OIID 600

February 17, 2025

Innate Immunity to the Periodontal Biofilm

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Oral Immunology and Infectious Diseases

Biofilms and Periodontal disease

Periodontal health has been traditionally characterized by a predominance of Gram-positive, facultative, and fermentative bacteria (*e.g.*, streptococci and actinomyces), which form a biofilm on tooth surfaces in an orderly ecological succession.

The onset of periodontal disease is traditionally associated with a significant shift to Gram-negative, anaerobic, and proteolytic bacteria.

(Contemporary microbiome studies are challenging this)

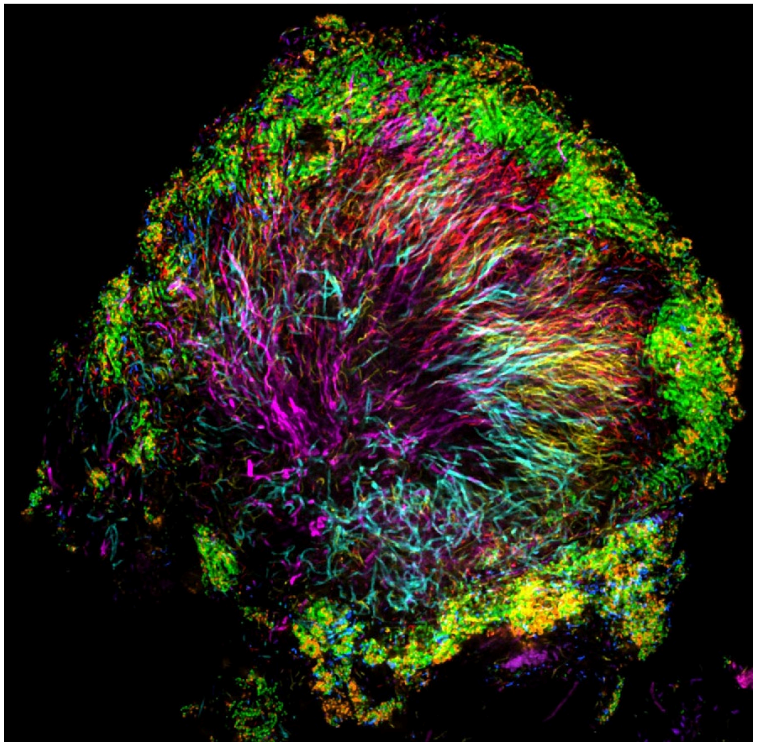
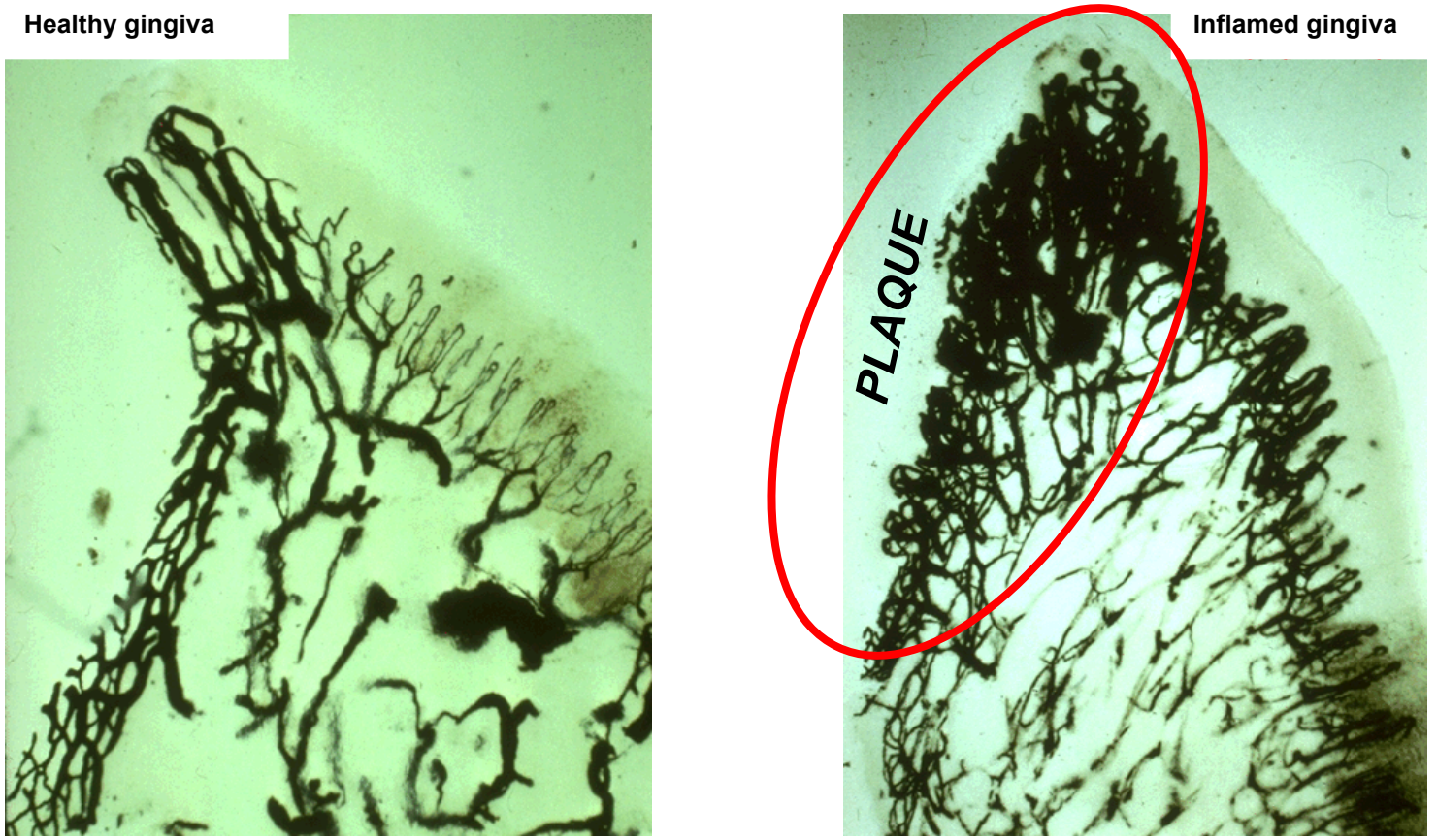


Image credit: Steven Wilbert and Gary Borisy, The Forsyth Institute

Innate Immunity from the Oral Perspective

(1) Vascular response
(2) Epithelium
(3) GCF
(4) Saliva
(5) Neutrophils
(6) Monocytes / Macrophages

(1) The vascular response



- Vessels of the gingival microvasculature enlarge and proliferate during inflammation.
- Alterations most pronounced adjacent to pocket epithelium, close to subgingival plaque.

Histamine

- Histamine….induces endothelial contraction and endothelial gaps (edema)
  - Distributed in tissues but primary source is mast cells adjacent to vessels
  - Released on mast cell stimulation with:
    - Physical trauma
    - Epithelial breach
    - Host-derived stimuli
      - Anaphylatoxins (C5a and C3a)
      - Cytokines, e.g., IL-1, IL-8
    - Bacterial-derived stimuli
      - Fimbriae
      - LPS

Histamine (red granules) in mast cell

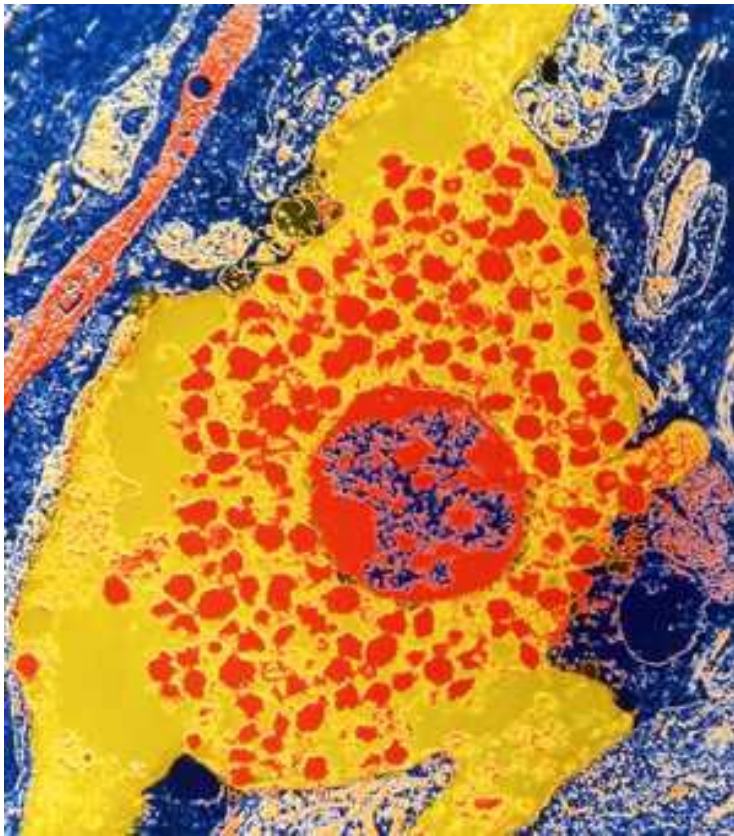


Image taken from: encarta.msn.com

Reflected in increased edema and bleeding on probing

(2) The epithelium

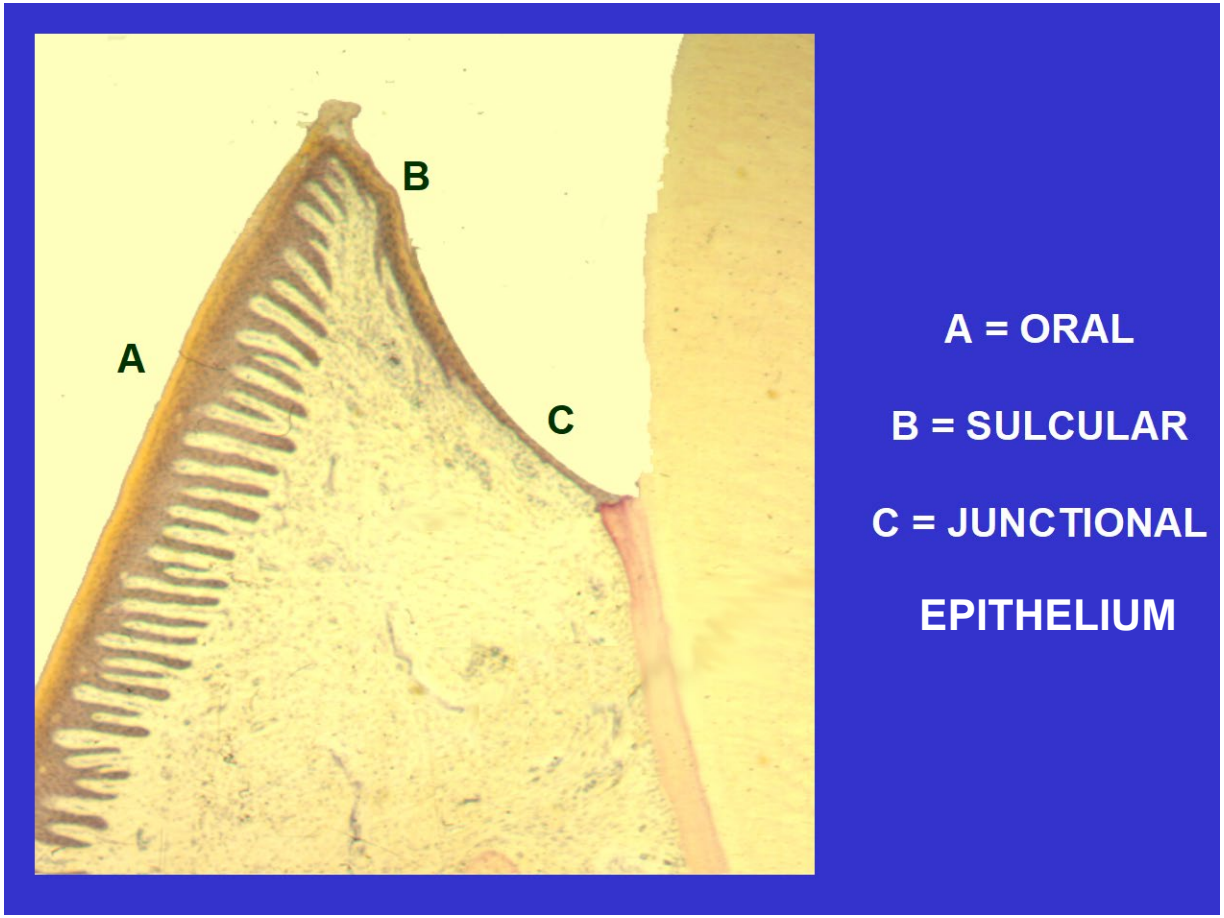


Image: www.uwcm.ac.uk/study/dentistry/courses/lectures/perioa.ppt

The junctional epithelium….

- The sulcular / junctional epithelium represents a physical barrier to plaque bacteria

- Dynamically facilitates leukocyte infiltration and migration to the gingival sulcus

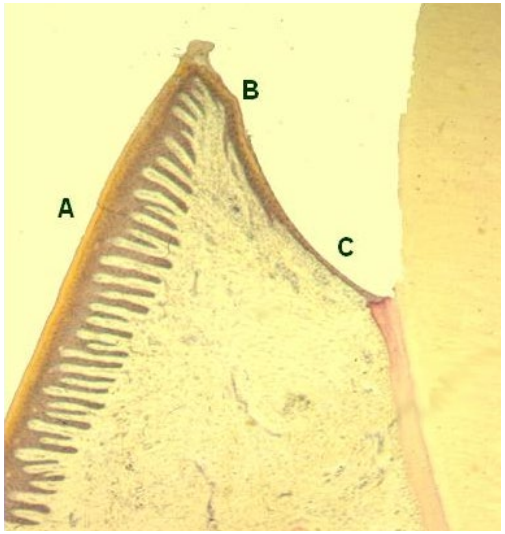
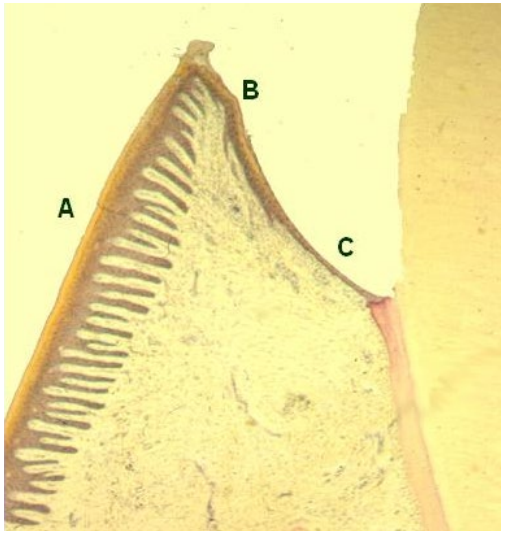


Image: www.uwcm.ac.uk/study/dentistry/courses/lectures/perioa.ppt

The junctional epithelium….

- **Portal for an important inflammatory exudate (gingival crevicular fluid; GCF)**



The junctional epithelium is not passive

- **Recognizes microbial challenges**

  The junctional epithelium expresses multiple receptors that recognize common molecular structures that are found in microbes but not humans.

  Engagement of these receptors initiates the inflammatory response.

The junctional epithelium is not passive

- **Recognizes microbial challenges (e.g., Toll-like receptors)**

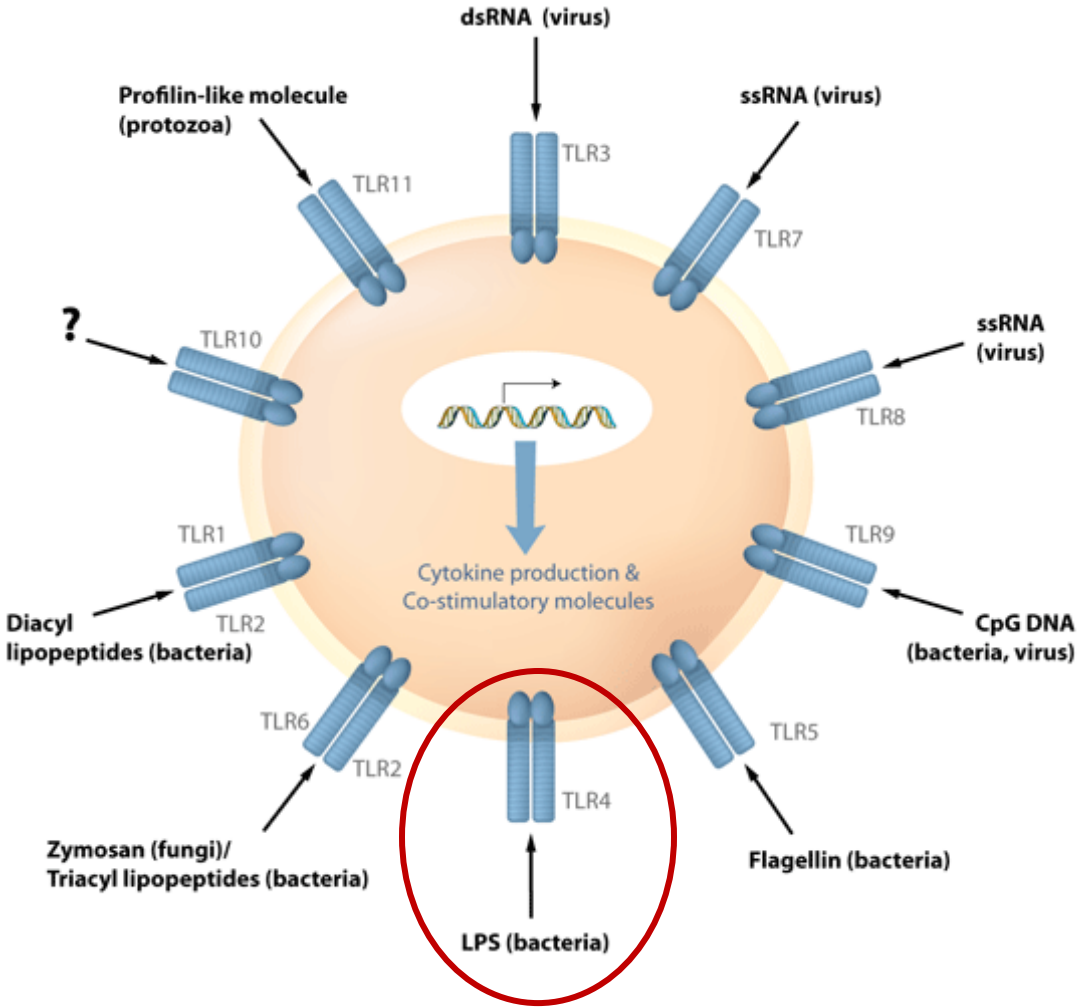


Fig source unknown.

The junctional epithelium is not passive

- **Produces potent antimicrobial proteins called defensins**
- **Defensins are antimicrobial molecules / “human antibiotics”.**
- **Defensins are chemotactic to leukocytes.**

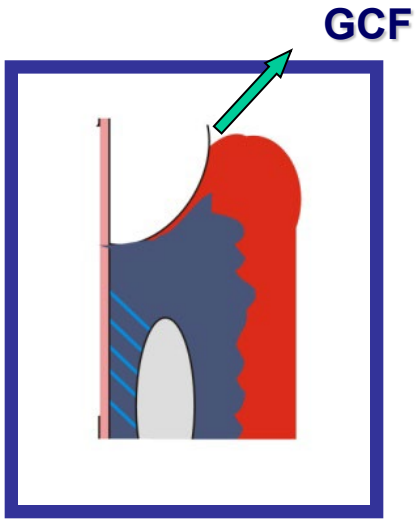
The junctional epithelium is not passive

- **Produces small, chemotactic proteins called cytokines (IL-1, IL-8)**
  - **Pro-inflammatory**
  - **Chemotactic to leukocytes**
- **Large regenerative capacity (heals easily)**

(3) Gingival crevicular fluid (GCF)

Contains soluble molecules derived from….

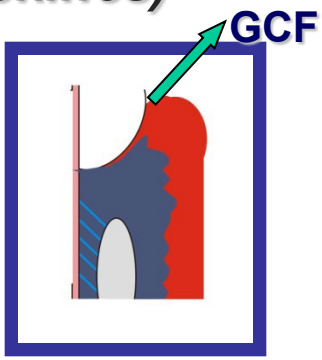
- ***Plasma***
- ***Connective tissue fluid***
- ***White blood cells***
- ***Junctional epithelium***
- ***Bacteria***



GCF

Contains…….

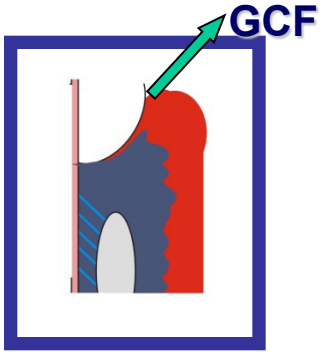
- ***Leukocytes** (>90% neutrophils)*
- ***Inflammatory mediators** (e.g. cytokines)*
- ***Bacterial metabolites***
- ***Tissue breakdown products***
- ***Systemic biomarkers***



GCF

Contains…….

- ***Antibodies*** *(from B cells…help kill microbes)*
- ***Lysozyme*** *(attacks bacterial cell walls)*
- ***Ferritin*** *(binds iron that bacteria require to survive)*
- ***Lactoferrin*** *(binds iron that bacteria require)*
- ***Complement***
- ***Many other components***



GCF collection

Aguiar et al,
Biomed Int Res, 2017

Measurement of GCF flow

Periotron

(we have one at ULSD)

GCF analysis

- Volume
- Cytokines
- Tissue breakdown products
- Systemic disease correlates
- Etc.

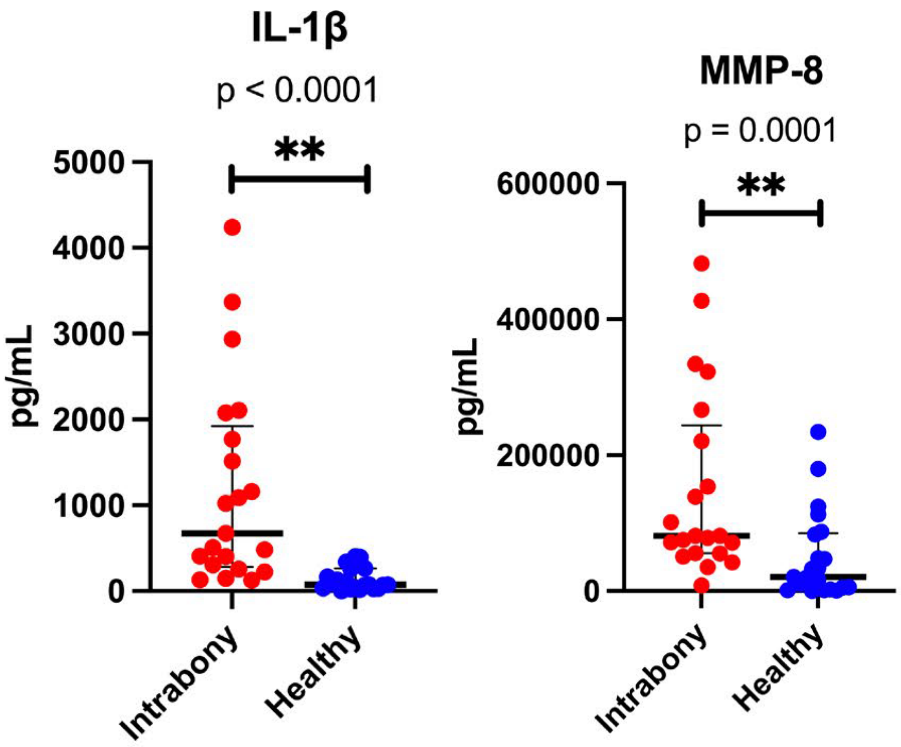
Received: 26 June 2021 | Revised: 7 October 2021 | Accepted: 12 October 2021
DOI: 10.1111/jre.12948

ORIGINAL ARTICLE

JOURNAL OF PERIODONTAL RESEARCH WILEY

Molecular profiling of intrabony defects' gingival crevicular fluid

Vasiliki P. Koidou[1,2] | Eleni Hagi-Pavli[2] | Samantha Cross[3] | Luigi Nibali[1,4] | Nikolaos Donos[1,2]



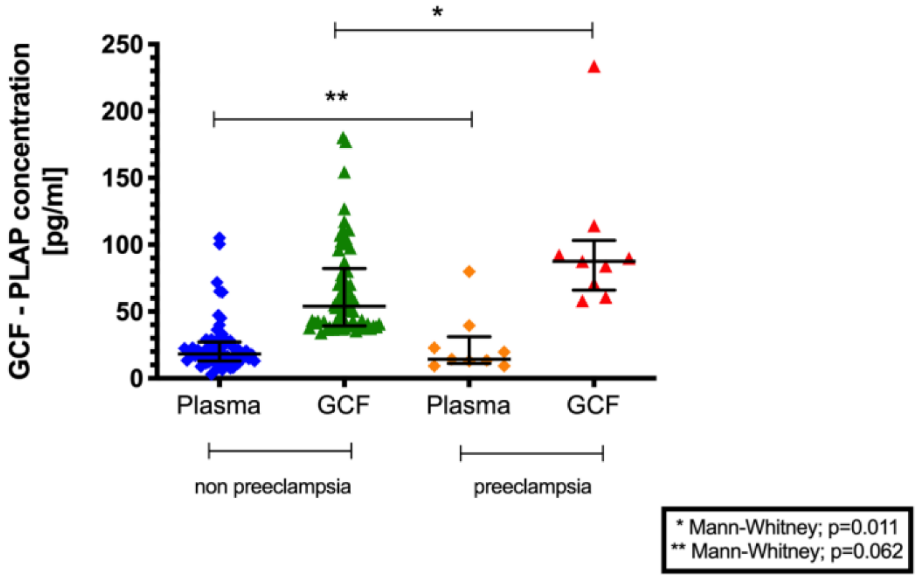
Inflammatory and destructive disease markers

diagnostics MDPI

Article

Gingival Crevicular Placental Alkaline Phosphatase Is an Early Pregnancy Biomarker for Pre-Eclampsia

Alejandra Chaparro [1,*], Maximiliano Monckeberg [2], Ornella Realini [1], Marcela Hernández [3], Fernanda Param [1], Daniela Albers [4], Valeria Ramírez [5], Juan Pedro Kusanovic [6,7], Roberto Romero [8,9,10,11,12,13], Gregory Rice [14,15] and Sebastián E. Illanes [2,16]



Pre-eclampsia marker

Complement-mediated amplification of inflammation (C3a and C5a)

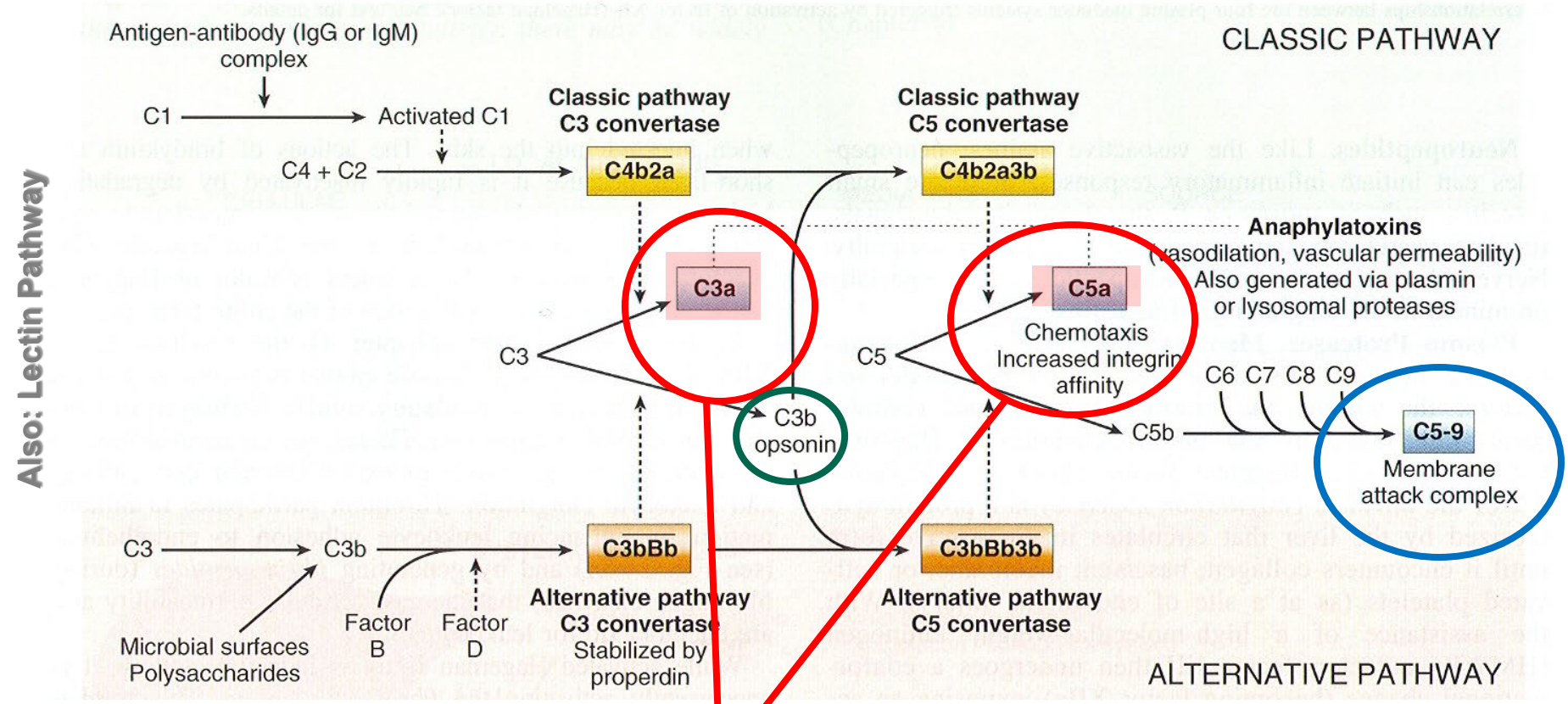
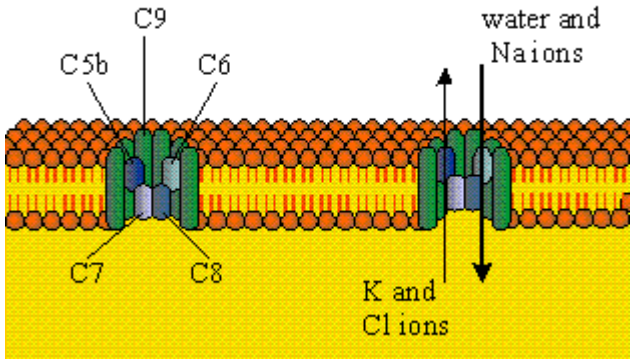


Fig: Robbins Basic Pathology

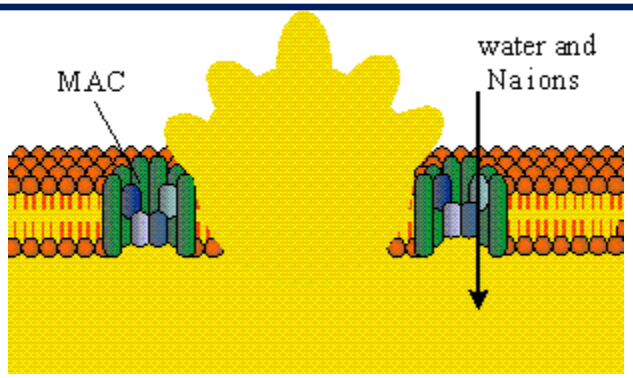
C3a and C5a are anaphylatoxins

- induce histamine release from mast cells
- activate leukocytes
- chemotactic to leukocytes

Complement-mediated bacterial killing

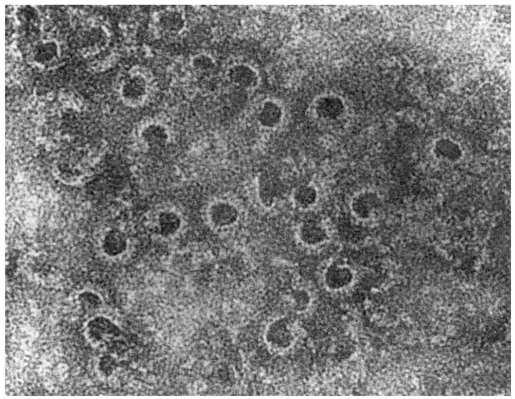


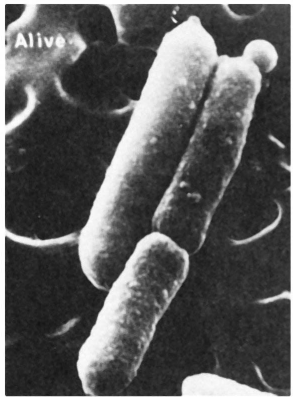
MAC is a “hole puncher”



Lyses bacterial cells

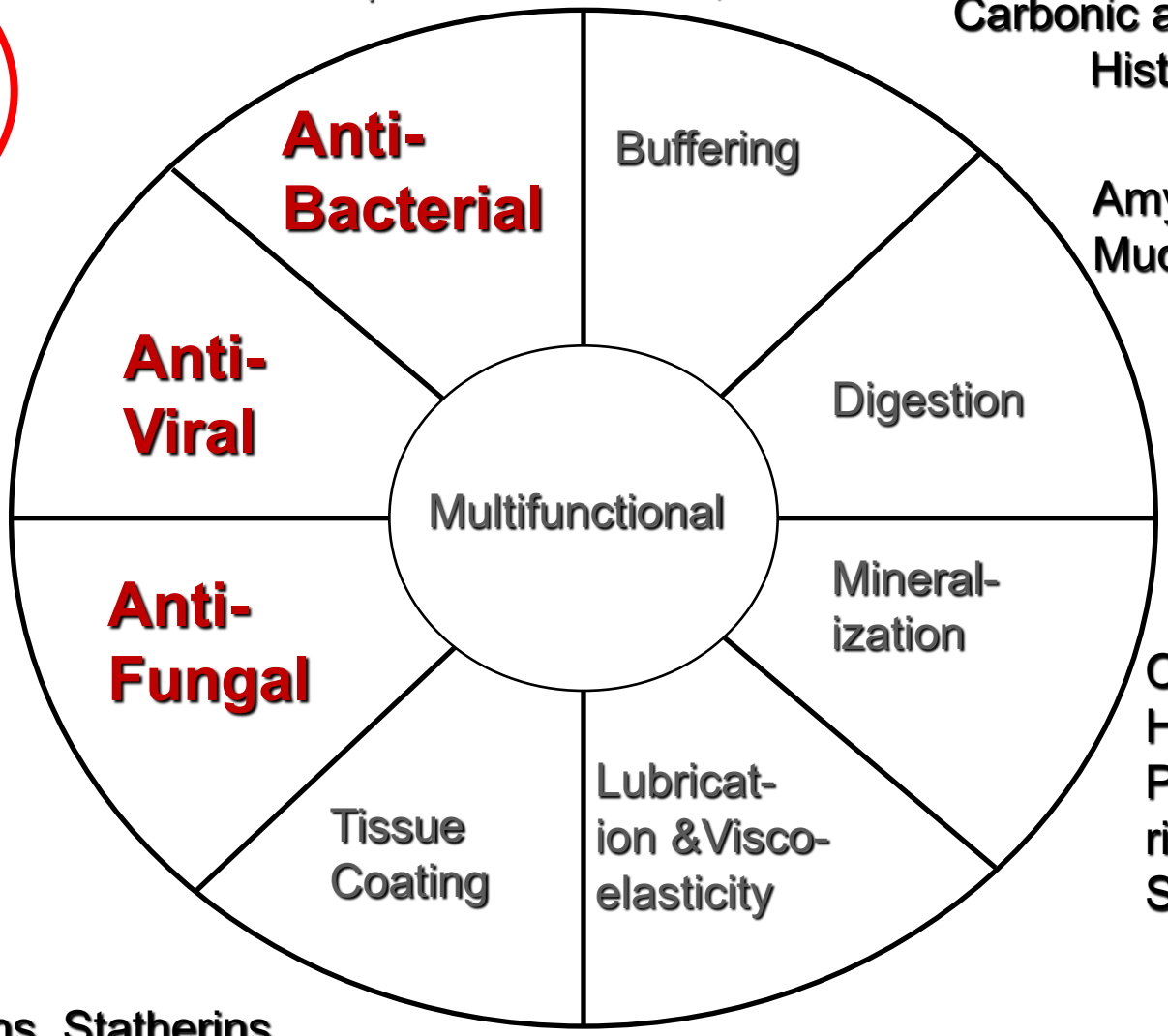
Example of complement-dependent bacterial killing. Membrane pores formed by the membrane attack complex (left) (transmission EM). Complement killed bacteria (right) compared to live ones (centre) (scanning EM)





Figures and text: webmed.unipv.it/ immunology/compl.html

(4) Saliva



Cystatins, Peroxidases, Antibodies

Carbonic anhydrases, Histatins

Amylases, Mucins, Lipase

Cystatins, Antibodies

Histatins

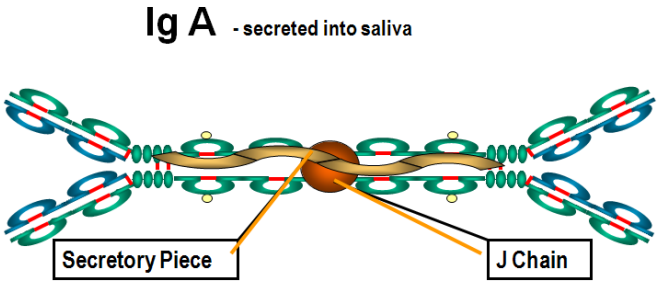
Cystatins, Histatins, Proline-rich proteins, Statherins

Amylases, Cystatins, Mucins, Proline-rich proteins, Statherins

Mucins, Statherins

Antimicrobial proteins of saliva

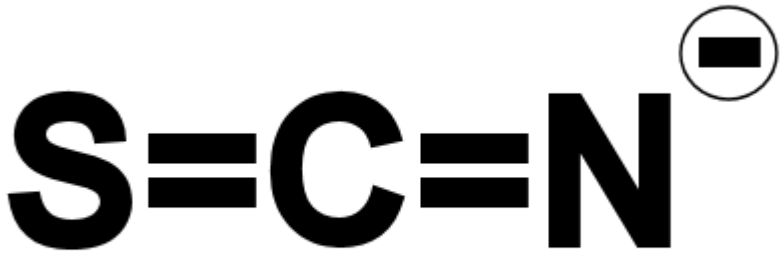
- **Secretory IgA**



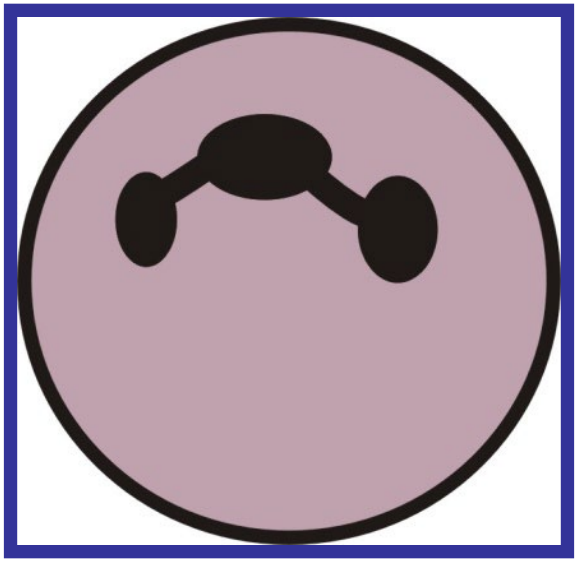
- **Lactoferrin**
- **Lysozyme**
- **Cystatins** (inhibit activity of several bacterial proteases)
- **Histatins** (histidine-rich proteins that inhibit *C. albicans* and several bacteria and viruses)

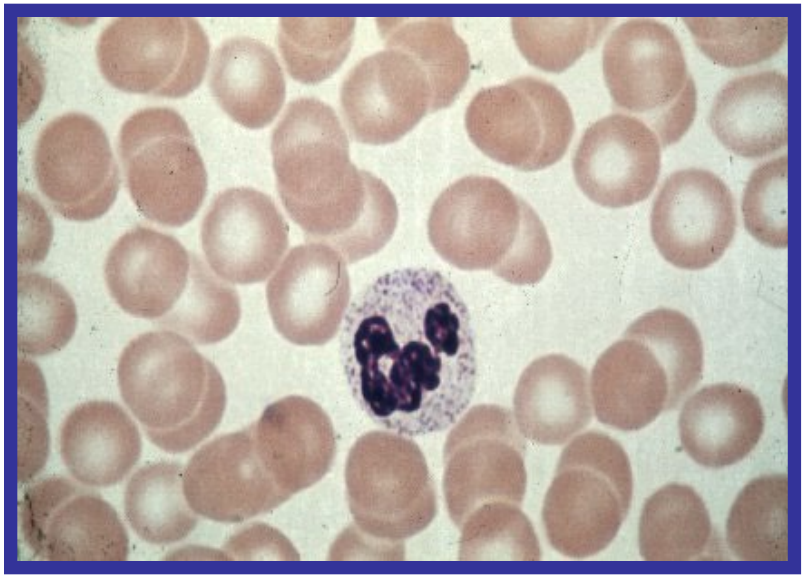
Thiocyanate

- Anti-bacterial
- Considered key anti-cariogenic agent



(5) Neutrophils





- Rapidly respond to the injury / infection

- Contain large amounts of pre-formed anti-microbial and pro-inflammatory agents

Neutrophil ultrastructure

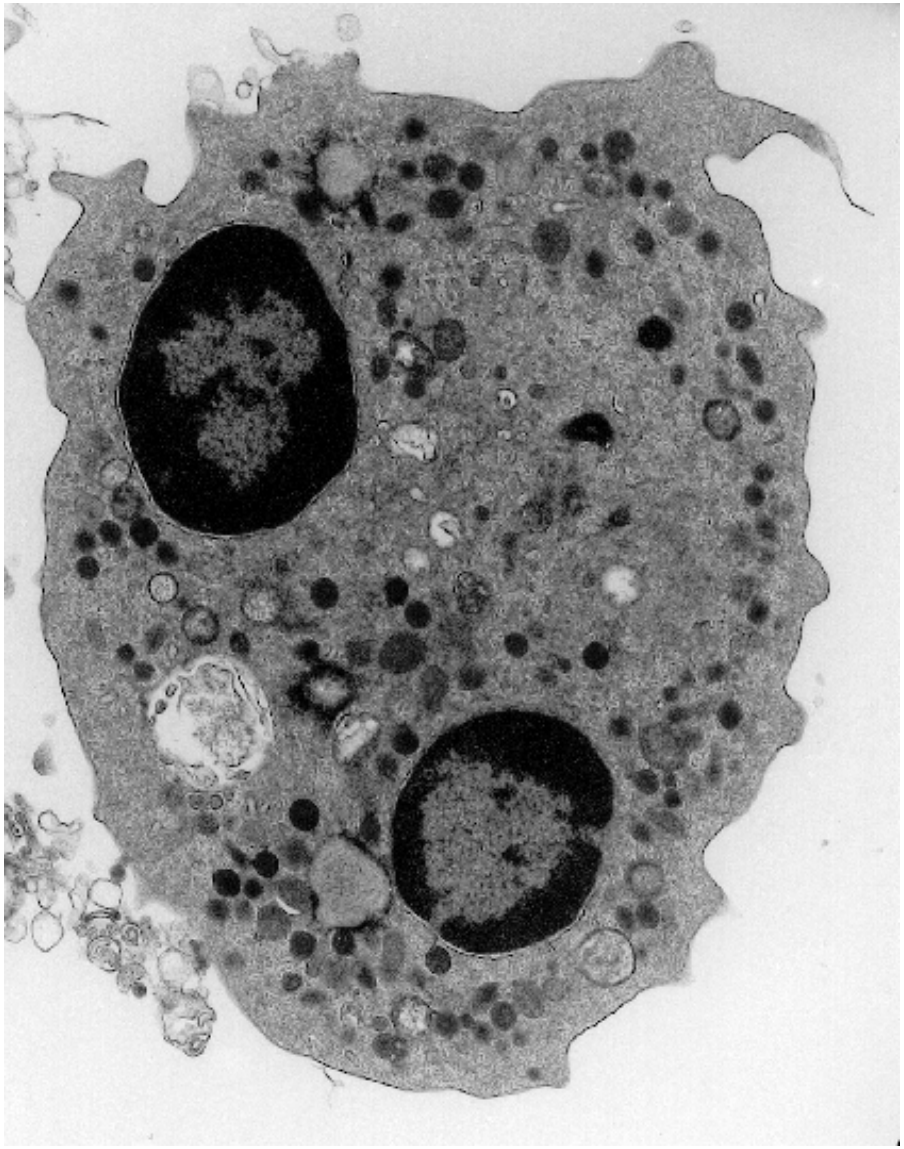


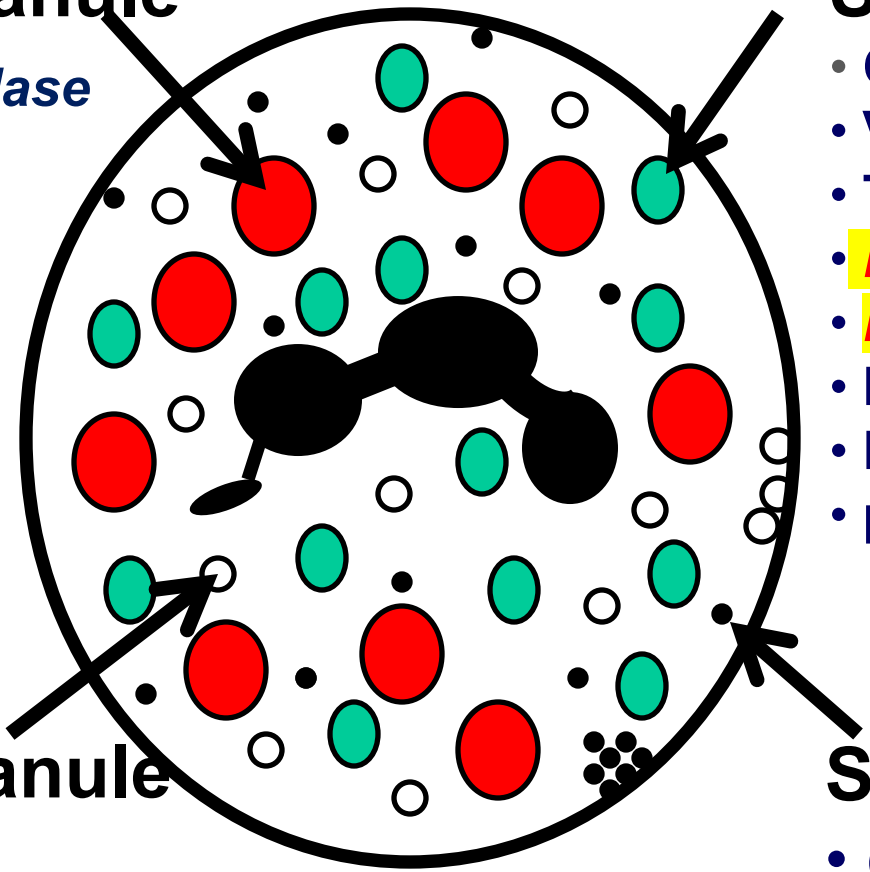
Image taken from: Krauss & Scott, *Frontiers Oral Biol*, 2012

Primary granule

- *myeloperoxidase*
- *elastase*
- CD63, CD68[a]
- cathepsin G
- PR3[b]
- *defensins*
- azurocidin
- BPI protein

Secondary granule

- CD11/CD18
- VAMP2[c]
- TNF-R
- *MMP-8, -9*
- *lactoferrin*
- hCAP18
- NGAL
- phox proteins[d]



Tertiary granule

- CD11/CD18
- VAMP2
- TNF-R
- MMP-9, -25
- *lysozyme*
- phox proteins

Secretory vesicle

- CD11/CD18
- VAMP2
- TNF-R
- *fMLP-R*
- MMP-25
- phox proteins
- TLRs[e]
- Fcγ-Rs
- CXC-Rs[f]
- CRs[g]

***Figure 3*: Contents of the intracellular membrane-bound compartments of human neutrophils**

[a]CD63 and CD68 are degranulation markers; [b]Proteinase 3; [c]VAMP2 is key in granule membrane-cell membrane fusion and exocytosis; [d]NADPH oxidase subunits involved in ROS production (Gp91phox; p22phox); [e]TLR1, -2, -4, -6 and -8; [f]CRCX1, -2 and -4 as well as chemokine receptors 1, -2 and -3; [g]complement receptor 1 (CD35), c1qR .

Image taken from: Krauss & Scott, *Frontiers Oral Biol*, 2012

Neutrophil chemotaxis

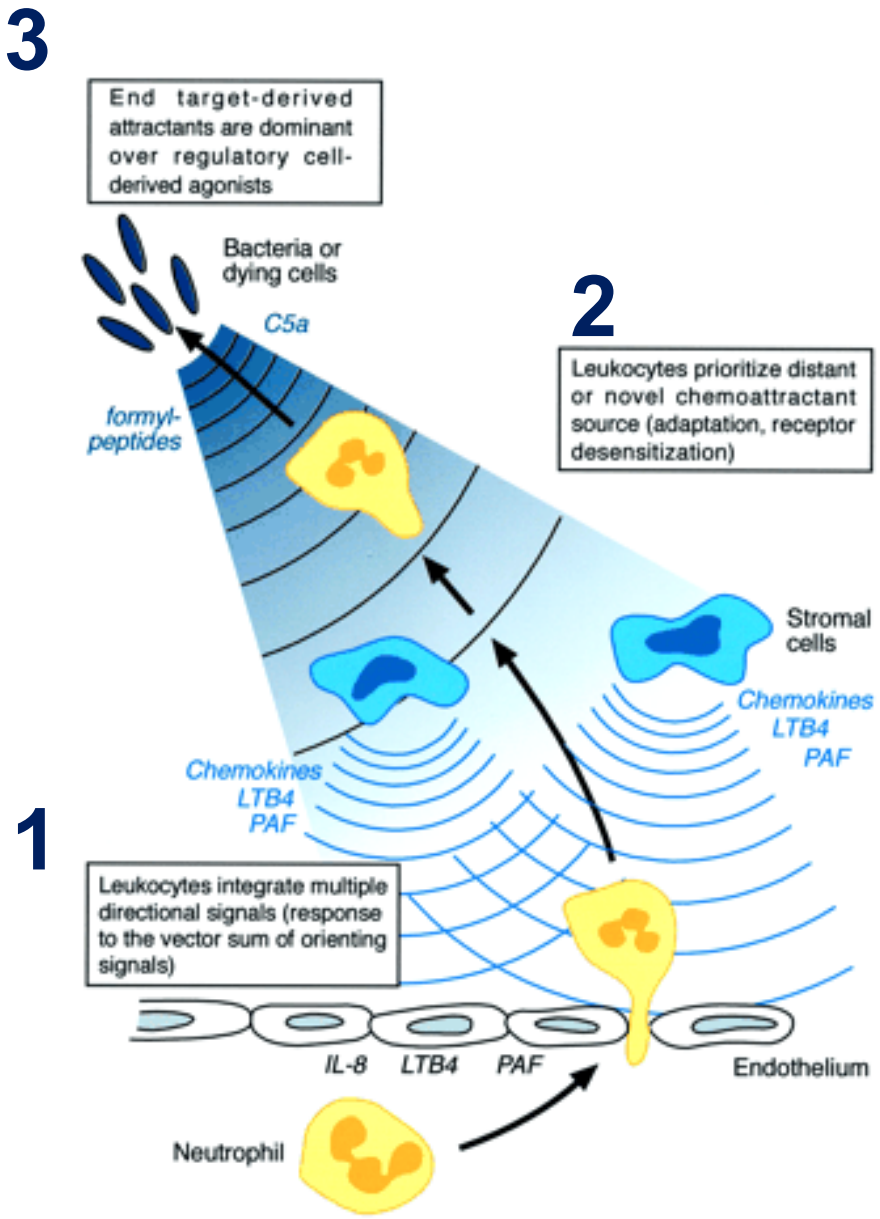


Image: Witko-Sarsat et al. Lab Invest 2000; 80: 615-653.

Exogenous chemotactic signals prioritized

- **Endogenous chemoattractants**
  - **Complement proteins (C3a, C5a)**
  - **Arachidonic acid metabolites**
  - **Cytokines, esp. IL-8**

- **Exogenous chemoattractants**
  - **Microbial products**
    - **LPS**
    - **fMLP (bacterial peptides)**

Diapedesis

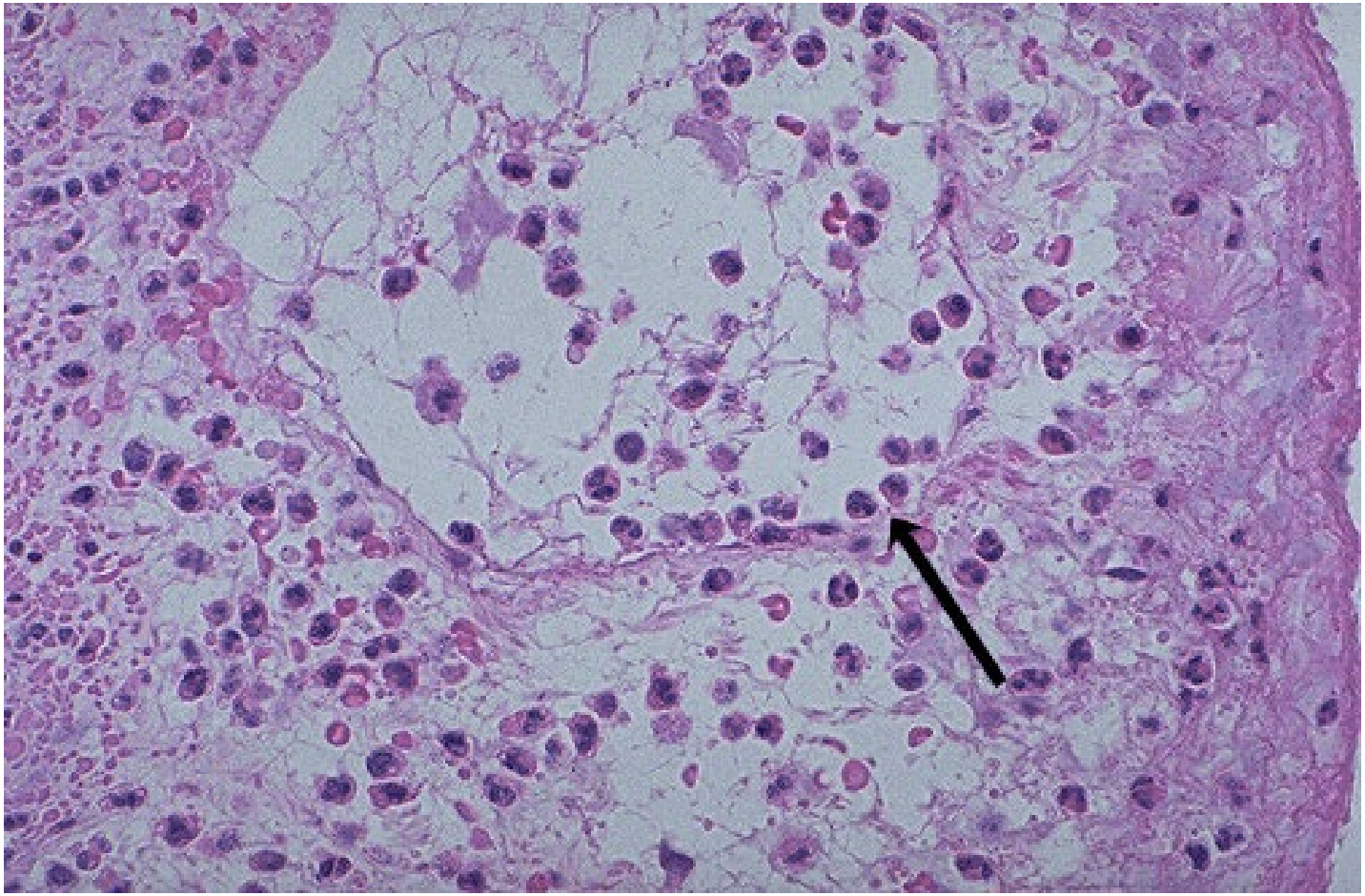


Image: medicine.skku.ac.kr/dataroom/2inflammation-content.ppt

Neutrophils (and other leukocytes) are marginated along the dilated venule wall (arrow) are squeezing through (diapedesis) into extravascular space at sites of microvasculature activation (e.g. by histamine).

The Neutrophil “Wall”

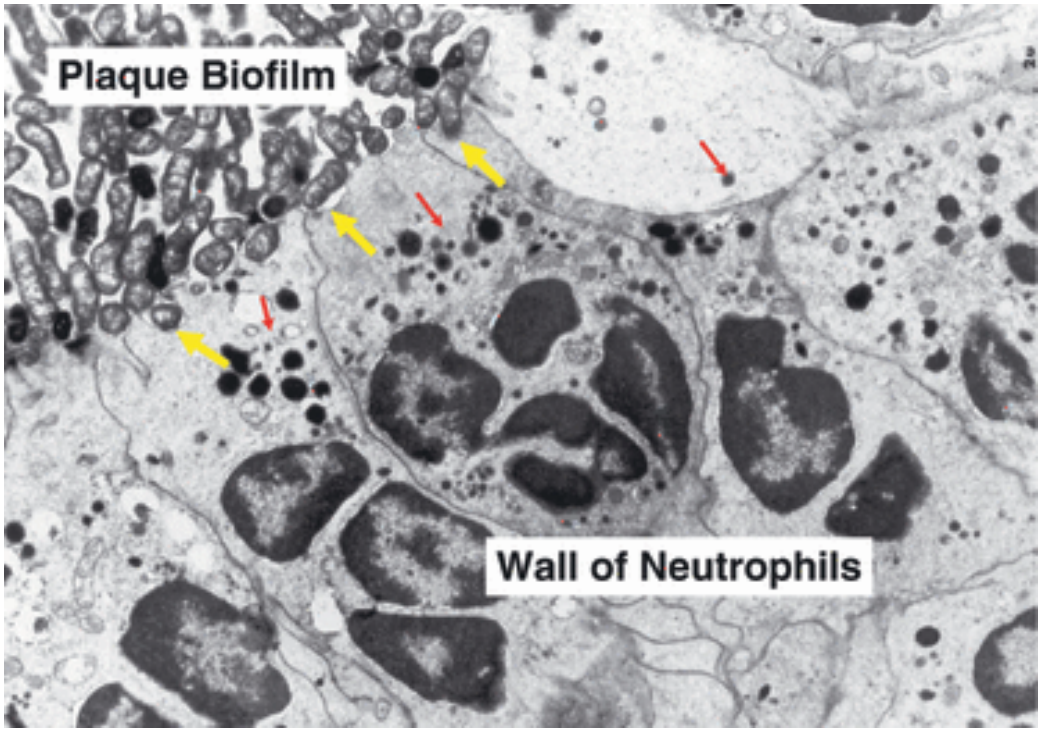
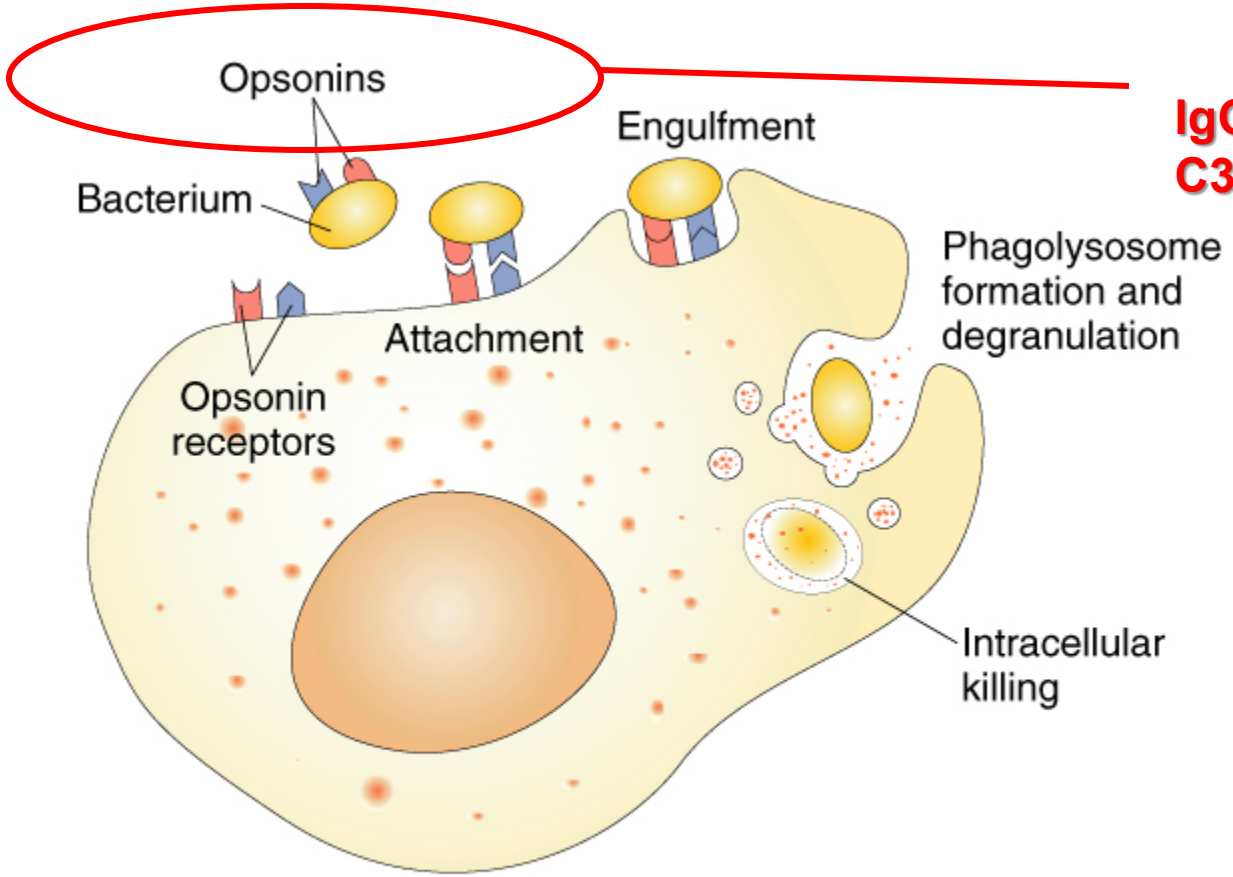


Image taken from: Krauss & Scott, *Frontiers Oral Biol*, 2012

Phagocytosis

- **1. Recognition and attachment of particle/bacterium to leukocyte**
- **2. Engulfment of particle and formation of phagocytic vacuole**
- **3. Killing and degradation of ingested material**



IgG (antibody)
C3b (complement)

Figure 20-4 Phagocytosis of a particle (*e.g.*, bacterium): opsonization, attachment, engulfment, and intracellular killing.

Phagocytosis

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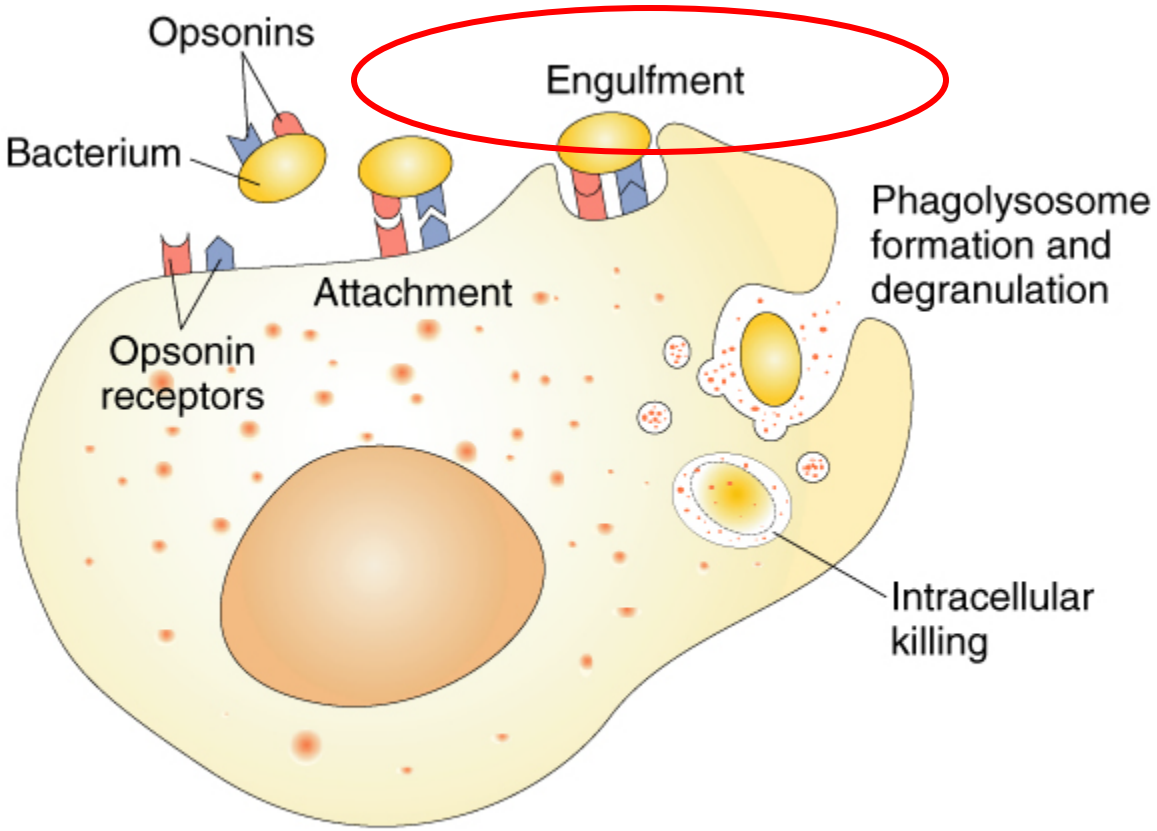

Figure 20-4 Phagocytosis of a particle (*e.g.*, bacterium): opsonization, attachment, engulfment, and intracellular killing.

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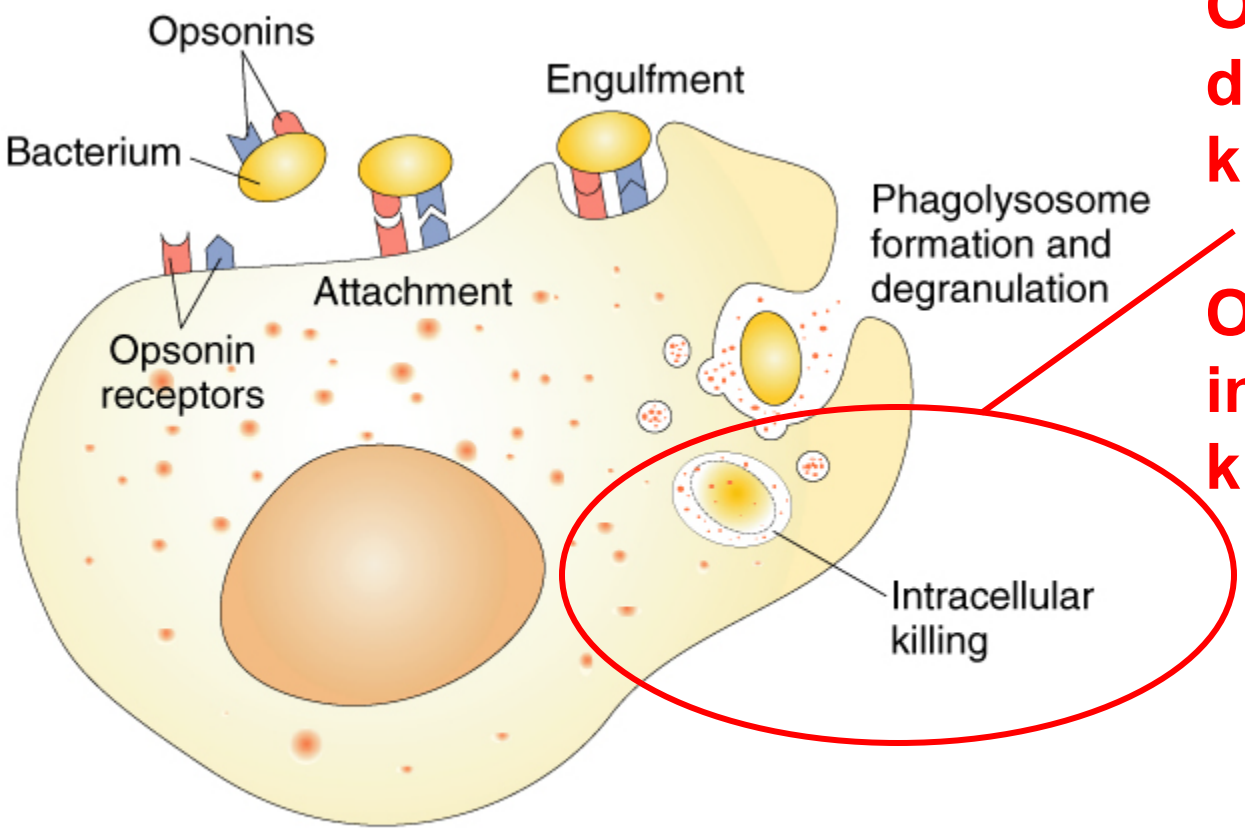


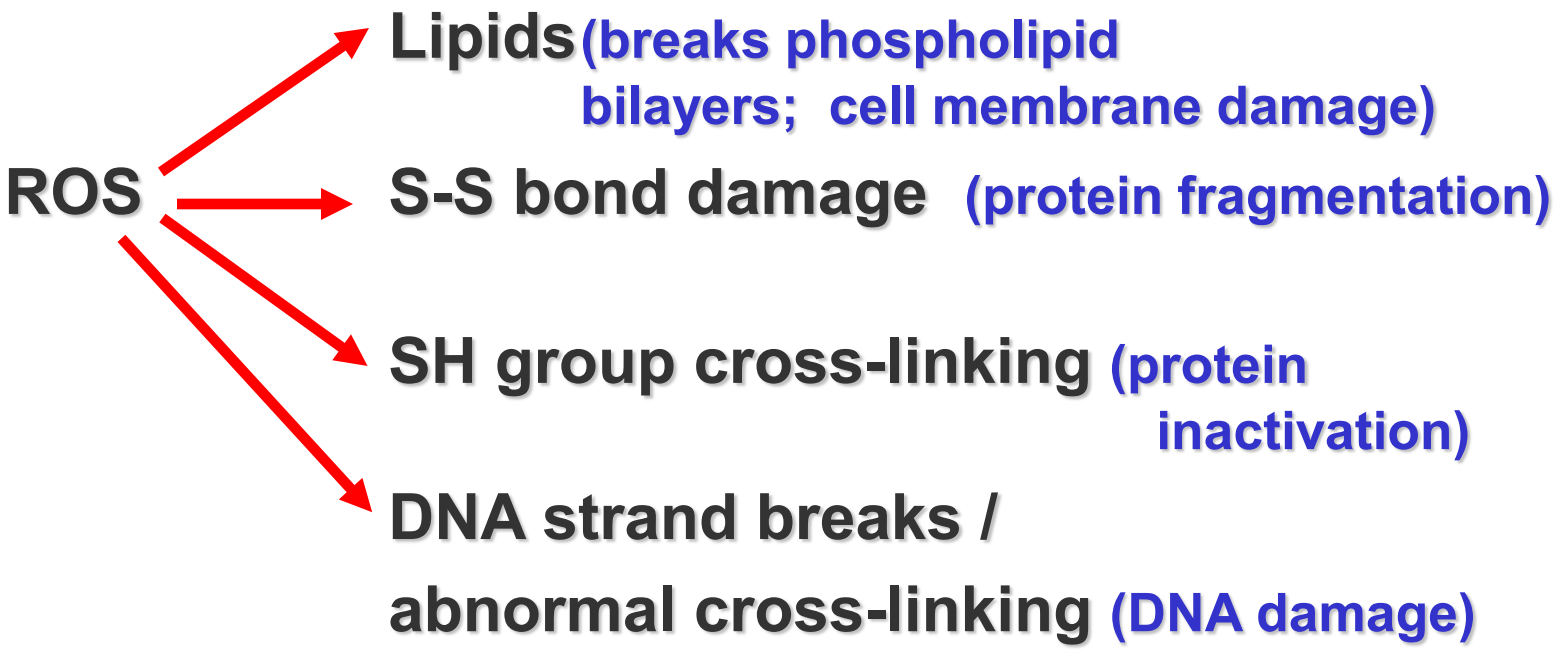
Figure 20-4 Phagocytosis of a particle (*e.g.*, bacterium): opsonization, attachment, engulfment, and intracellular killing.

Reactive Oxygen Species (ROS) are generated by neutrophils from oxygen:

- Superoxide radical ($O_2^{\cdot -}$)
- Hydroxyl radical ($\cdot OH$)
- Nitric oxide radical ($NO^{\cdot}$)
- Hydrogen peroxide (H_2O_2)
- Hypochlorous acid (HOCl…**bleach**)

Oxygen-dependent killing

Target molecules of ROS



Oxygen-dependent killing

NEUTROPHILS:

OXYGEN-*IN*DEPENDENT KILLING MECHANISMS

Will still work efficiently in low oxygen / anaerobic environments such as deep periodontal pockets

NEUTROPHILS: OXYGEN-INDEPENDENT KILLING MECHANISMS

Defensins

- Small peptide molecules that form transmembrane channels that allow the free flow of electrolytes, metabolites and water across bacterial cytoplasmic membranes
- Defensins are like “hole punchers”

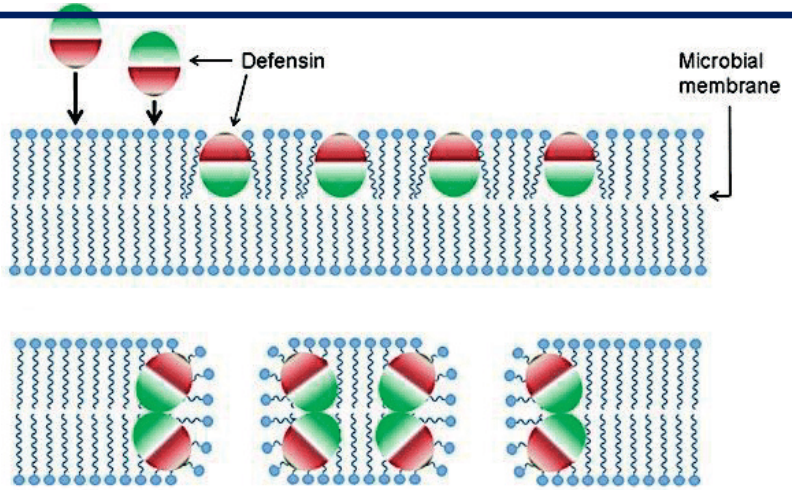


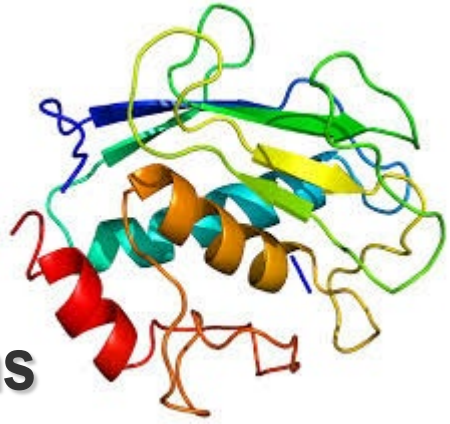
Image taken from: researchnet.com (2025)

Oxygen-independent killing

NEUTROPHILS: OXYGEN-INDEPENDENT KILLING MECHANISMS

Matrix metalloproteinases

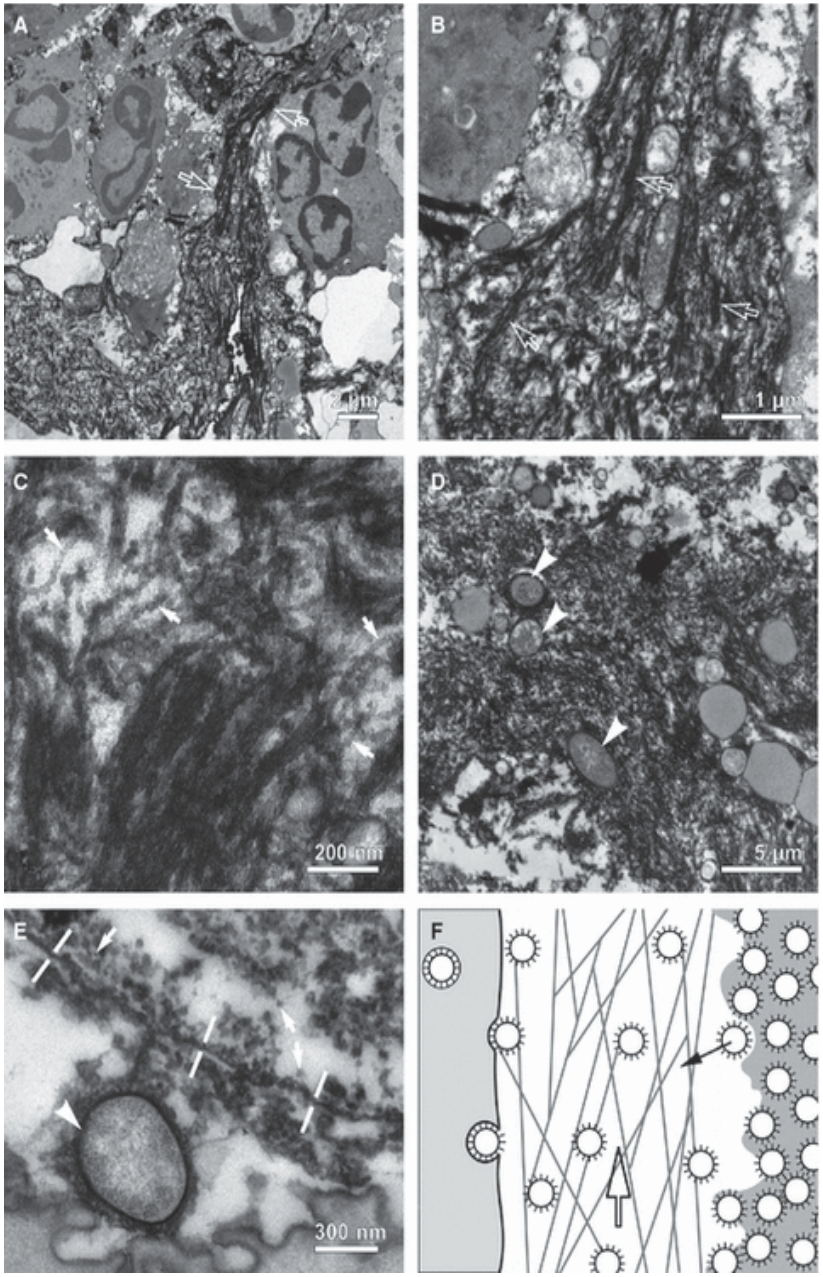
(MMP-8, MMP-9)



- Proteases that digest killed organisms

Lactoferrin

- Sequesters free iron, depriving bacteria of this essential element



Extracellular neutrophil traps (NETs) in periodontitis.

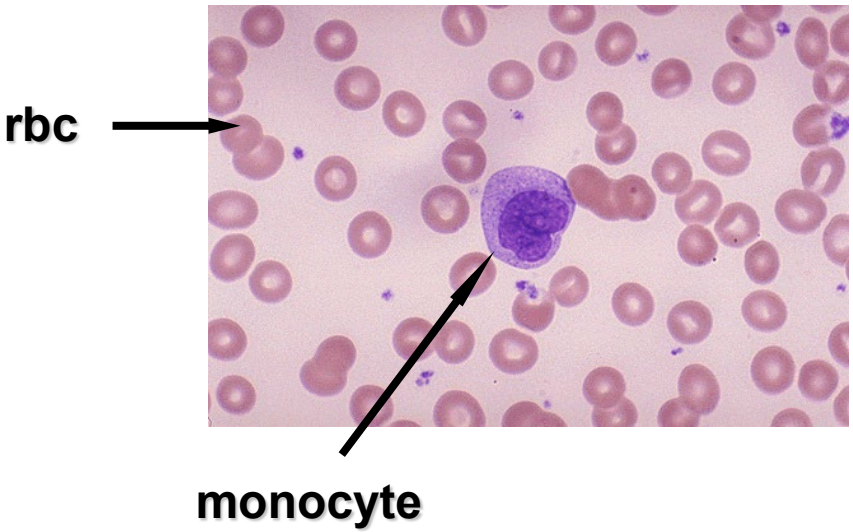
(A/B) Neutrophil extracellular traps on the pocket epithelium surface. The pronounced blackening is characteristic for neutrophil extracellular traps. The open arrows indicate neutrophil extracellular traps threads.

(E) A bacterium (solid white arrowhead) adherent to the epithelium surface is entrapped by neutrophil extracellular traps.

Images taken from: Krauss & Scott, *Frontiers Oral Biol*, 2012

MONOCYTIC CELLS

- Monocytes & macrophages perform many of the same functions as neutrophils
- However, they are slower to react….
- But are longer lived



Images:
http://medlib.med.utah.edu/WebPath/HEMEHTML/HEME003.html;
http://cellbio.utmb.edu/microanatomy;

Questions?