointestinal disorders.

Montalto M1, Gallo A, Santoro L, D'Onofrio F, Landolfi R, Gasbarrini A.

cal calprotectin (FC) has been proposed as a useful and n
invasive marker of acute intestinal inflammation.

nmarize recent evidences on FC, providing practical perspectives on its diagno
tic role in different gastrointestinal conditions.

S:

relevant data derived from studies on inflammatory bowel disease (IBD). FC concer
howed a good diagnostic precision for separating organic and functional intestinal
l correlated with IBD activity. FCCs were higher in subjects with NSAID enteropathy
orrelation between FC and endoscopy is under investigation.

USIONS:

been widely proposed as a filter to avoid unnecessary endoscopies. Nevertheless, i
considered as a marker of organic intestinal disease at all; rather it represents a m
philic intestinal inflammation". In IBD, more and larger studies are needed to con
to correlate with IBD extent, to predict response to therapy and relapse, and the
clinical intestinal inflammation in asymptomatic first-degree relatives of patients.

di infiammazione
stinale
. I test per individuare pazienti con possibile
e dell'intestino: scopri i test disponibili e il loro
o

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

Dipartimento Assistenziale Integrato
Medicina Diagnostica

U.O. IMMUNOLOGIA- IMMUNOPATOLOGIA DLC05
Responsabile F.F Prof. Fabrizio Maiuzzo
Tel: 06-49970066

Roma, ...

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

Prelievo del .....

Provenienza ...DAI Pediatria.....

DOSAGGIO CAPROTECTINA FECALE

< 50 mg/kg di feci Ne

50 - 100 mg/kg di feci
Zona Grigia, si consi

> 100 mg/kg di feci P

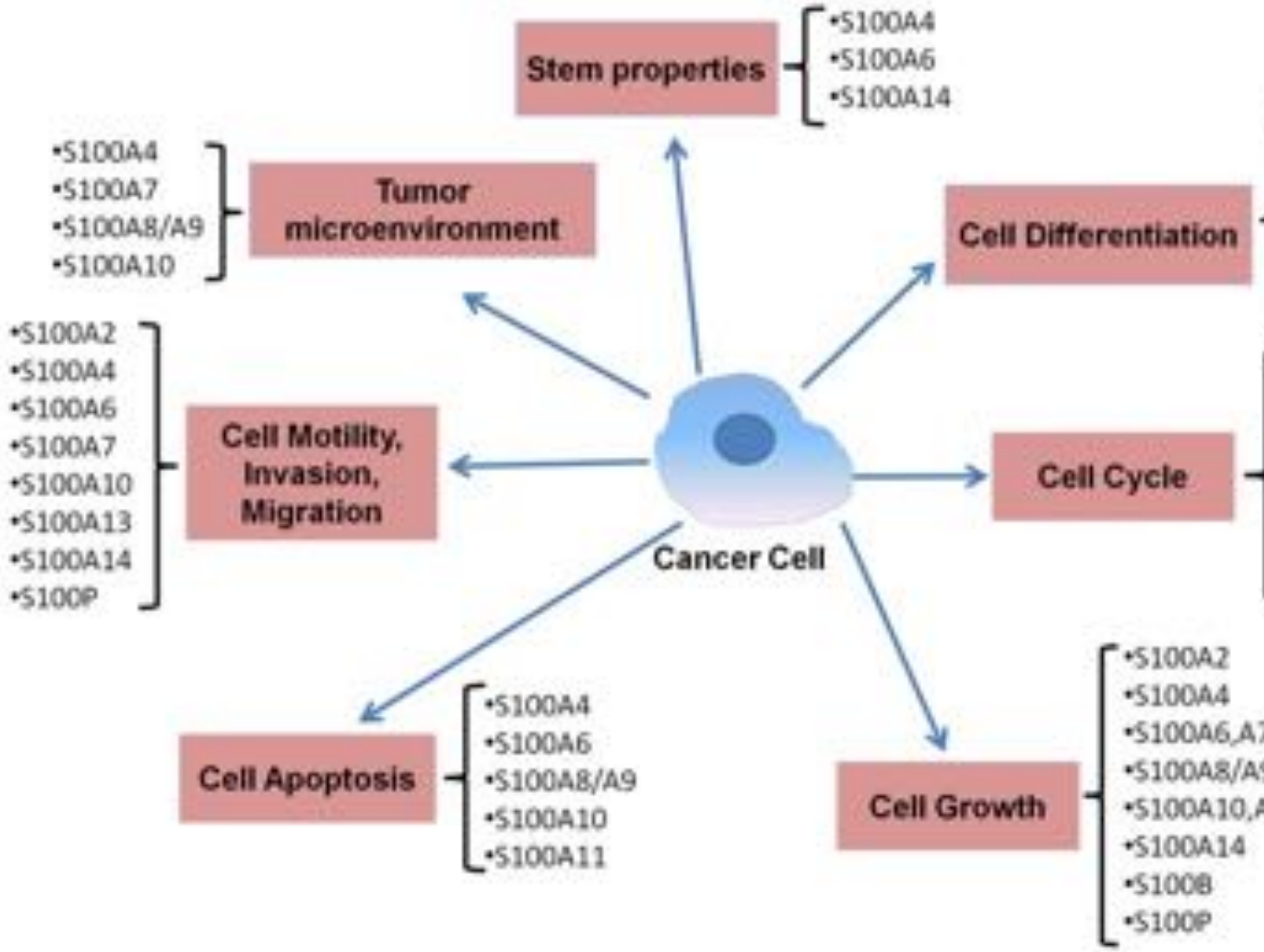
Il kit Calprest (Eurospital, Trieste, Italia) è utilizzato x l'analisi della calprot
fecale. Calprest è un test immunoenzimatico che sfrutta l'uso di anticorpi poli
(riconoscimento del massimo numero di epitopi) diretti contro la calprotectina e per
un dosaggio quantitativo di essa.

Il Responsabile

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.

cated in multiple stages of
rigenesis and progression.
ng the S100 genes, 22 are
ered at chromosome locus
, a region frequently
anged in cancers. S100
in possesses a wide range
racellular and extracellular
ions such as regulation of
um homeostasis, cell
feration, apoptosis, cell
sion and motility,
skeleton interactions,
ein phosphorylation,
ation of transcriptional
ors, autoimmunity,
otaxis, inflammation and
potency. Many lines of
nce suggest that altered
ession of S100 proteins
associated with tumor
ression and prognosis.
efore, S100 proteins might



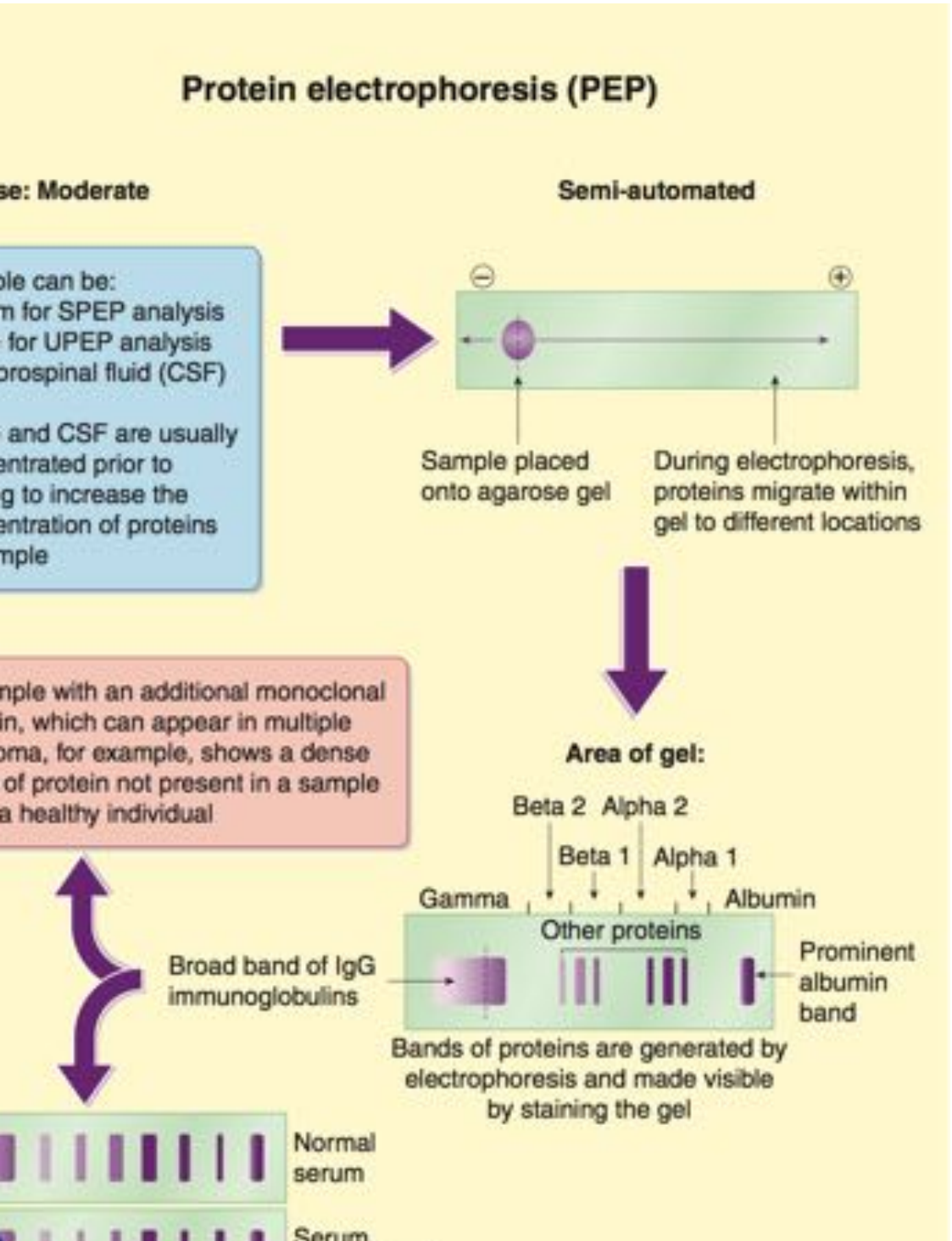
ctrophoresis is the diagnostic tool for the detection of APP

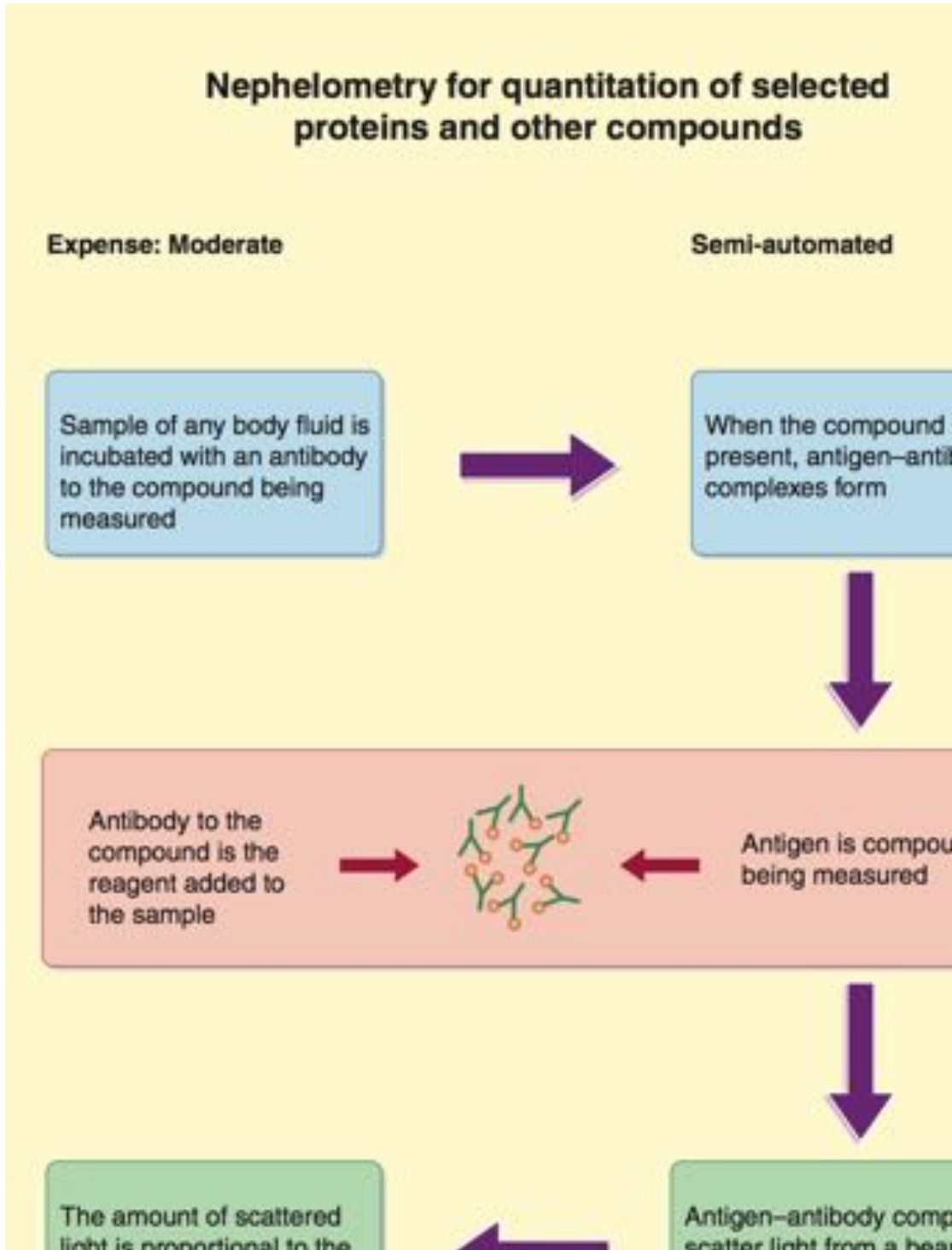
ey can also be detected by ELISA, nephelometry,
munoturbidimetry radioimmunoassay and molecular biolo

hnical problems with the testing of APP include change
concentrations of APP observed with the use of diffe
icoagulants and in the presence of hemolysis and lipemia.

hough APP are considered to be stable at -20 °C, the long t
rage at -70 °C is recommended.

Laboratory evaluation of APP!

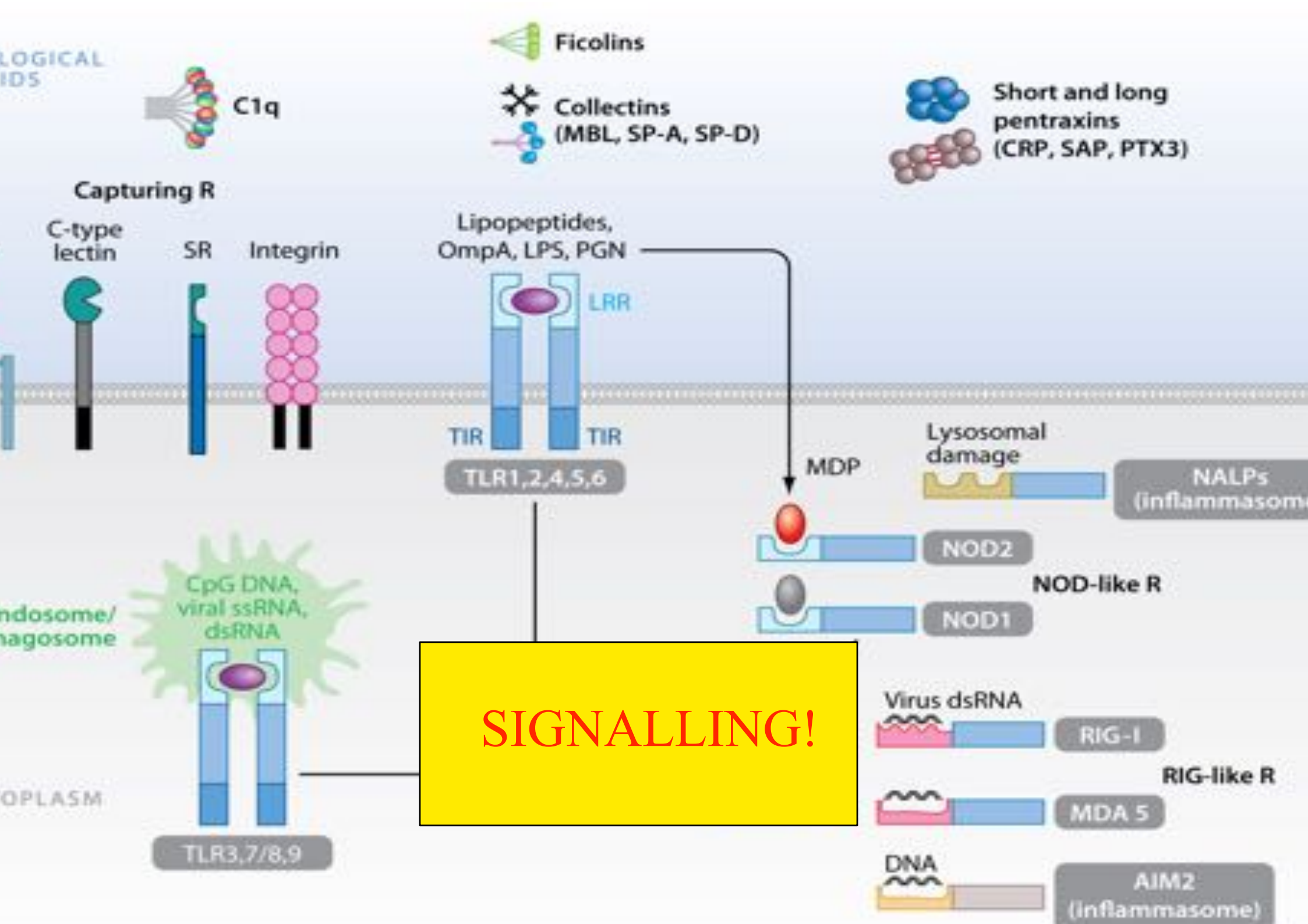




SINCE ONE OF THE MAIN FUNCTION OF THE APP POSITIVE IS TO ACTIVATE THE COMPLEMENT AND THE PHAGOCYTOSIS,

THEY CAN BE NAMED:

THE SOLUBLE DAMAGE RECEPTORS OF NATURAL IMMUNITY AND INFLAMMATION!


Ficolins
C1q
Collectins
(MBL, SP-A, SP-D)
Short and long
pentraxins
(CRP, SAP, PTX3)
Capturing R
C-type
lectin
SR
Integrin
Lipopeptides,
OmpA, LPS, PGN
LRR
TIR
TIR
TLR1,2,4,5,6
MDP
Lysosomal
damage
NALPs
(inflammasome)
NOD2
NOD-like R
NOD1
CpG DNA,
viral ssRNA,
dsRNA
SIGNALLING!
Virus dsRNA
RIG-I
RIG-like R
MDA 5
DNA
AIM2
(inflammasome)
TLR3,7/8,9

THE APP POSITIVE AS THE SOLUBLE DAMAGE RECEPTORS

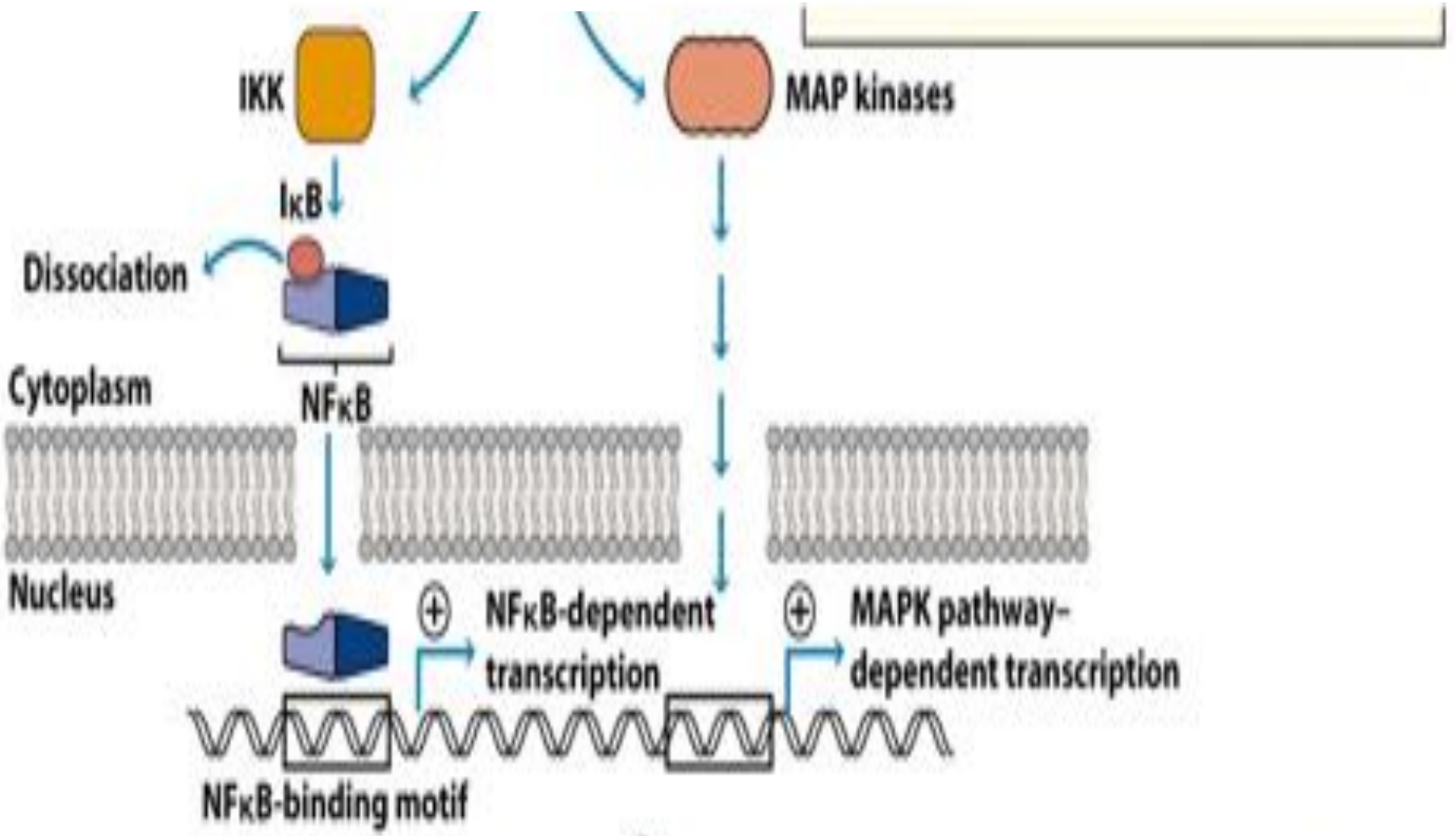
CAN COOPERATE WITH THE CYTOPLASMIC AND MEMBRANE RECEPTORS

TO ACTIVATE

THE TRANSCRIPTIONAL PROGRAM OF NATURAL IMMUNITY AND INFLAMMATION!

INVOLVE

NFKB AND MAPK ACTIVATION!




ШАРККК

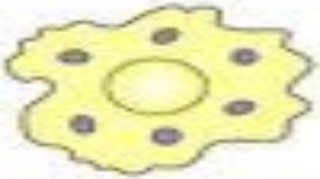
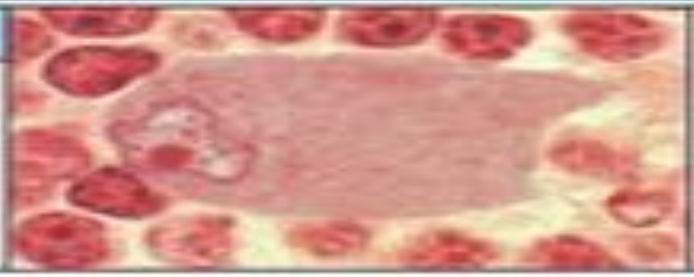
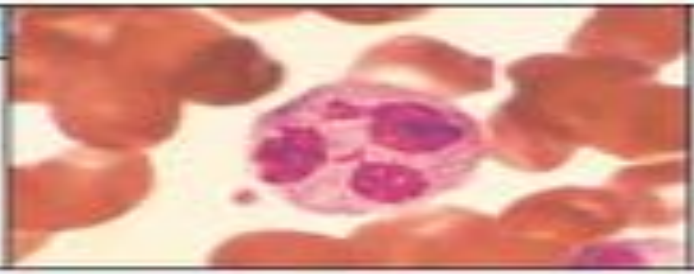
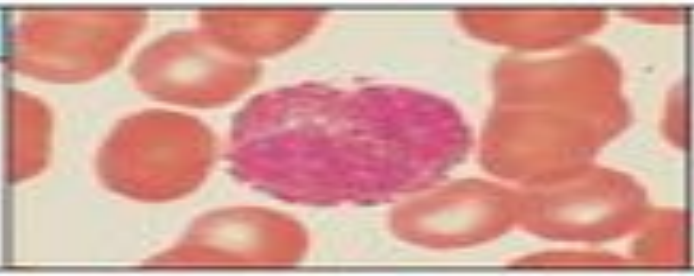
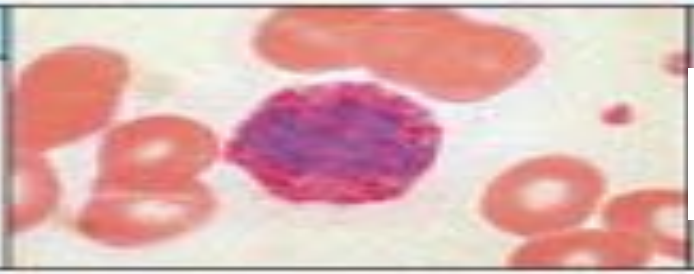
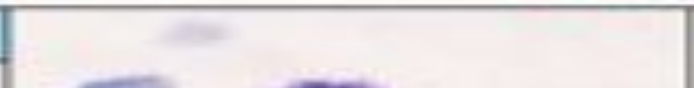
THE TRANSCRIPTIONAL
OGRAM OF NATURAL IMMUNITY AN
INFLAMMATION
CONTROLS

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

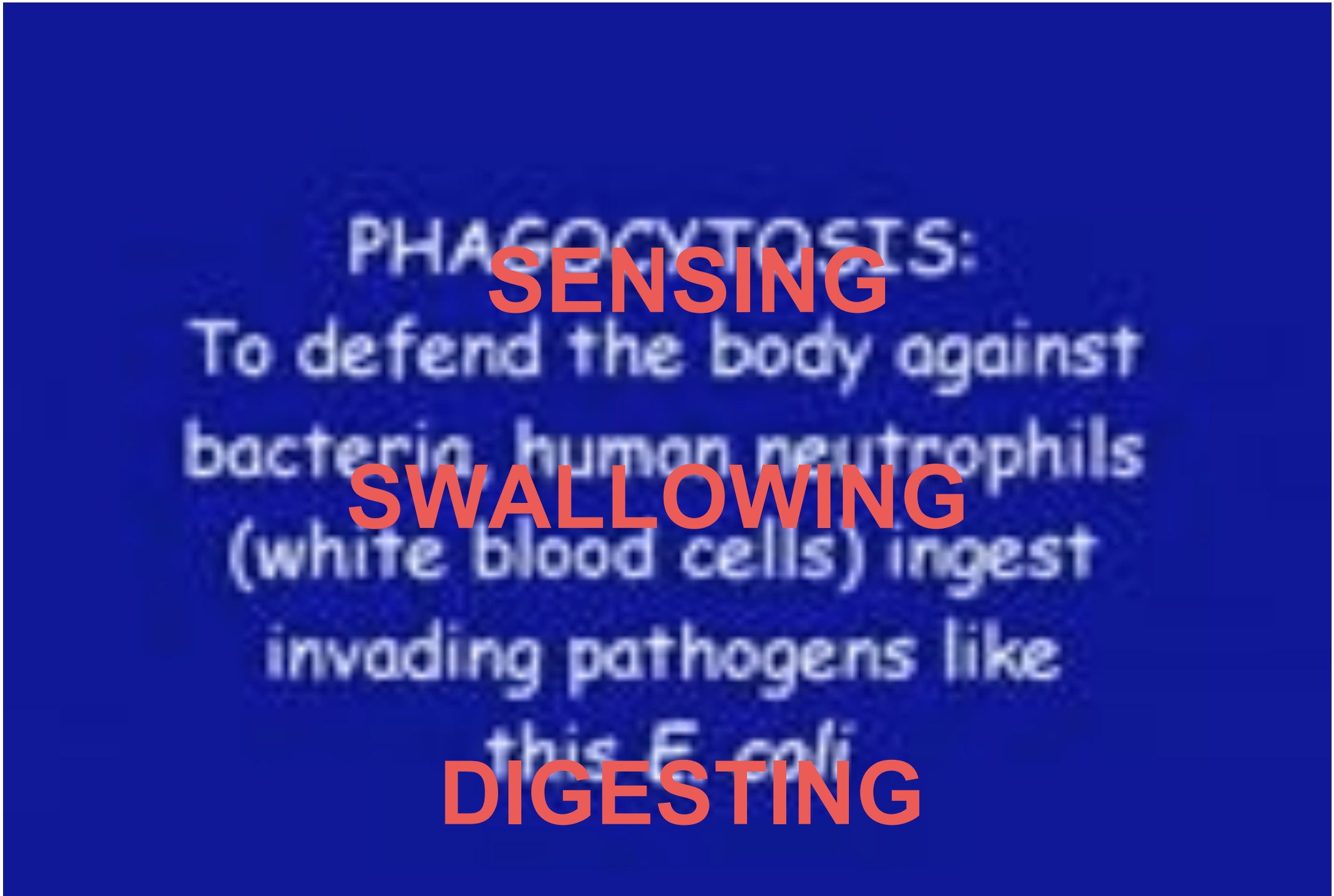
Monocytes/

DEGRANULAZIONE

DEGRANULATION

Cell		Activated function
Macrophage 		Phagocytosis and activation of bactericidal mechanisms Antigen presentation
Dendritic cell 		Antigen uptake in peripheral sites Antigen presentation in lymph nodes
Neutrophil 		Phagocytosis and activation of bactericidal mechanisms
Eosinophil 		Killing of antibody-coated parasites antiviral
Basophil 		Release of histamine
Mast cell		Release of

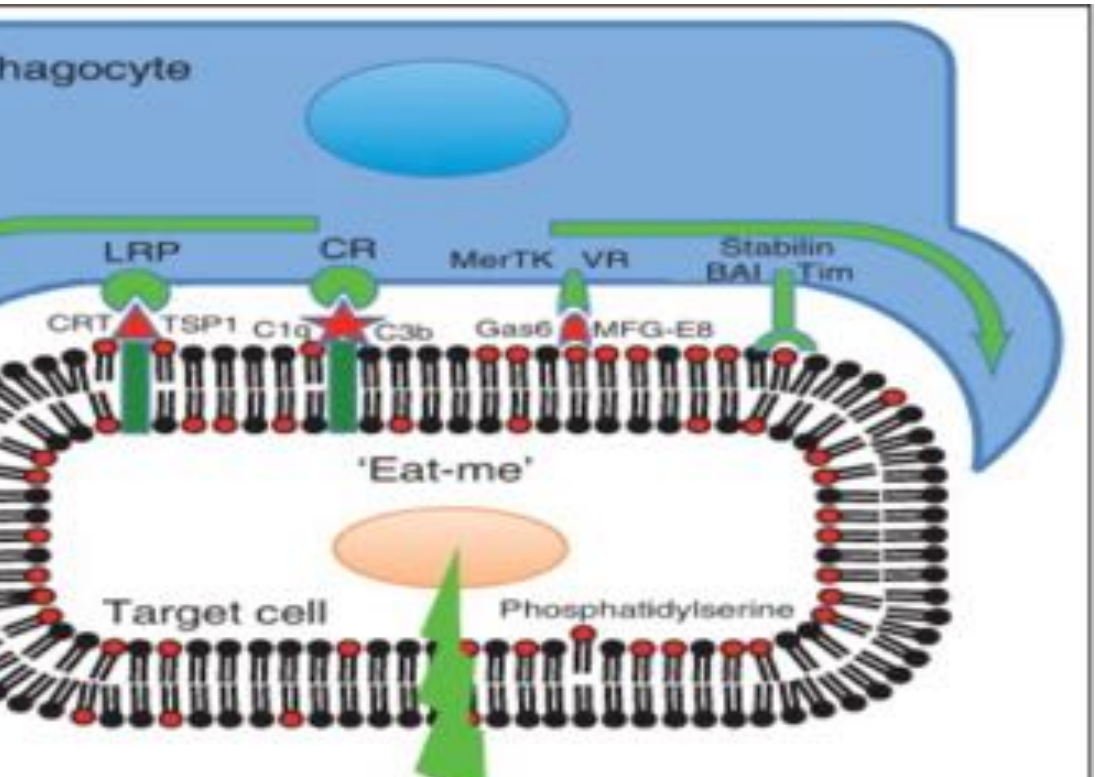
NAMICSDINAMICITA' DELLA FAGOCITO



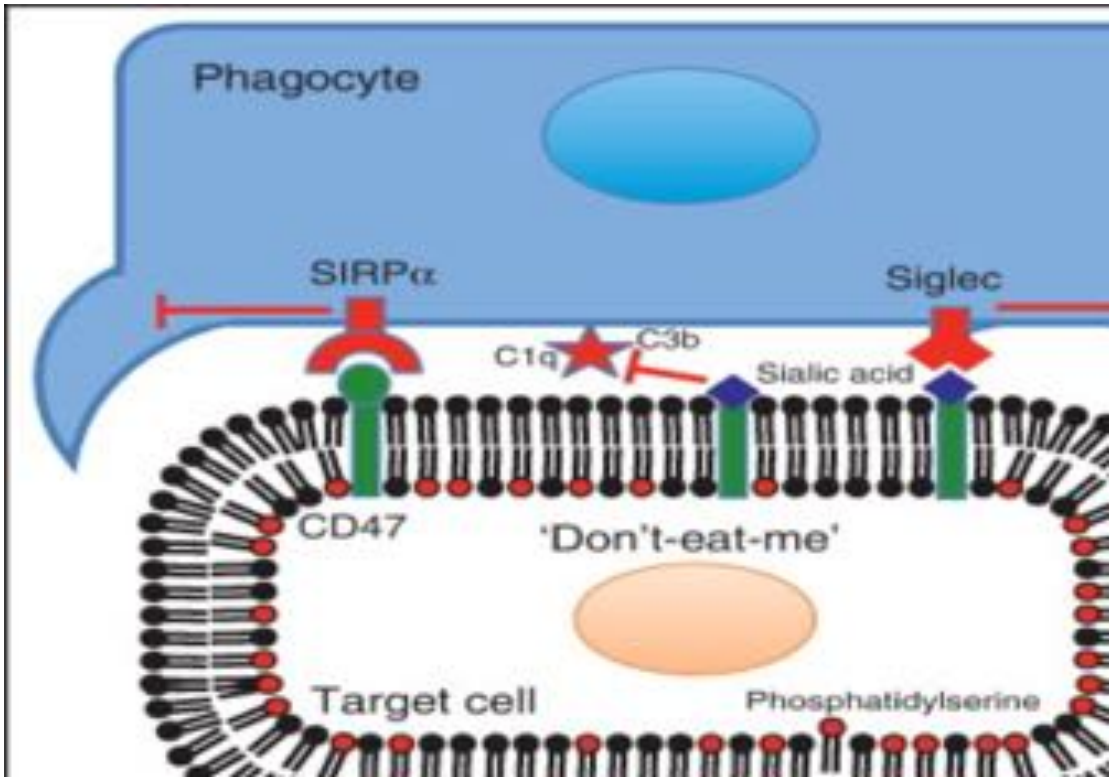
RECENTLY IT HAS BEEN DEMOMSTRATED THAT A TYPE OF
GOCYTOSIS, CALLED PHAGOPTOSIS, ELIMINATES ALSO CE
ALIVE!

**m is created by combining phago-, which is derived from the ancient Greek 'p
g to devour, and -ptosis, which is from the ancient Greek 'ptosis' meaning to fall; us
connotation of dying; therefore, phagoptosis would connote 'devouring-induced d
aused by being devoured'.**

'Eat-me' signalling!



Don't eat-me' signalli

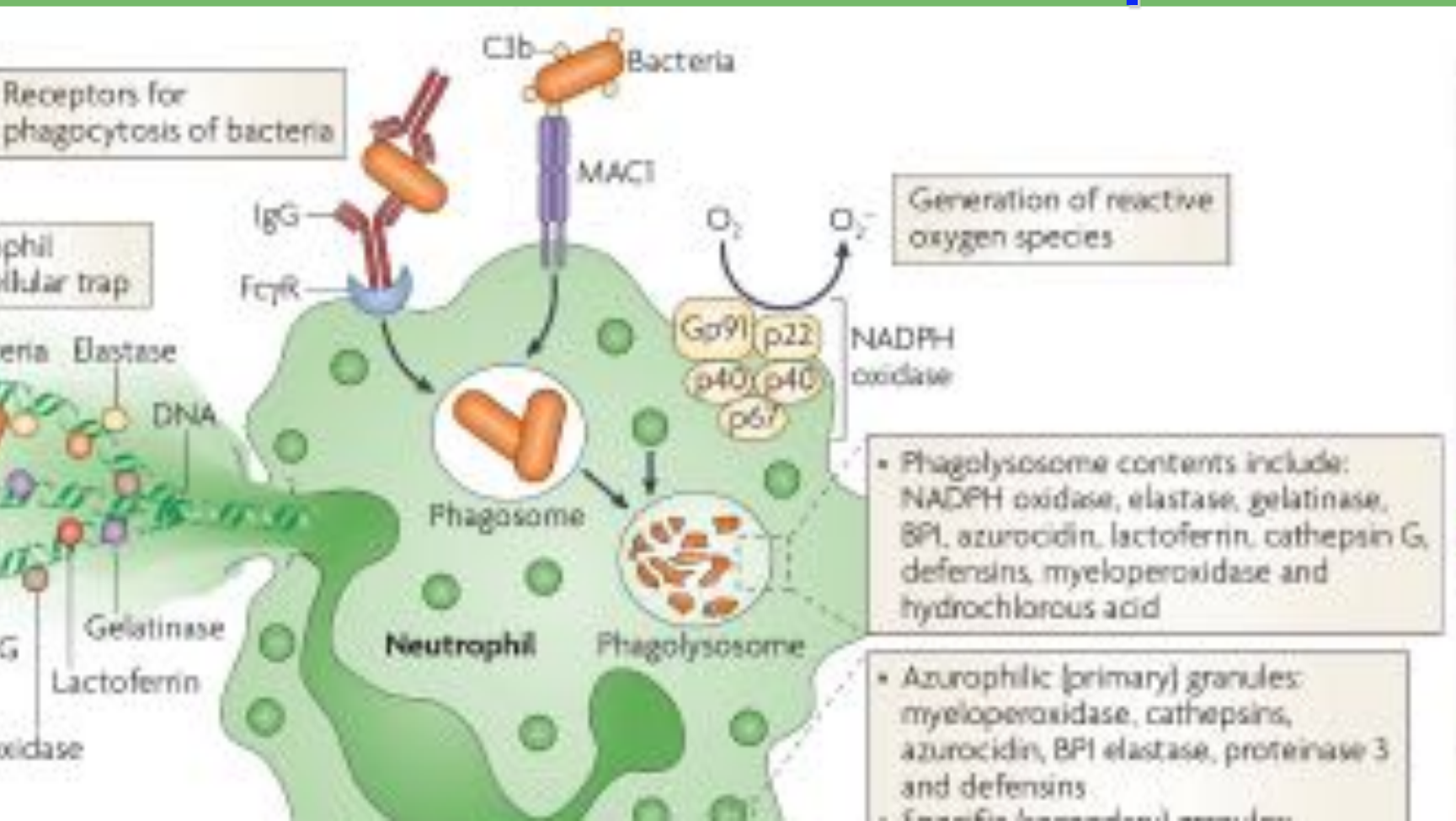


utrophils and other cells, and thus is quantitatively one of the main forms of cell death in the body!

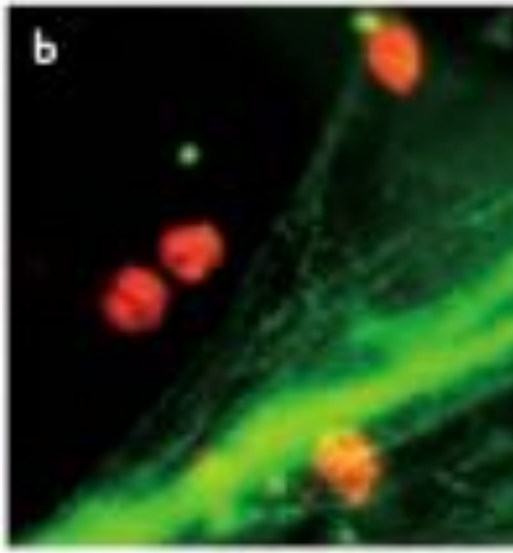
Table I. Rough estimates of the physiological rates of cell turnover by different forms of cell death in humans

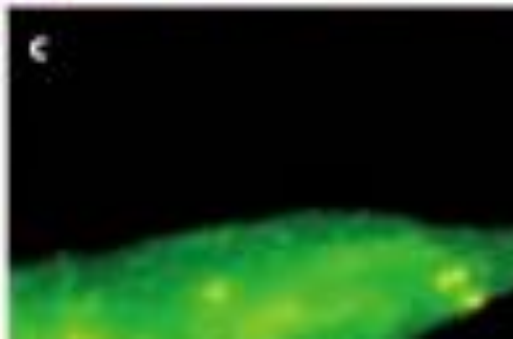
Type of cell death	Cells	Rate (thousands of cells/second)
Phagoptosis	Erythrocytes	2000
	Neutrophils	500–1000
Shedding	Enterocytes	80
Cornification	Keratinocytes	40
Necrosis	Enterocytes	10
Apoptosis	T cells and B cells	1
Autophagy		None known

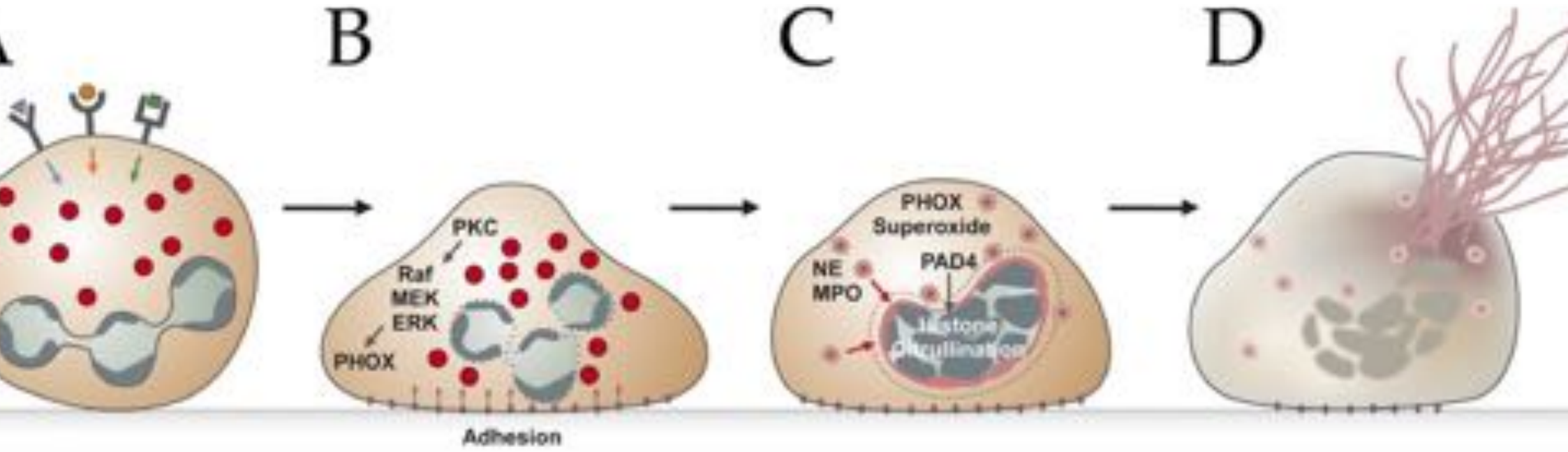
Neutrophil Extracellular Traps: an additional antibacterial weapon!



DNA







stimulation of receptors (A), neutrophils adhere to the substrate (B) and mo
ule components, namely NE and MPO (C). Granules are depicted as red ci
nes in the nucleus get processed, and the intracellular membranes disinteg
ly, the cell membrane ruptures, and the mixture of cytoplasm and nucleoplasm
lled to form NETs (D).
s been reported that peptidylarginine deiminase 4 (PAD4), an enzyme that con
r monomethyl-Arg to citrulline in histones, is essential for NET formation. The a
tensive chromatin decondensation along the NETs were rich in histone citrullina

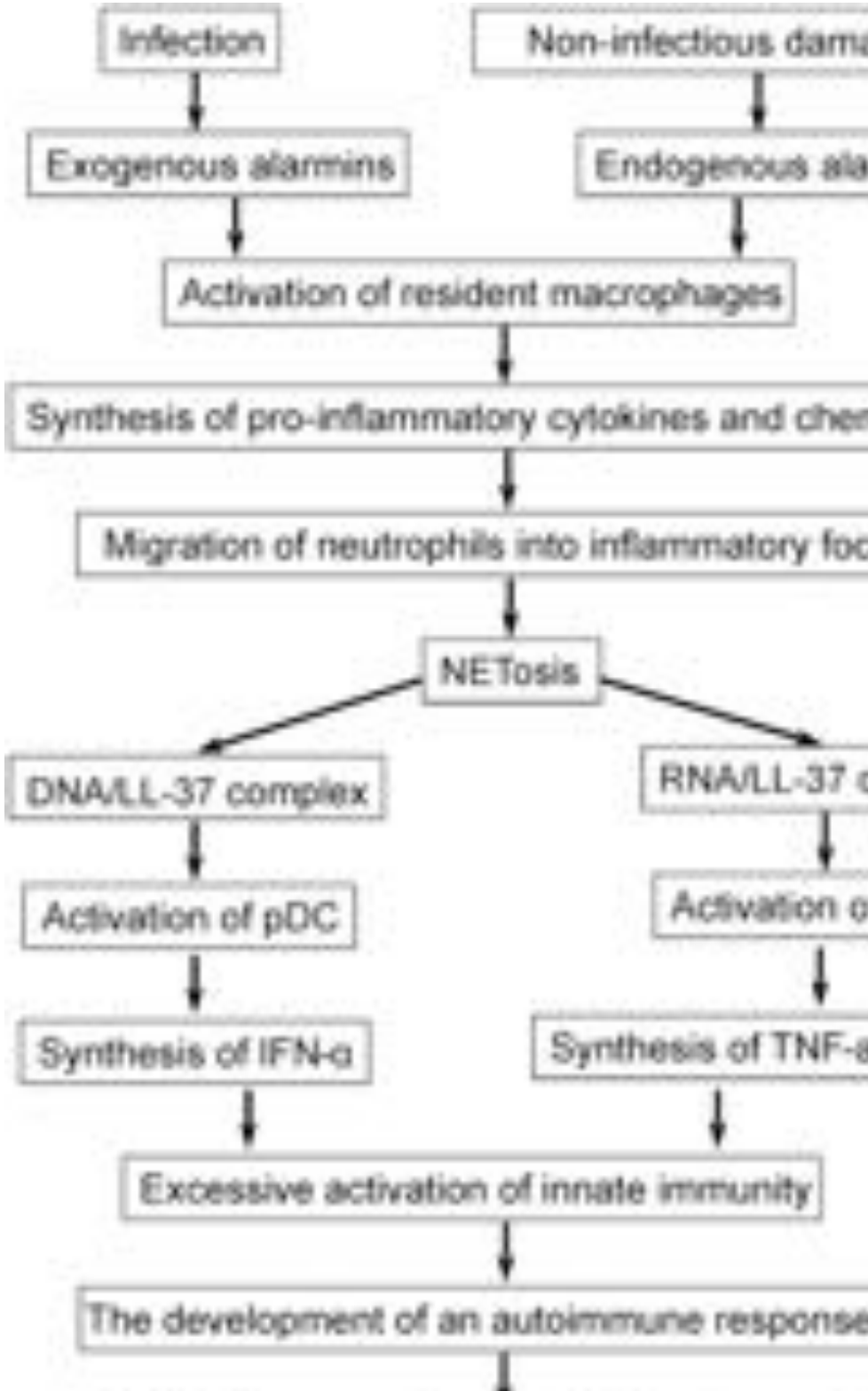
Front Immunol. 2012;3:307.

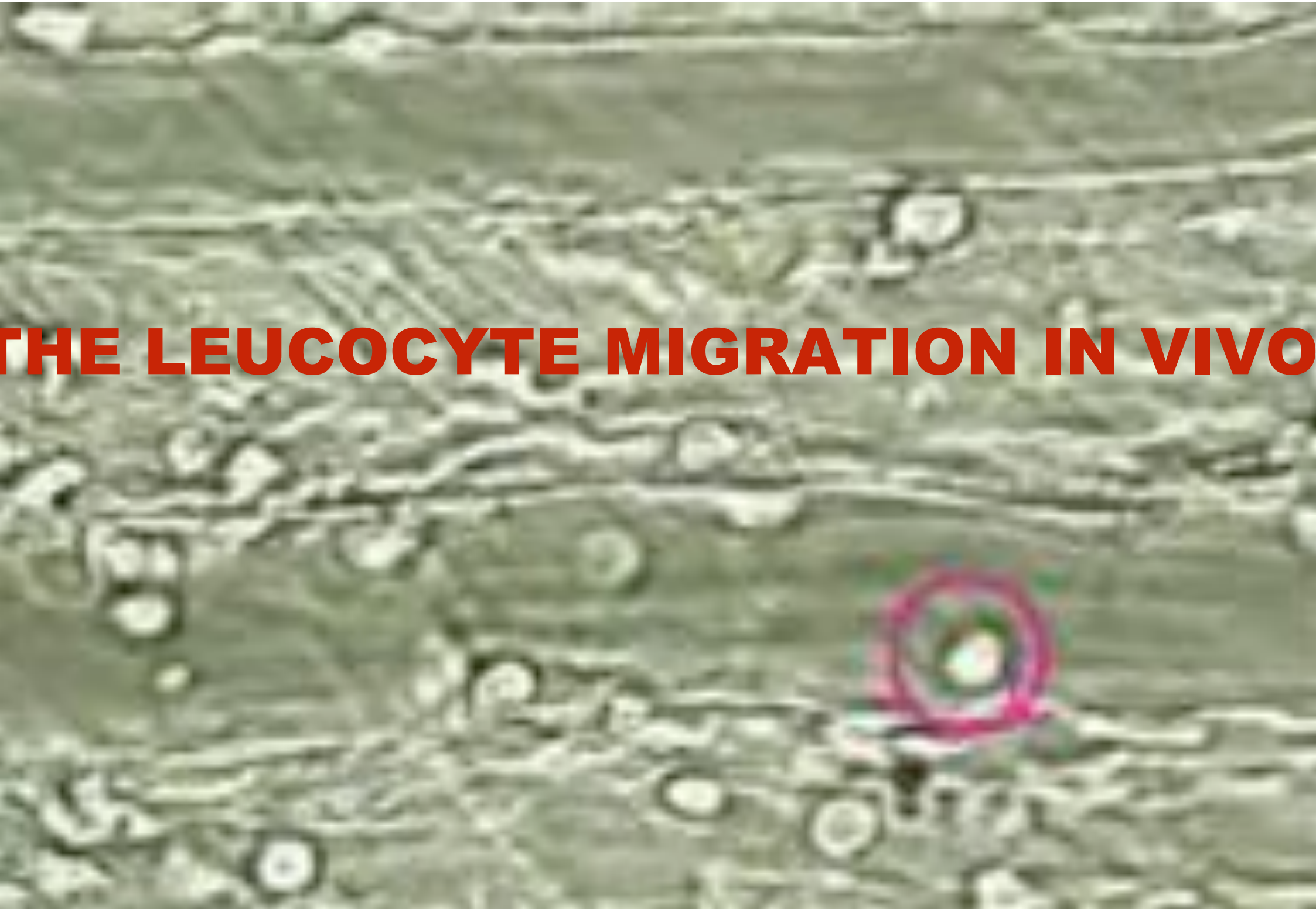
AD4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures

athogenesis of many autoimmune es is initially based on a redundant or ged activation of the innate immune . It was suggested that an excessive on of the innate immunity is often the of a chronic inflammatory process in the sm. This inflammation can be induced by ous and endogenous alarm factors, or ns. We believe that the recently ered neutrophil extracellular traps, or completely meet the criteria of alarmins.

eview summarizes current knowledge ning the general characteristics of NETs, ntimicrobial properties, and their role in evelopment of chronic inflammatory ses that underlie the pathogenesis of sis and atherosclerosis.

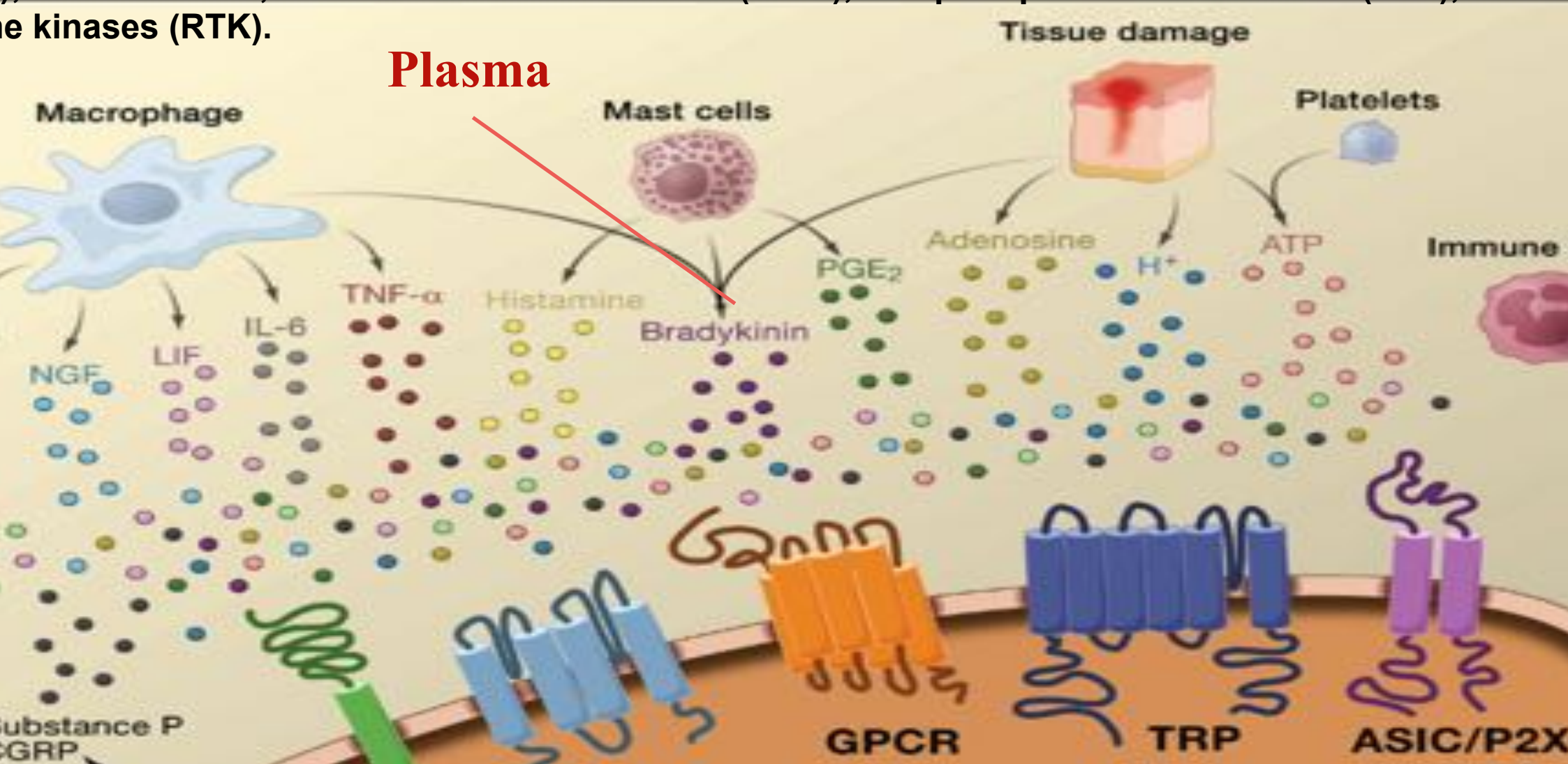
s on the NETosis can provide the




THE LEUCOCYTE MIGRATION IN VIVO

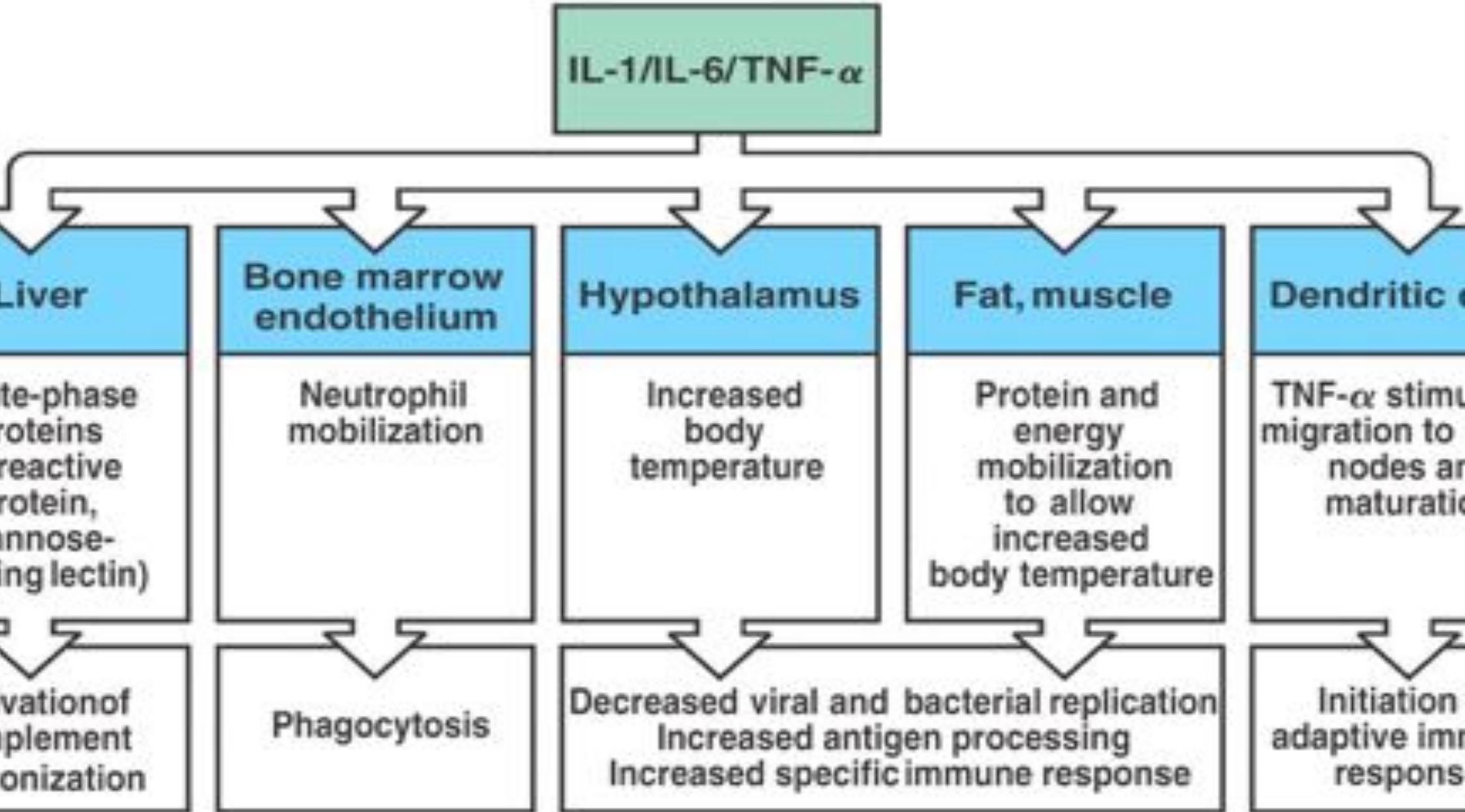
MEDIATORS….

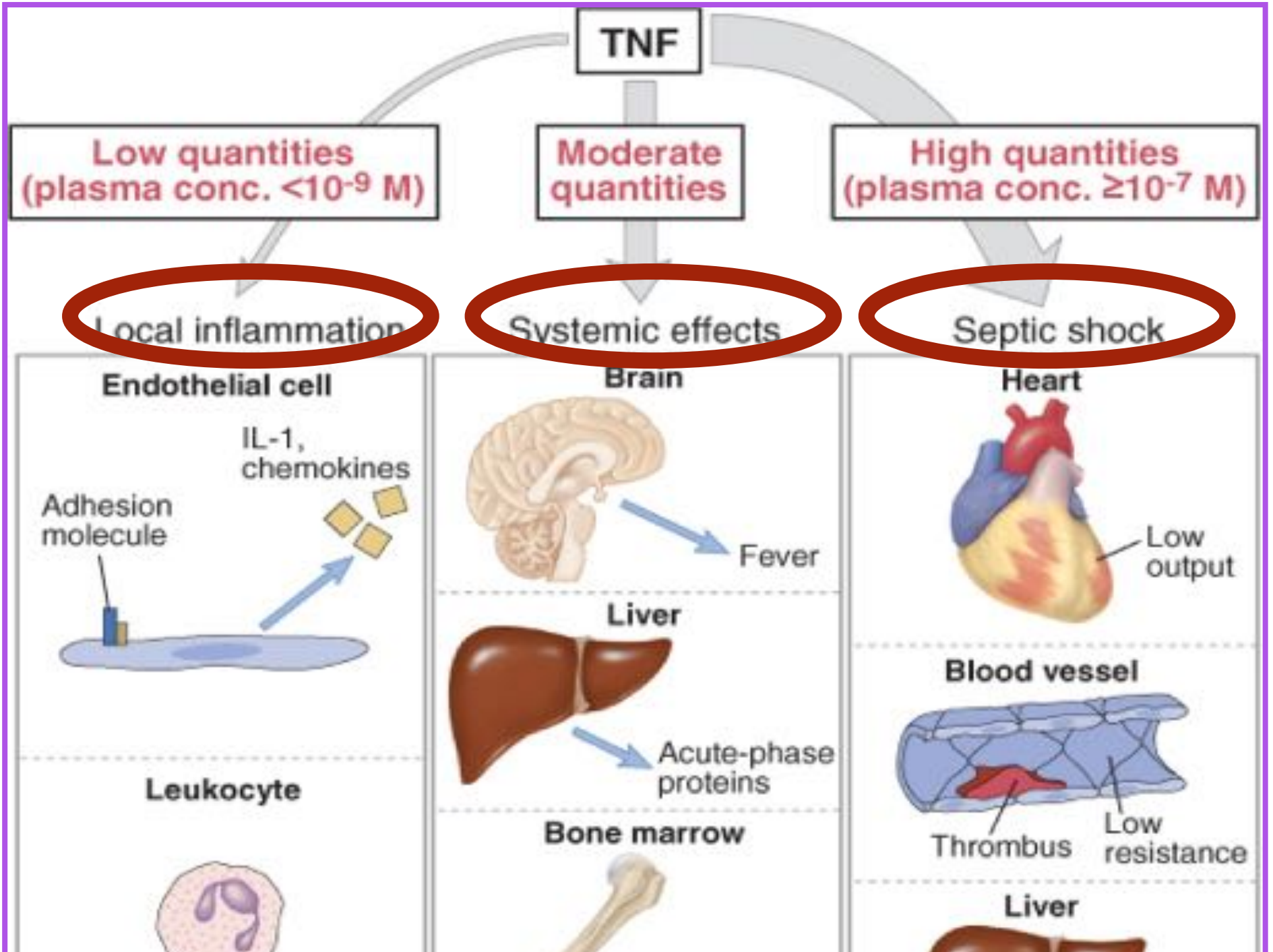
damage leads to the release of inflammatory mediators by activated nociceptors or nonneura
ng mast cells, basophils, platelets,macrophages, neutrophils, endothelial cells, keratinocyte
asts. This "inflammatory soup" of signaling molecules includes histamine, ATP, adenosine, sub
citonin-gene related peptide (CGRP), bradykinin, extracellular prostaglandins, thrombo
rienes, nerve growth factor (NGF), tumor necrosis factor a (TNF-a), interleukin-1β (IL-1β) etc.
s act directly by binding to one or more cell surface receptors, including G protein-coupled rec
), TRP channels, acid-sensitive ion channels (ASIC), two-pore potassium channels (K2P), and re
e kinases (RTK).



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

...WHICH CAN HAVE VARIOUS EFFECT...



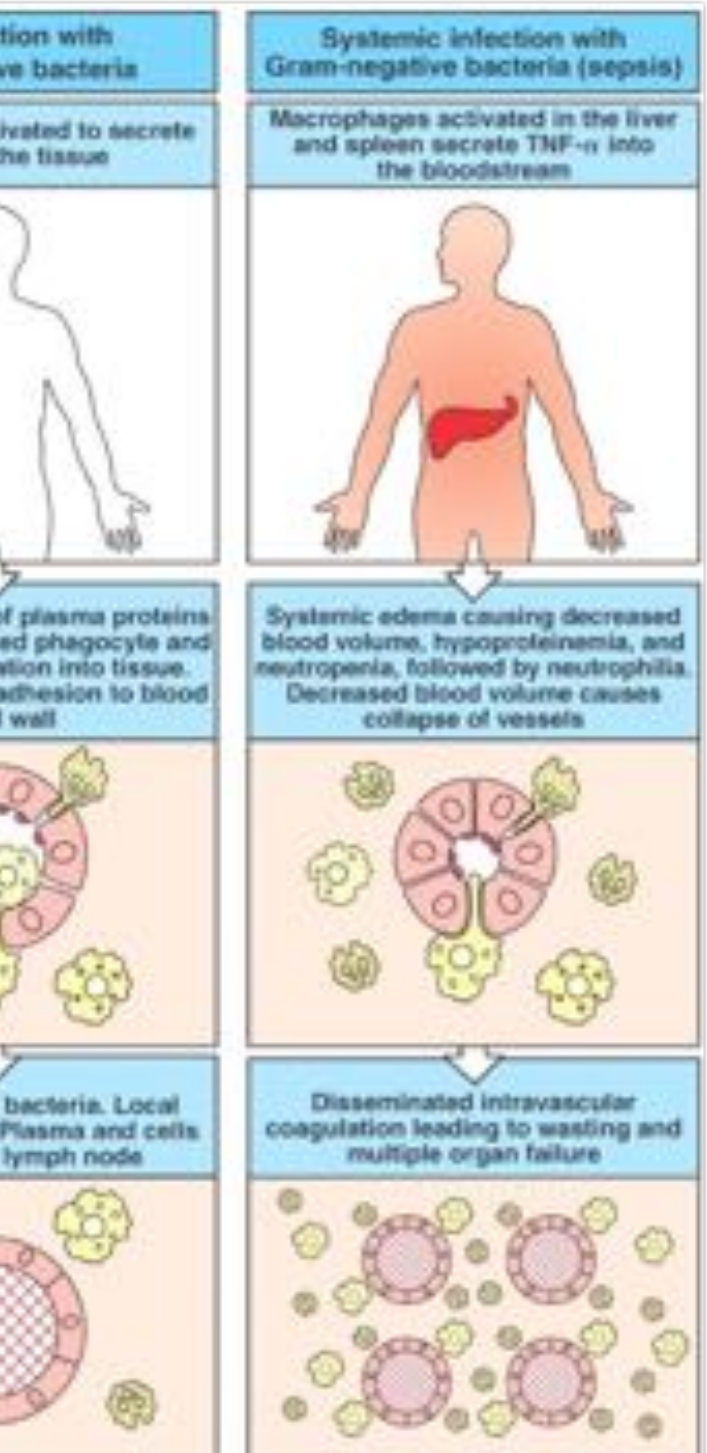

TNF
Low quantities (plasma conc. <10-9 M)
Moderate quantities
High quantities (plasma conc. ≥10-7 M)
Local inflammation
Systemic effects
Septic shock
Endothelial cell
IL-1, chemokines
Adhesion molecule
Leukocyte
Brain
Fever
Liver
Acute-phase proteins
Bone marrow
Heart
Low output
Blood vessel
Thrombus
Low resistance
Liver

Pathological consequences of Systemic Inflammatory response (SIRS) are:

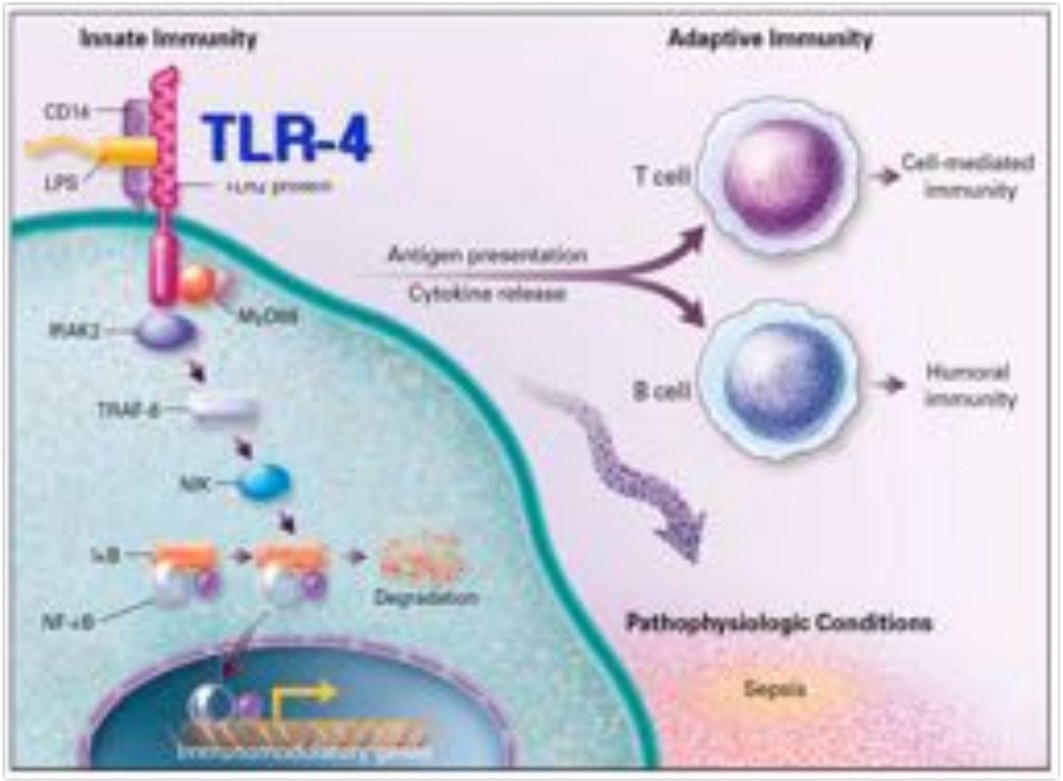
Septic shock;

ARDS;

Multiple organ dysfunction (MOD)

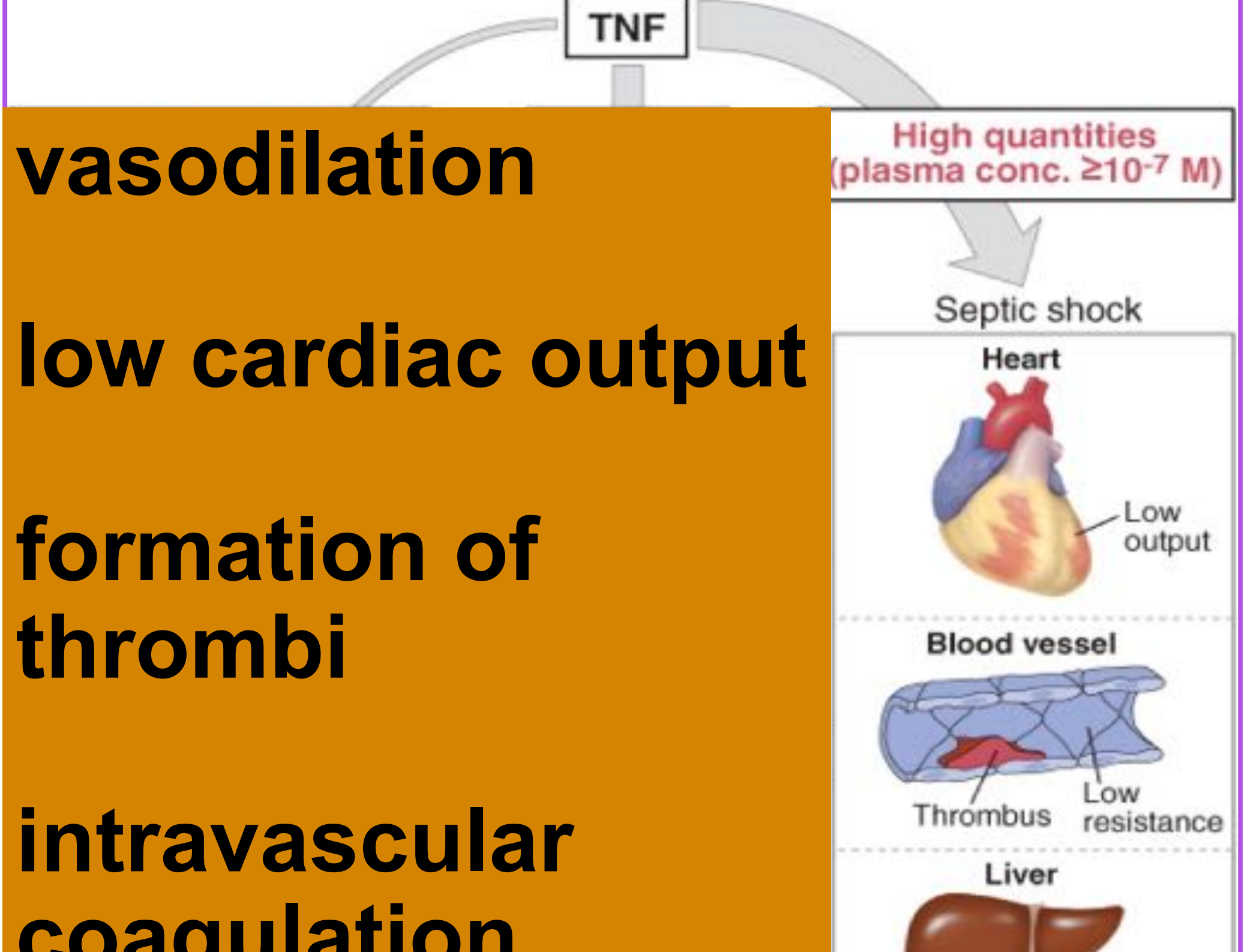


Pathological consequences of inflammatory response to systemic LPS: the septic shock



Septic shock is classically triggered by Gram- bacteria (TLR-4/LPS); Gram+ bacteria too can induce a systemic

- endototo
- esotos



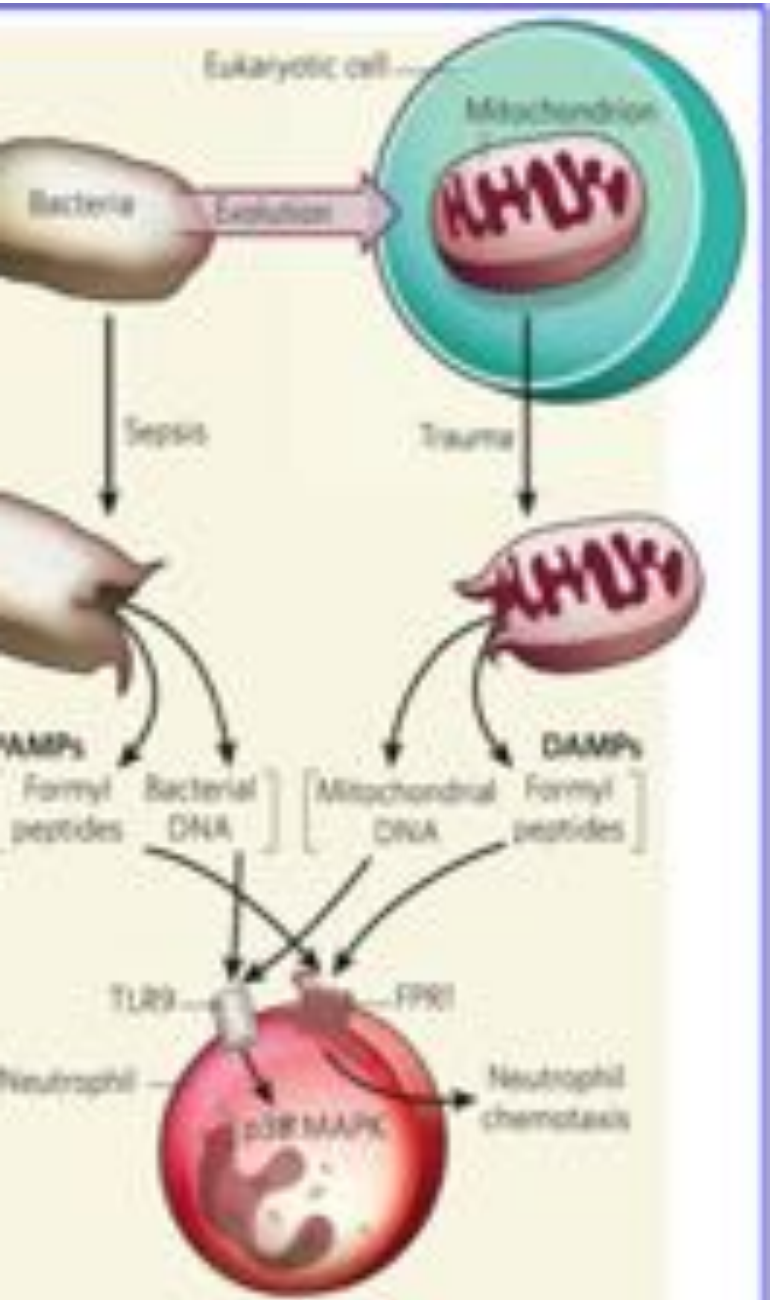
vasodilation

low cardiac output

formation of thrombi

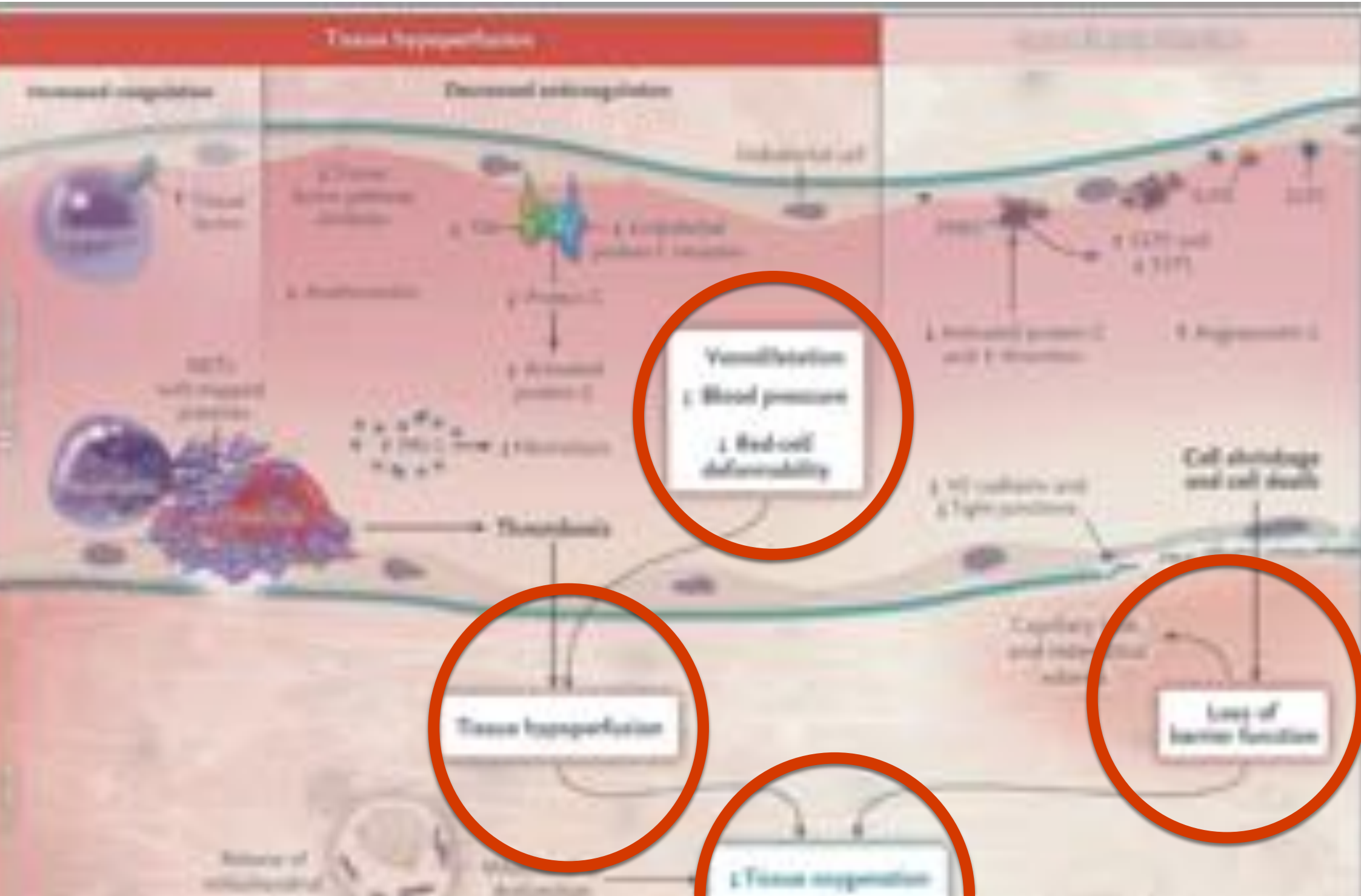
intravascular coagulation

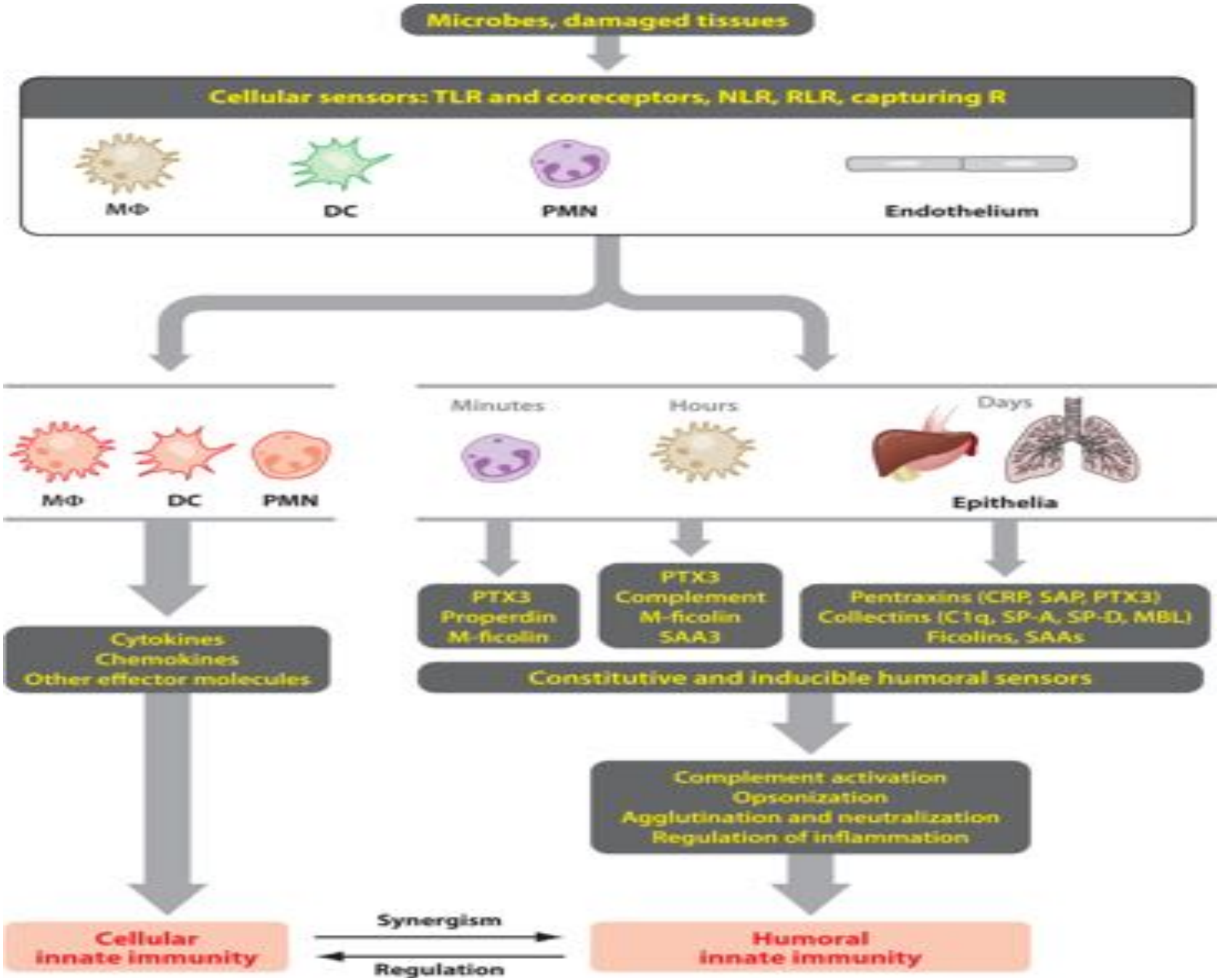
ESPIRATORY DISTRESS SYNDROME (ARDS)



	ARDS		
	Mild	Moderate	Se
Timing	Acute onset within 1 week of a known clinical in new/worsening respiratory symptoms		
Hypoxemia	PaO_2/FiO_2 201–300 with PEEP/CPAP ≥ 5	PaO_2/FiO_2 ≤ 200 with PEEP ≥ 5	PaO_2/Fi with PE
Origin of Edema	Respiratory failure associated to known risk factor fully explained by cardiac failure or fluid overloa objective assessment of cardiac failure or fluid ov no risk factor are present		
Radiological Abnormalities	Bilateral opacities*	Bilateral opacities*	Opacities at lea quadr
Additional Physiological Derangement	N/A	N/A	$V_{E\,Corr}$ > 10 o C_{RS}<40 m

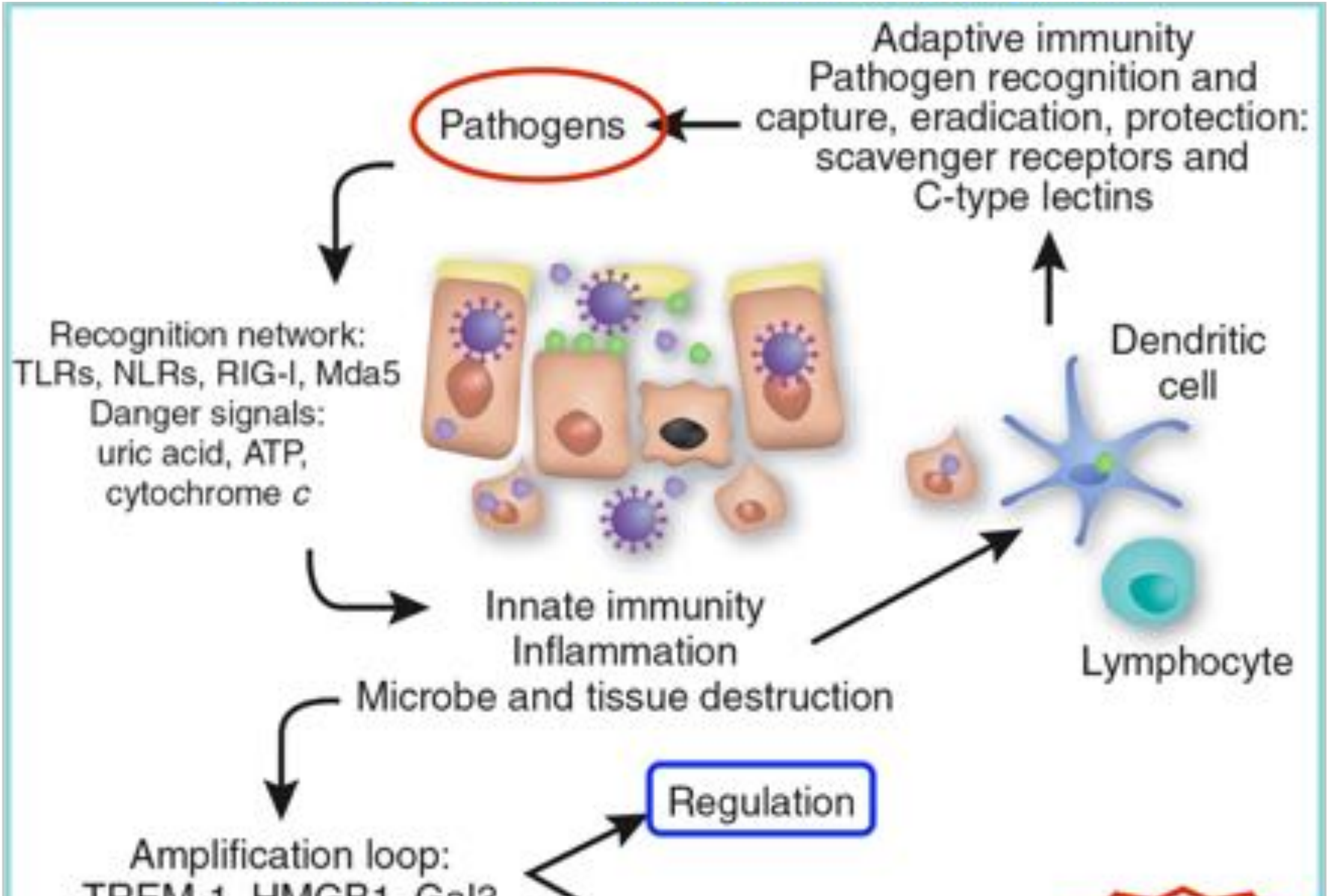
Multiple organ failure (MOF)!





umoral and Cellular sensors share fundamental mechanis
of effector function: complement activation and regulatio

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!