Basic Immunology

23-24.

Immunology of dental caries

Immunology of periodontal diseases

Oral mucosal diseases

Zoltán Kellermayer

Dental caries

Latin, *decay*

Localized **decay** of tooth tissue

Sugar + bacteria = acid, dissolving the hard tissue of tooth

Prevalence: 95% in developed countries

Leads to loss of ~ **US$ 27 billion** annually worldwide

Prevention!!! **Physical** (brushing teeth) + **chemical** (fluoride)

Dental caries



Pathophysiology

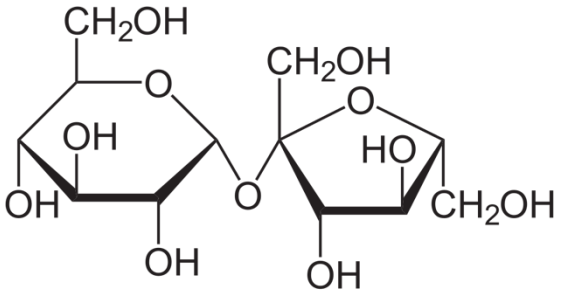
-Carbohydrates are fermented into acid by bacteria
-Acid (~*pH* < *5.2*) leads to demineralization of enamel and dentin

Carbohydrates

- Glucose
- Fructose
- Sucrose

Bacteria

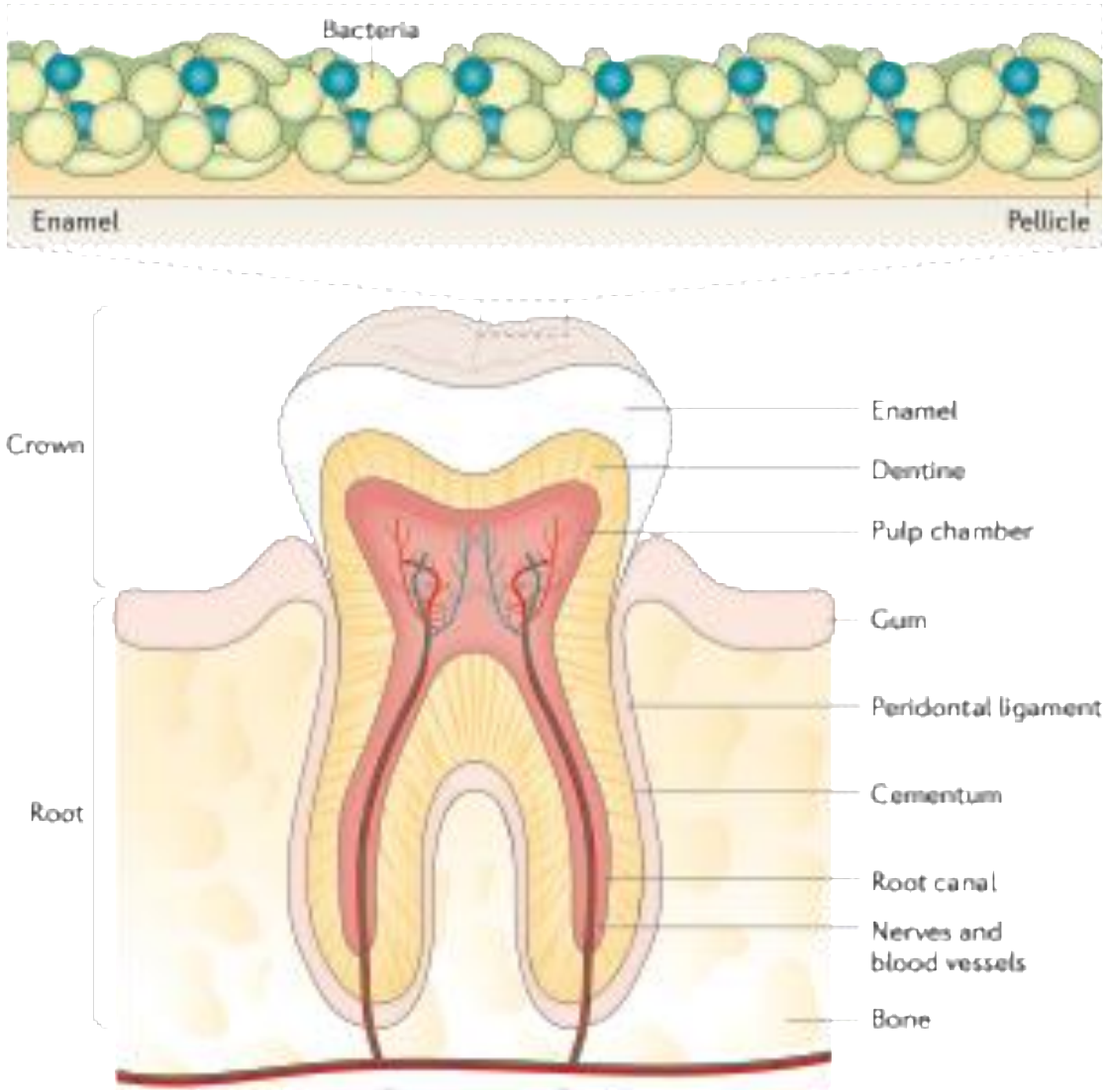
- Streptococci
- Lactobacilli
- Actinomyces
- *Most important: S. mutans*



Sucrose

Pathophysiology: Biofilm

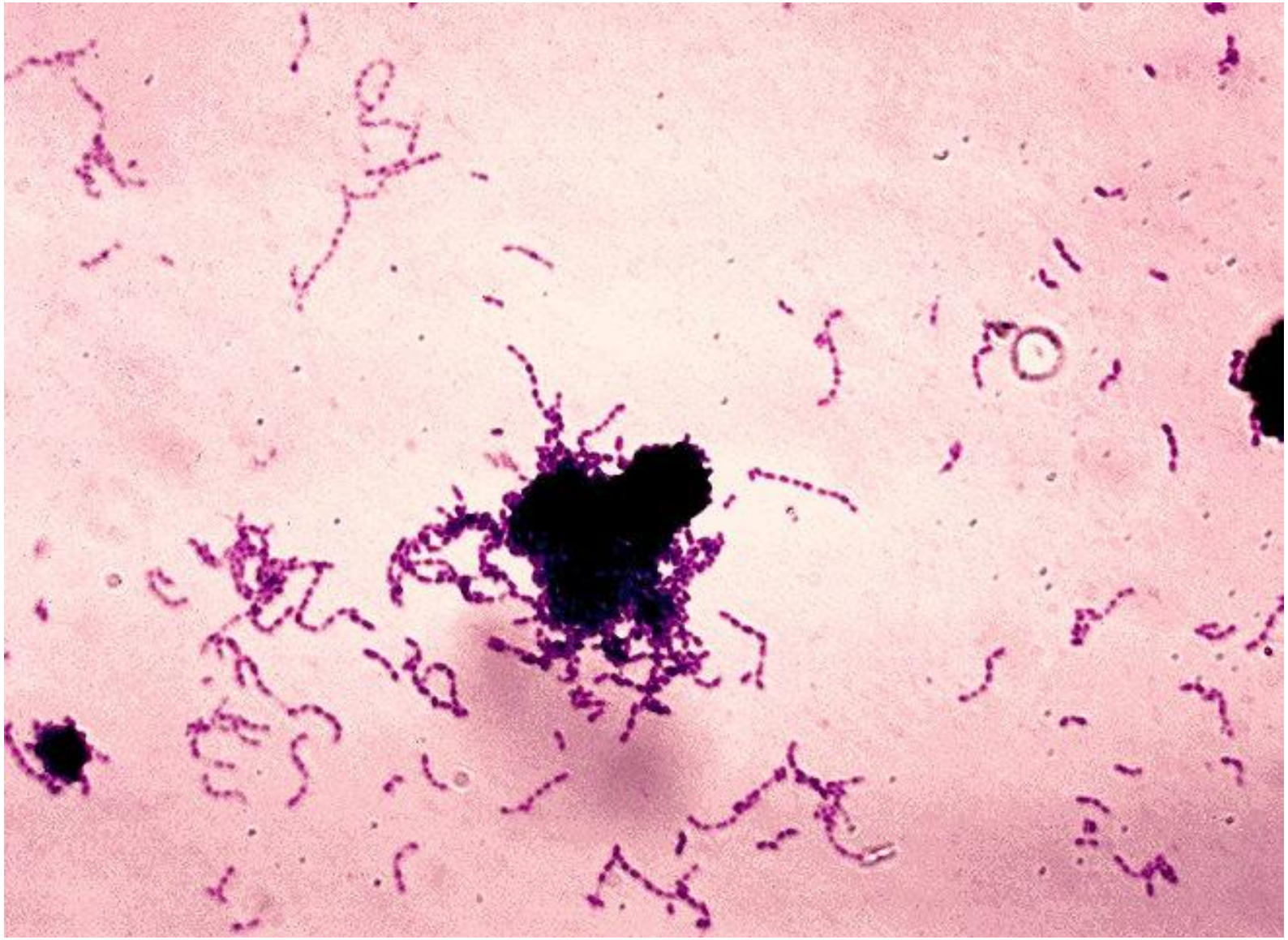
Dental Biofilm



Proteins+ glycoproteins

Dental Caries. Pitt NB et al 2017. Nature Reviews Disease Primers.

Streptococcus mutans



Streptococcus mutans

Gram-positive, facultative anaerobe bacterium

Produces high amounts of extracellular polysaccharide, enhancing adhesion to tooth surface

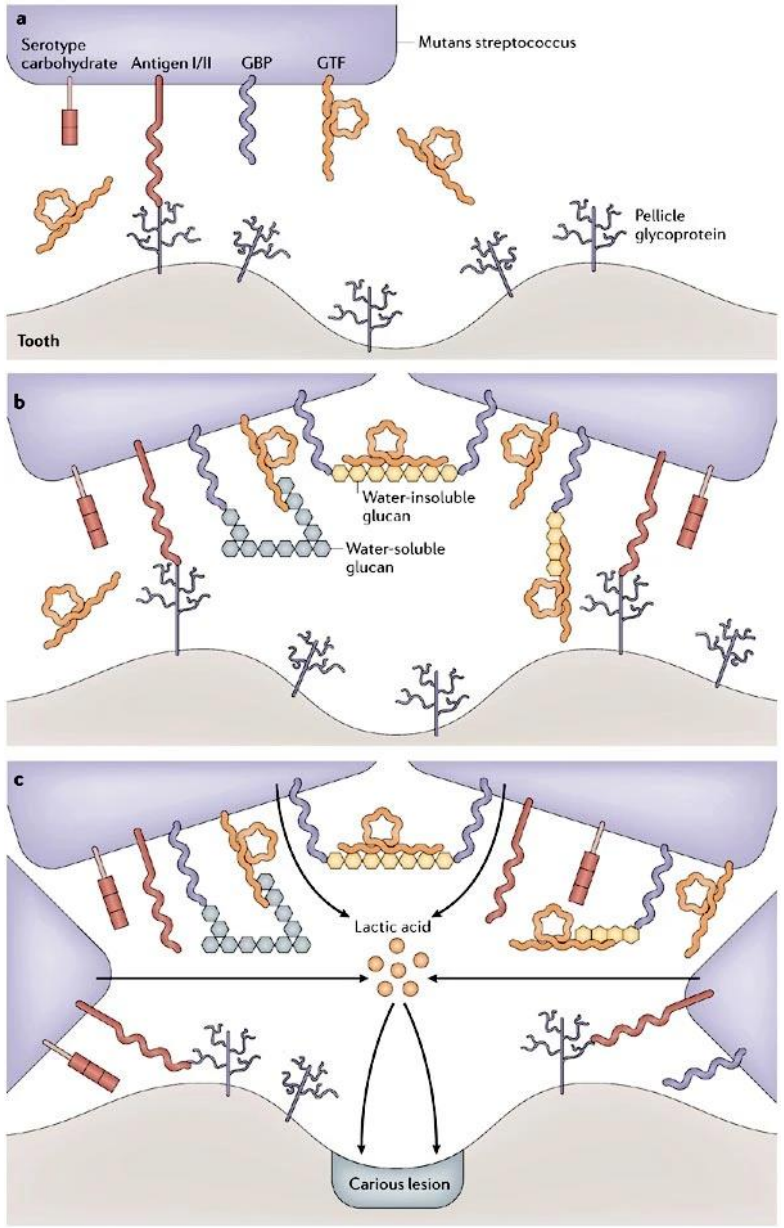
Metabolizes sucrose to lactic acid via glucansucrase

Tolerates low pH (active excretion of protons)

Antigens:

- -Glucosyltransferase (GTF)
  - *Synthesis of adhesive glucans*
- -Streptococcal antigen I/II (SA I/II)
  - *Adhesin, important in bacterial colonization*
  - *Vaccination against SA I/II antigen?*

Streptococcus mutans



The scientific and public-health imperative for a vaccine against dental caries. Taubman MA et al. Nat Rev Immunol 2006.

Immune response

Humoral response

-Elevated serum IgG to S. mutans

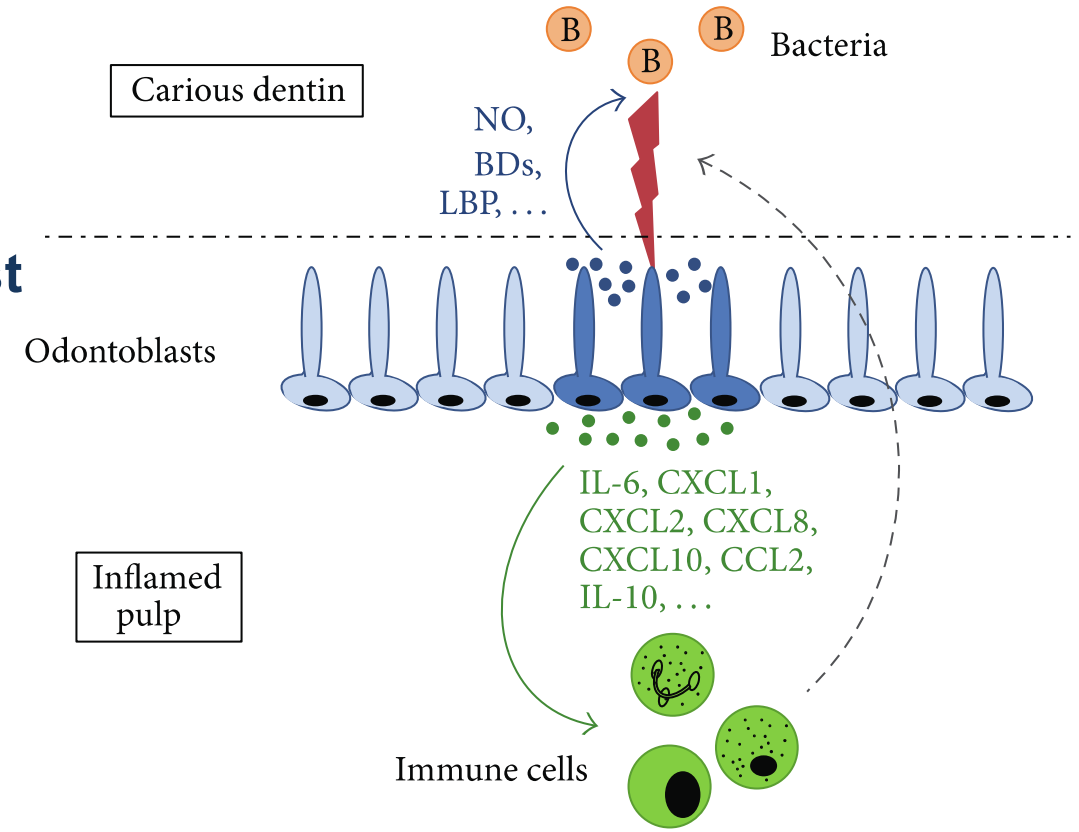
-Salivary antibody response less consistent

Odontoblast defense against dentin-invading bacteria

-TLR2, TLR4

-antibacterial molecules (blue)

-proinflammatory cytokines (green)



Dental pulp defence and repair mechanisms in dental caries. Farges JC et al 2015. Mediators Inflamm 2015:230251.

Immunization against caries

Active immunization

Mucosal (oral/nasal) or systemic immunization

Repeated immunizations needed

Few human studies

Passive immunization

mAb against SA I/II fragment 3 prevented colonization of S. mutans

Antibody in milk…?

Natural immunity to caries

Individuals with low caries experience had higher levels of serum IgG against SA I/II and salivary IgA against GTF

Genetic factors

HLA-DR6: low incidence of caries

HLA-DR6+ lymphocytes: stronger response against cariogenic bacteria (S. mutans)

HLA-DR4: higher risk of caries

Periodontal diseases

Inflammatory diseases affecting the gingiva and supporting structures of teeth

Results in attachment loss and destruction of alveolar bone

Etiology is important for proper treatment



Marginal gingivitis

Diagnosis and classification of periodontal disease. Highfield J, Aus Dent J. 2009.

Classification of periodontal diseases (AAP, 1999)

Most common:

-Chronic marginal gingivitis (CMG)

- Inflammatory reaction to plaques
- Reversible inflammation

-Chronic inflammatory periodontal disease (CIPD)

- Adult periodontitis
- Irreversible damage
- Smoking important exacerbating factor

Pathophysiology

Bacteria ("PSD" model: polymicrobial synergy and dysbiosis)

>600 species in the oral cavity
~200 detectable in an individual

8 bacterial species have been associated with periodontal disease
- e.g.: *Prevotella intermedia* – acute necrotizing ulcerative gingivitis
- ***Porphyromonas gingivalis*** – chronic inflammatory periodontal disease

Found in both healthy and diseased sites…
~ 50% of plaque bacteria can be cultured, rest are unknown!

Pathogenic factors:
- -leukotoxins
- -endotoxin
- -capsular products (activators of bone resorption)
- -hydrolytic enzymes (collagenases, phospholipases, proteases… etc)

Bacteria and bacterial toxins can invade the periodontal epithelium

Pathophysiology

Immunogenetic factors

-*HLA association* (animal and human studies)

HLA-A9: associated with higher risk for CIPD, juvenile periodontitis, rapidly progressing periodontitis

indicate that HLA-A9 is associated with periodontal destruction

-*Genotype variants*

IL-1α, IL-1β, TNFα (pro-inflammatory); IL-4, IL-10 (anti-inflammatory)

-*Twin studies*

No difference in gingivitis, probing depth, attachment loss, and plaque in monozygous twins raised apart or together

indicate that genetic component is more important than environment

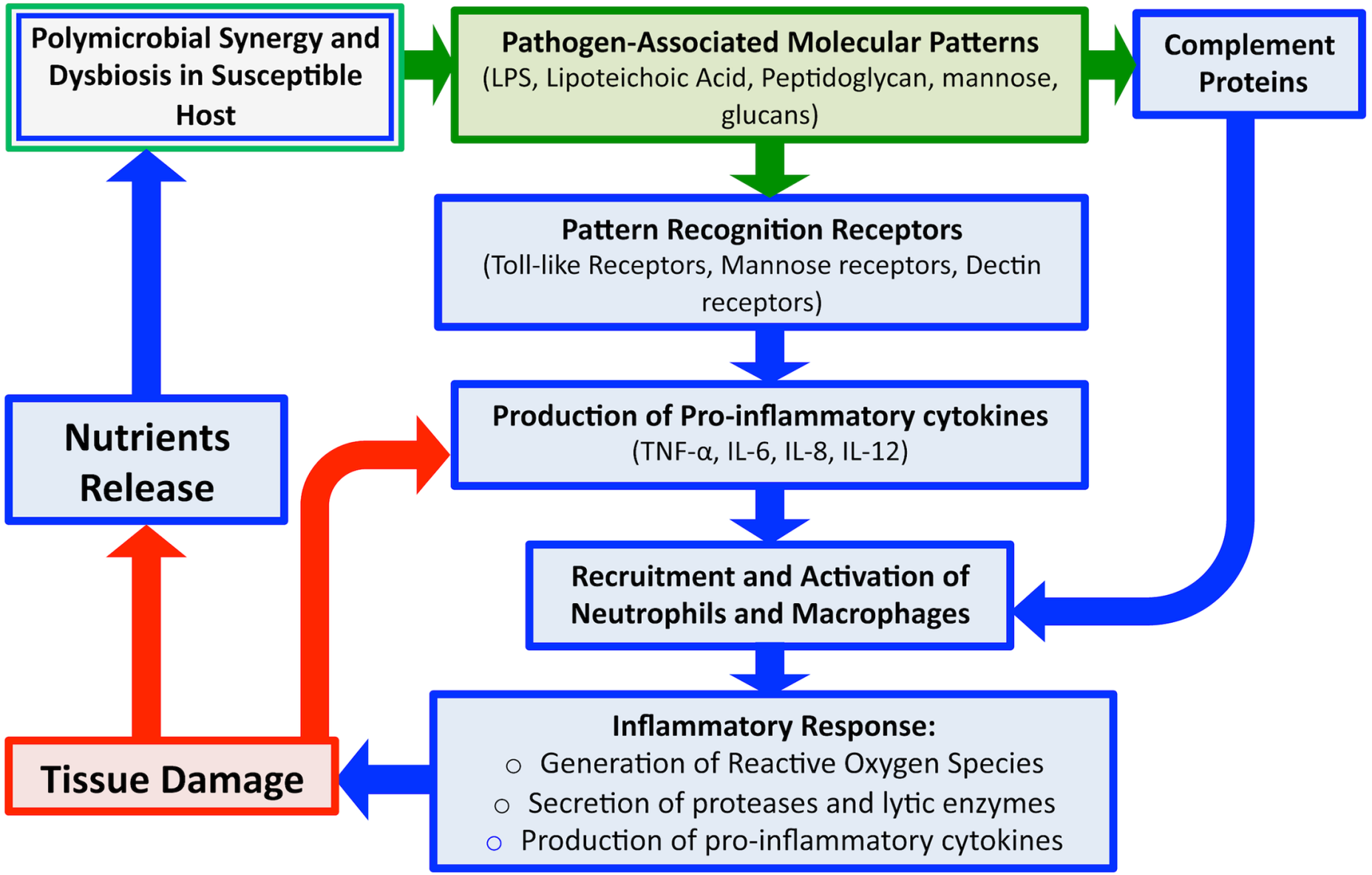
-*Antibody response*

Usually directed against Gram- bacteria; levels correlate with disease severity

e.g. increased antibody levels against *P. gingivalis* in CIPD

Both systemic and local

Pathophysiology



Periodontal diseases: bug induced, host promoted. Khan SA et al, PLOS Path. 2015.

Pathophysiology

Stages *(gingivitis always precedes periodontal disease!)*

I. Initial lesion: reversible damage to gingival sulcus, polymorphonuclear cell infiltration, complement activation

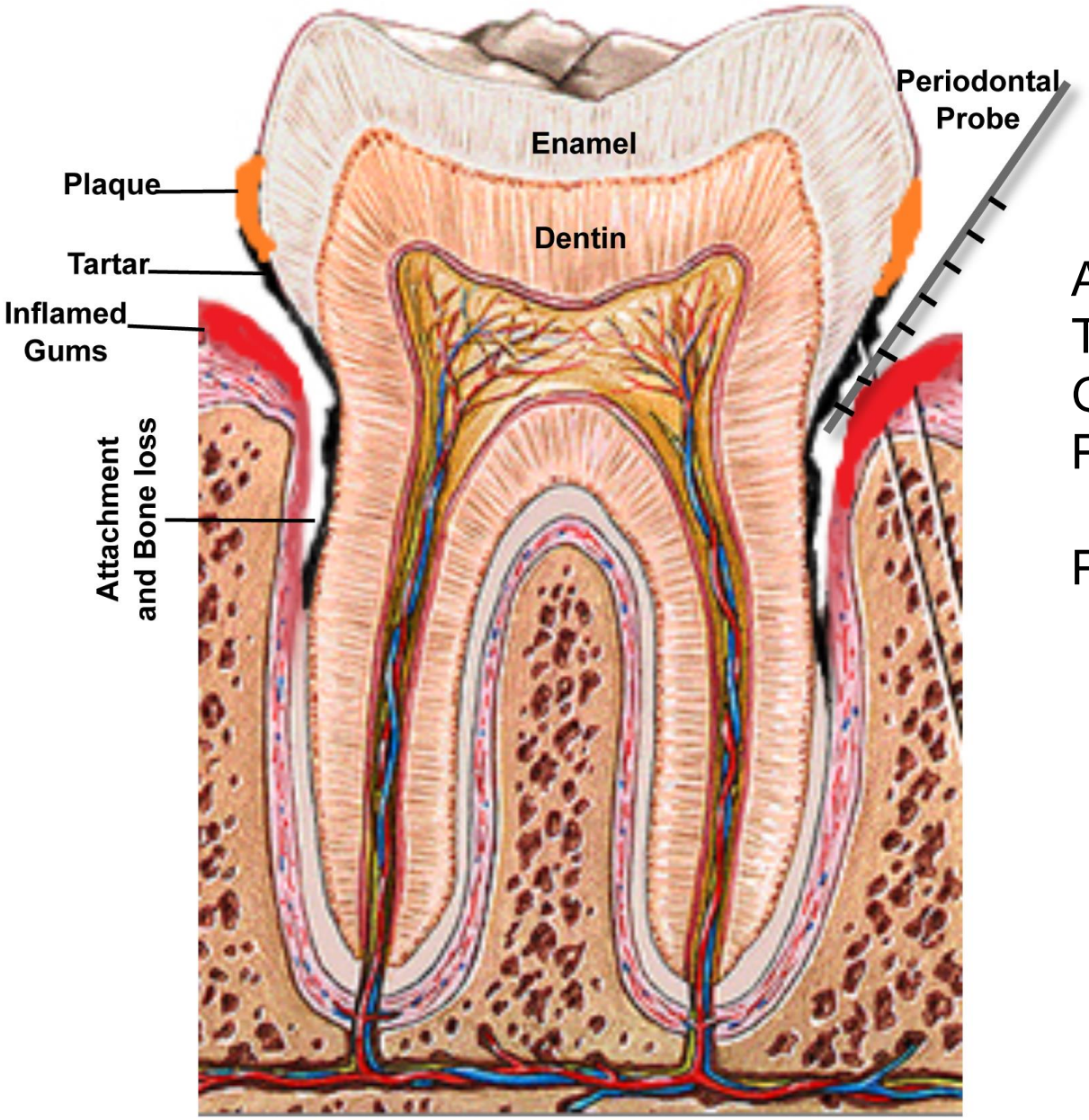
II. Early lesion: still reversible, lymphocytes replace polymorphonuclear cells. Mostly T cells (T_H17), few plasma cells

III. Established lesion: predominant plasma cell infiltration, mainly IgG^+

IV. Advanced lesion: destructive state; pocket formation, epithelial ulceration, periodontal ligament destruction, bone resorption

P. gingivalis important!

Pathophysiology

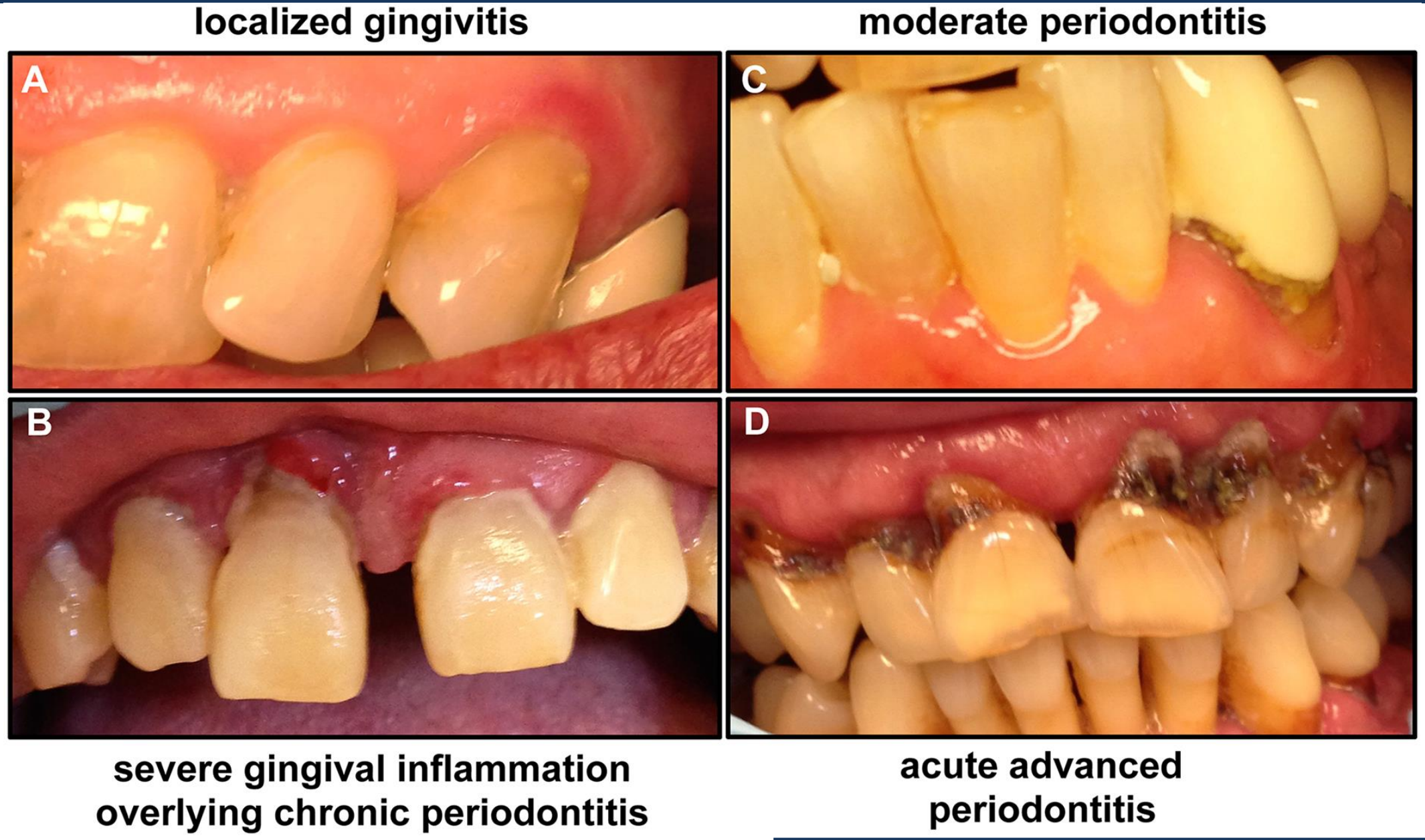


Accumulation of dental plaque
Tartar formation
Gingival inflammation
Periodontal pocket formation, loss of bone support

Pocket: 3mm< unhealthy
7mm< high risk of eventual tooth loss

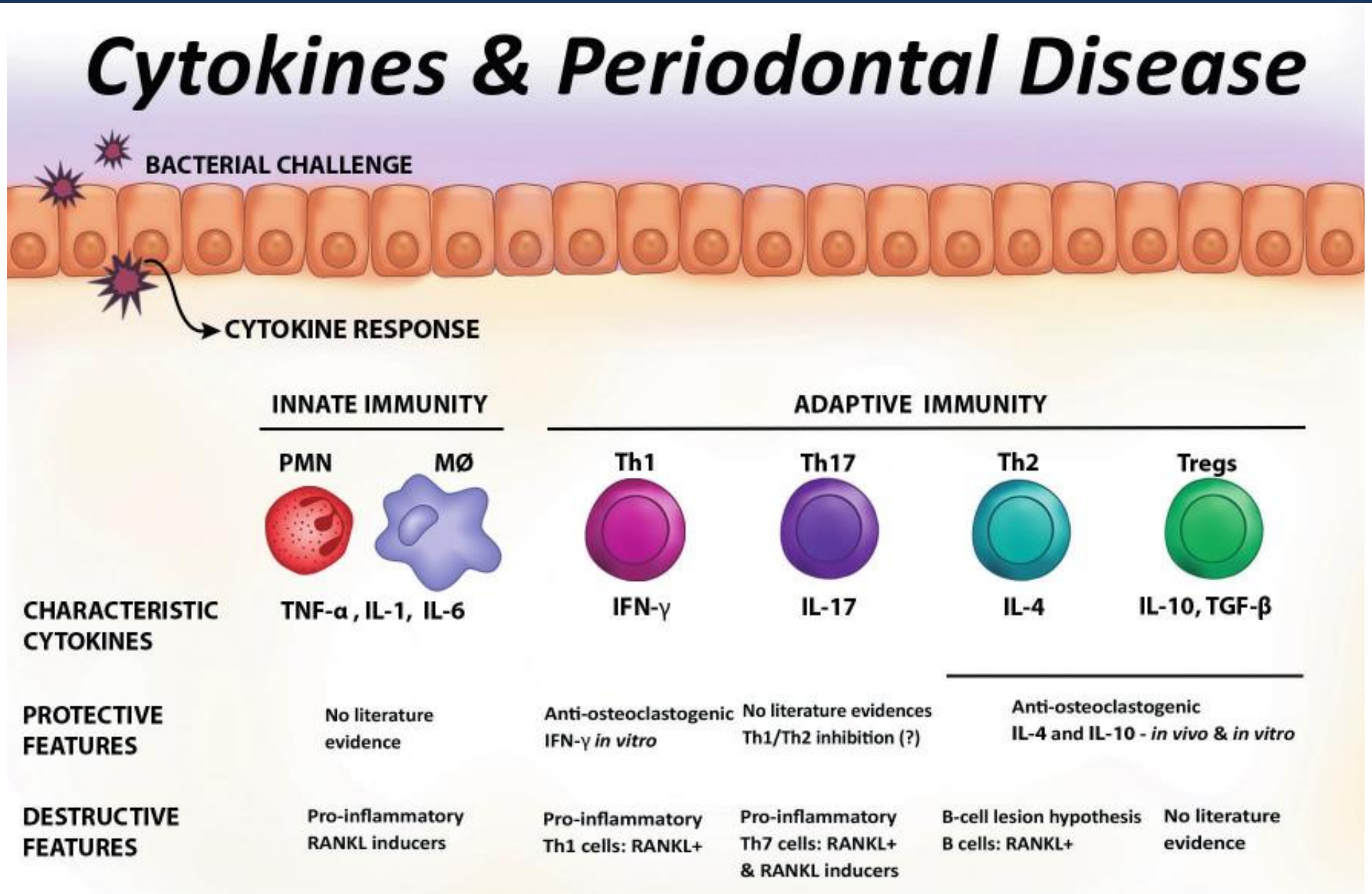
Periodontal diseases: bug induced, host promoted. Khan SA et al, PLOS Path. 2015.

Pathophysiology



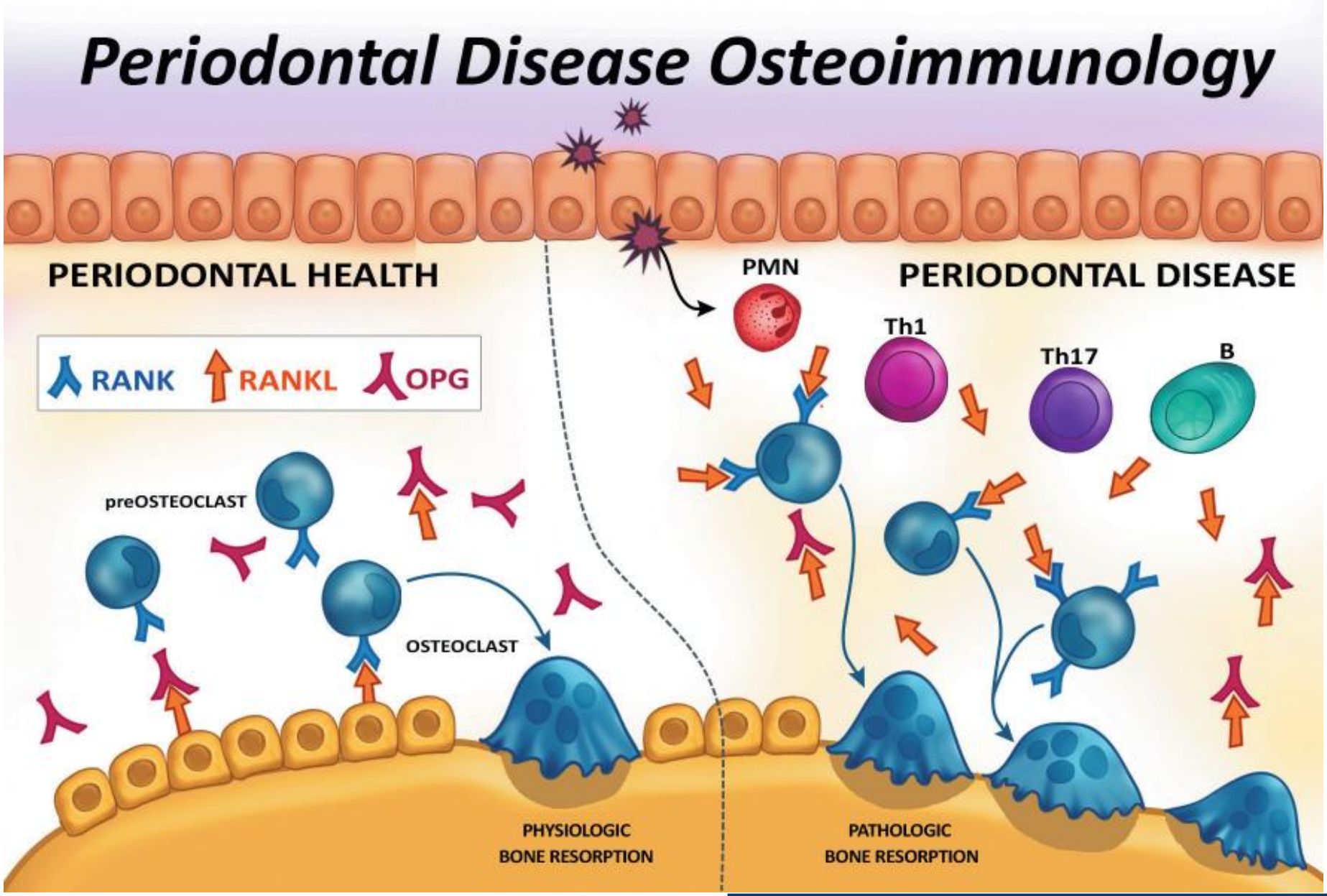
Periodontal diseases: bug induced, host promoted. Khan SA et al, PLOS Path. 2015.

Cytokines



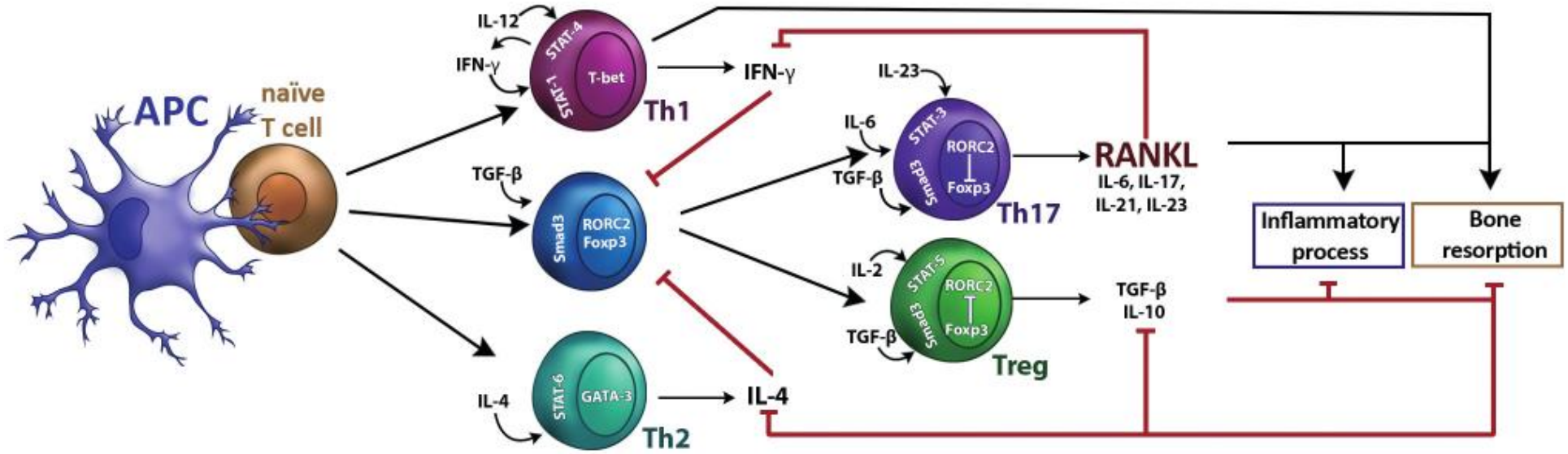
Host response mechanisms in periodontal diseases. Silva N et al, J Appl Oral Sci. 2015.

Osteoimmunology



Host response mechanisms in periodontal diseases. Silva N et al, J Appl Oral Sci. 2015.

Osteoimmunology



Osteoblast – Osteoclast balance:

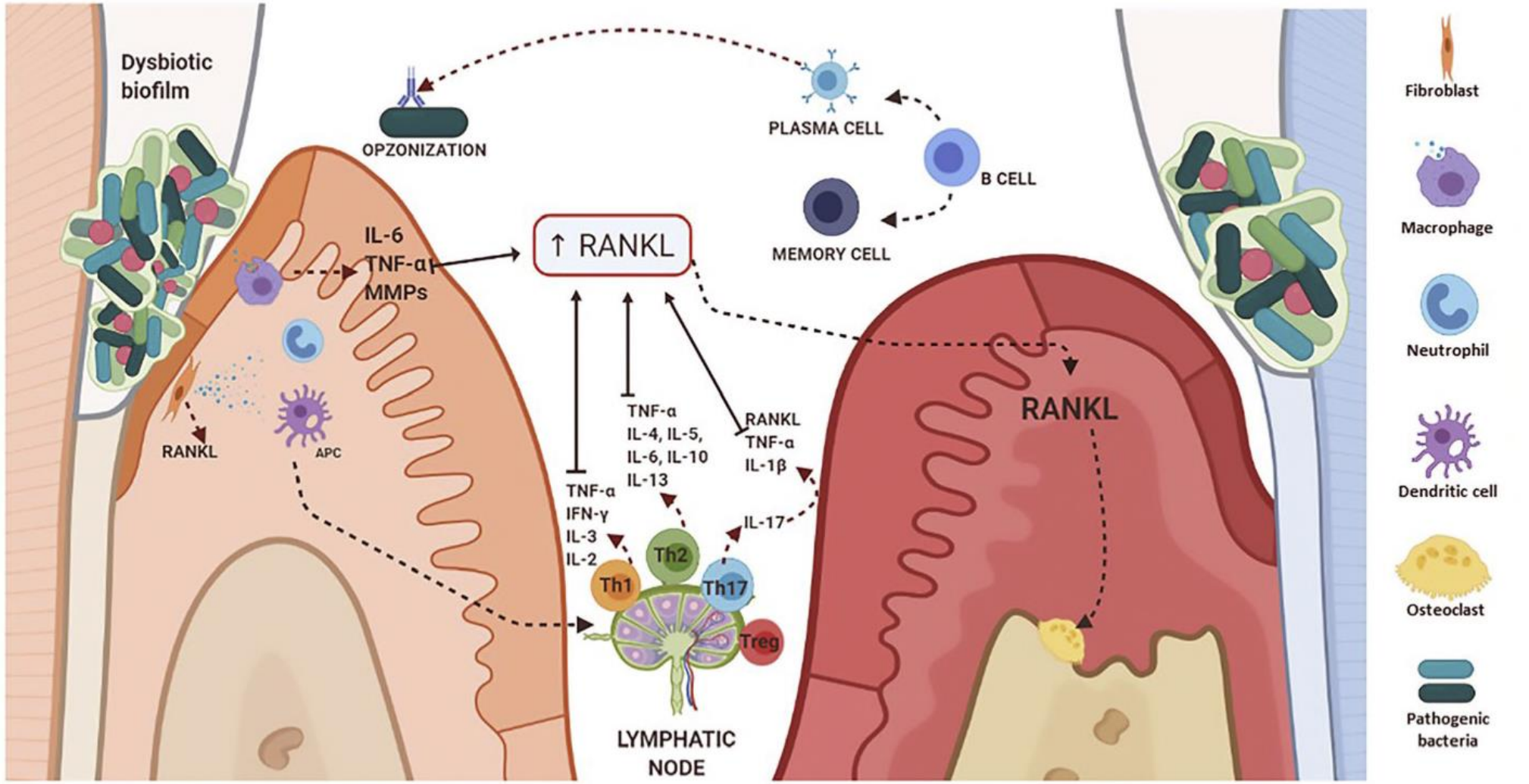
-RANKL: binds to RANK → Osteoclast differentiation, activation

-Osteoprotegerin: binds RANKL → inhibits osteoclast activation

-T_H17 cells can produce RANKL

Host response mechanisms in periodontal diseases. Silva N et al, J Appl Oral Sci. 2015.

Immunology of periodontitis



Most important:
T_H17
RANKL

Innate and adaptive immunity of periodontal disease. From etiology to alveolar bone loss. Becerra-Ruiz JS, Oral Dis. 2021.

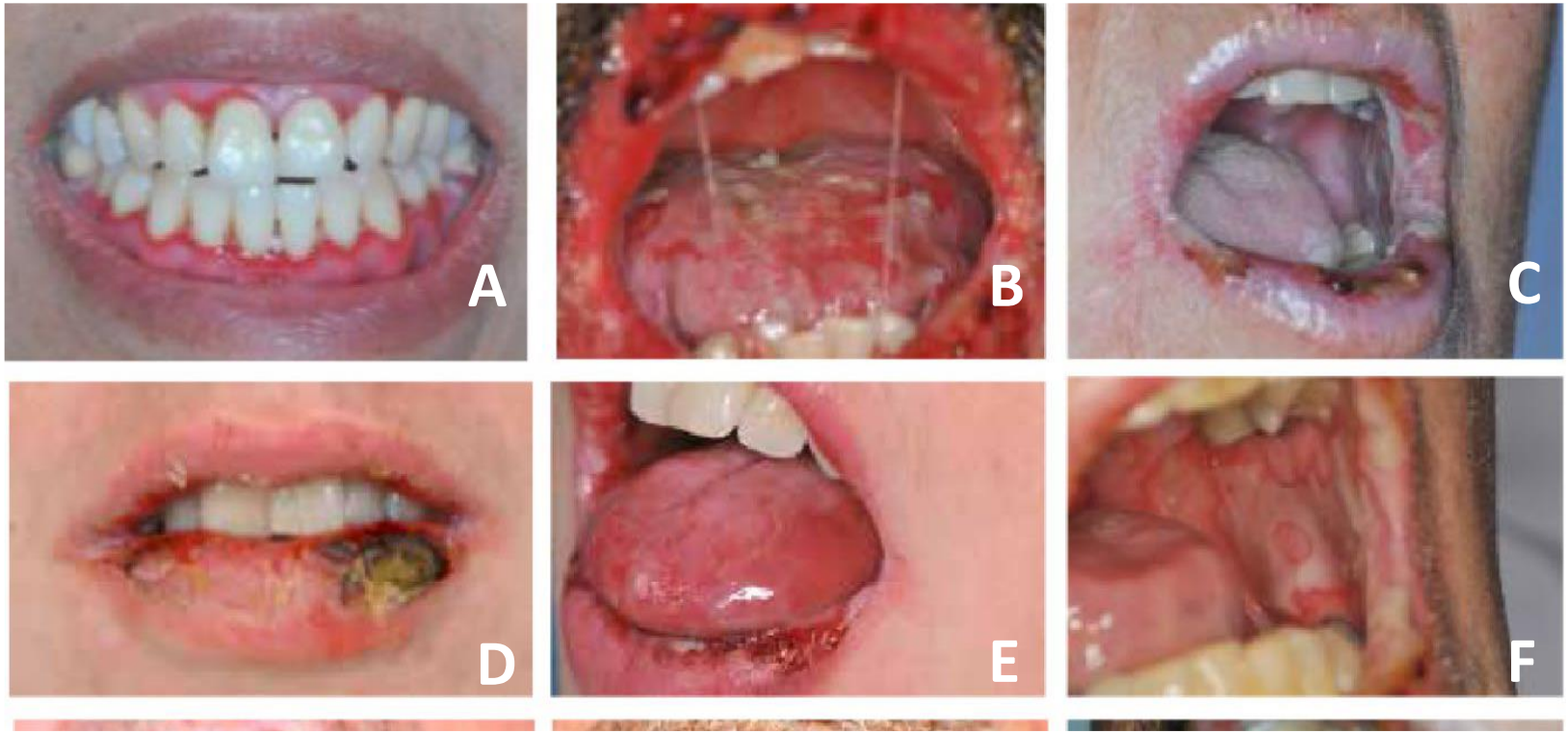
Oral mucosal diseases

1. Autoimmune ulcerative diseases
2. Recurrent aphthous stomatitis
3. Oral candidiasis
4. Herpes Simplex infection

Autoimmune ulcerative diseases

Mucous membrane pemphigoid

Pemphigus vulgaris



Rashid H et al. 2019. American Journal of Clinical Dermatology

Oral epithelium

Built up of cells (mainly keratinocytes) + Basement membrane

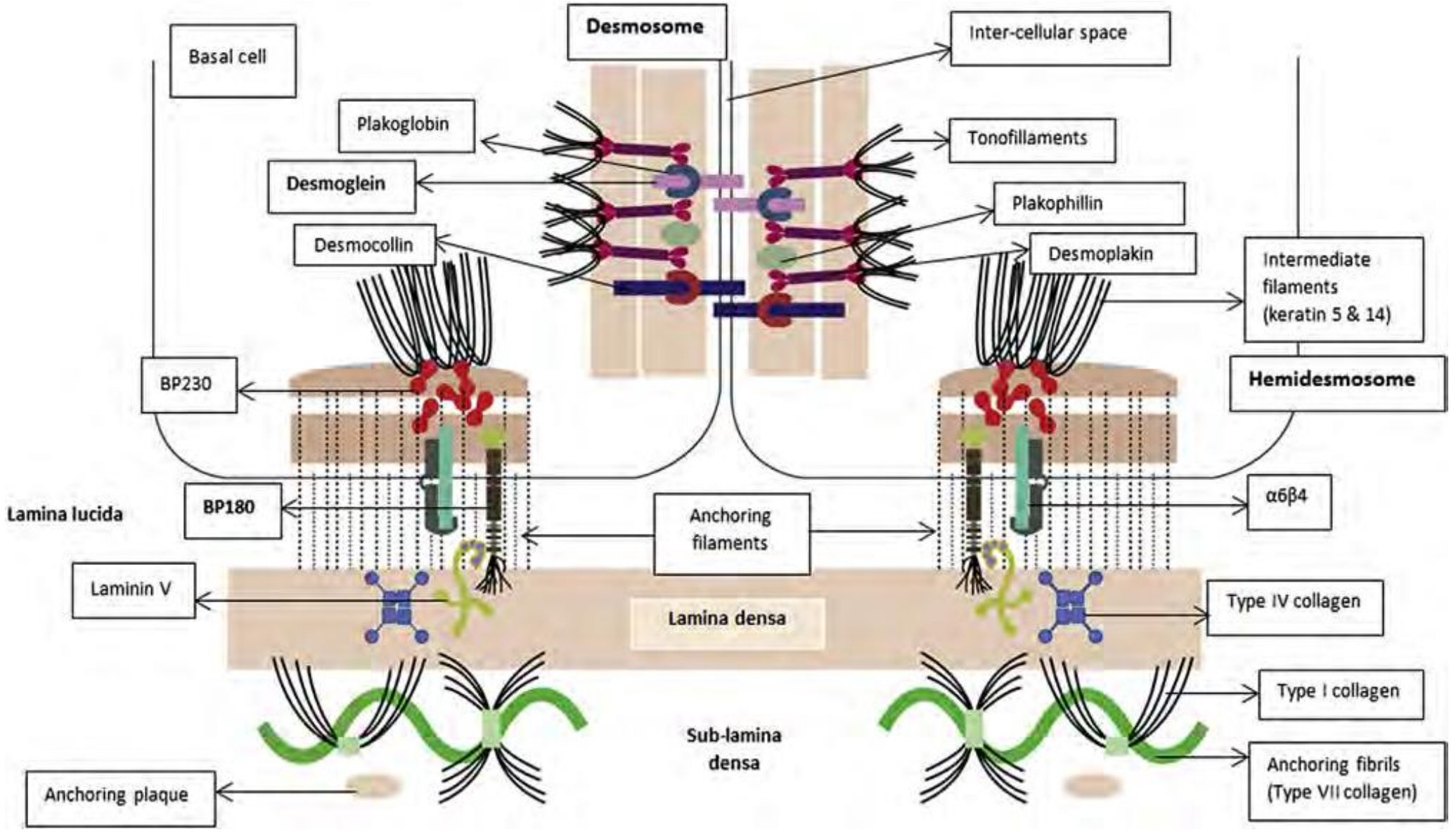
Basement membrane: connects epithelium to lamina propria

Consists of: basal cell plasma membrane + lamina lucida + lamina densa + sublamina densa

Cell – cell connections: *desmosomes* + gap junctions, tight junctions

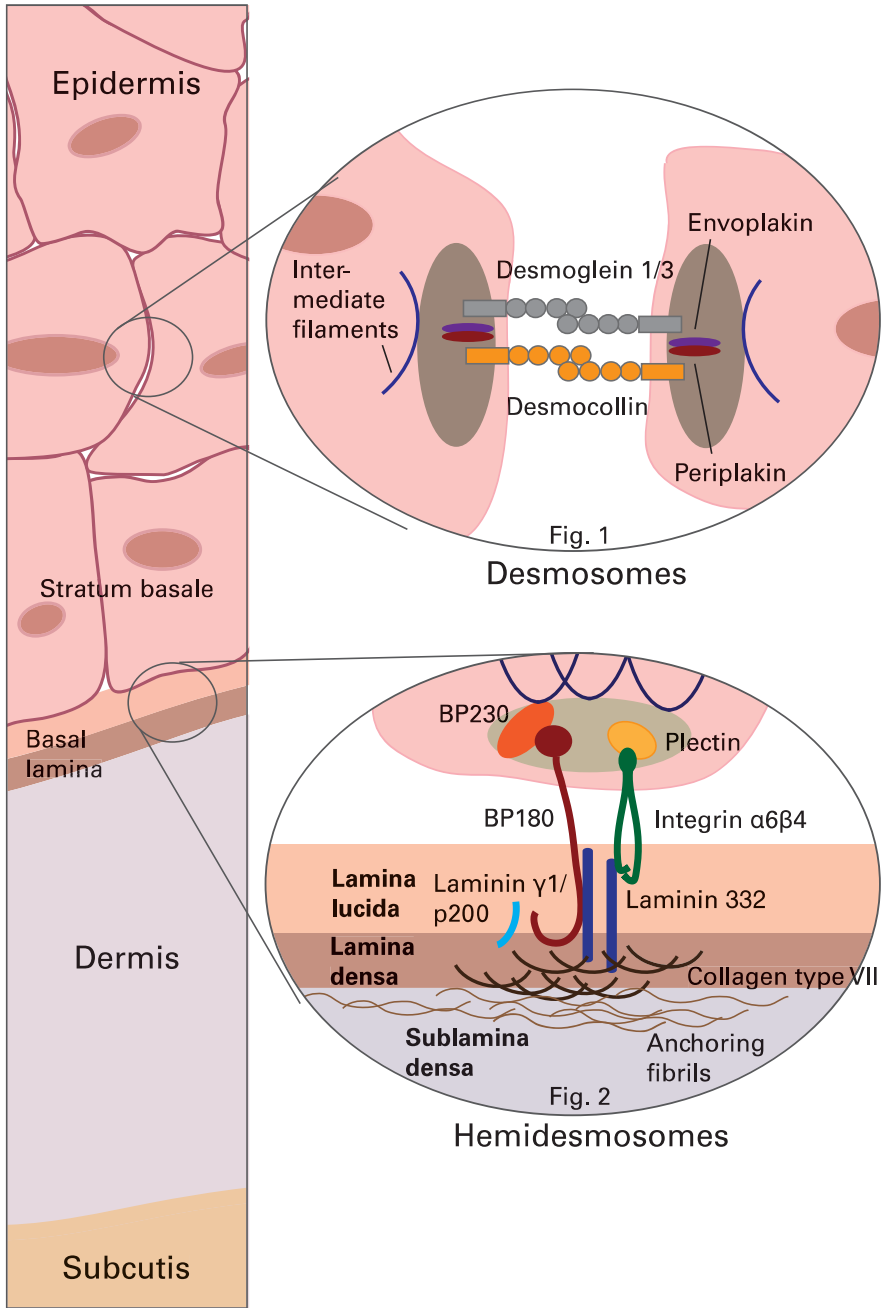
Cell – Basement membrane connection: *hemidesmosome*

Oral epithelium



Mestecky, Strober, Russell, Kelsall, Cheroutre, Lambrecht. Mucosal Immunology. 4th edition. Copyright © 2015 by Elsevier, Inc

Epithelial and Basement membrane (auto)antigens



Epithelial and Basement membrane (auto)antigens

Pemphigus vulgaris

Desmoglein 3 (important in desmosome)

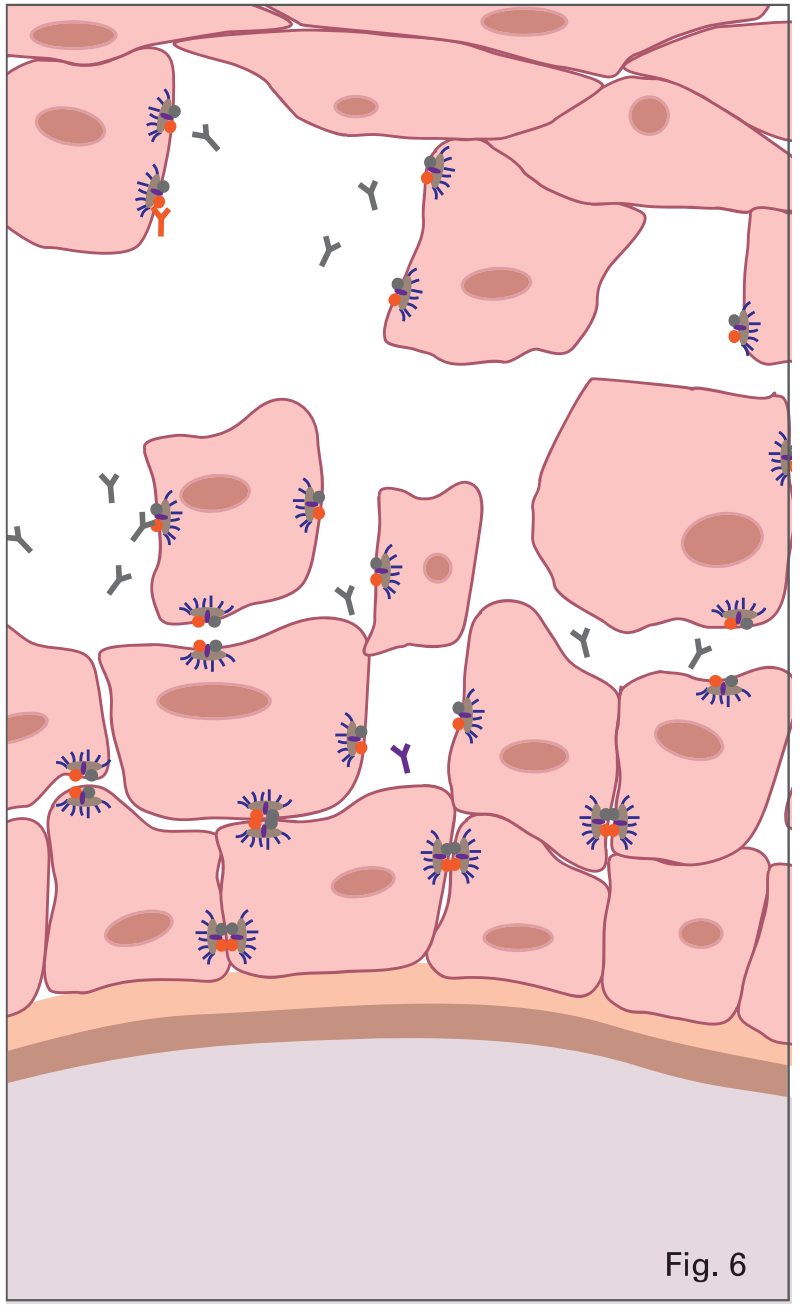


Fig. 6

Epithelial and Basement membrane (auto)antigens

Mucous membrane pemphigoid

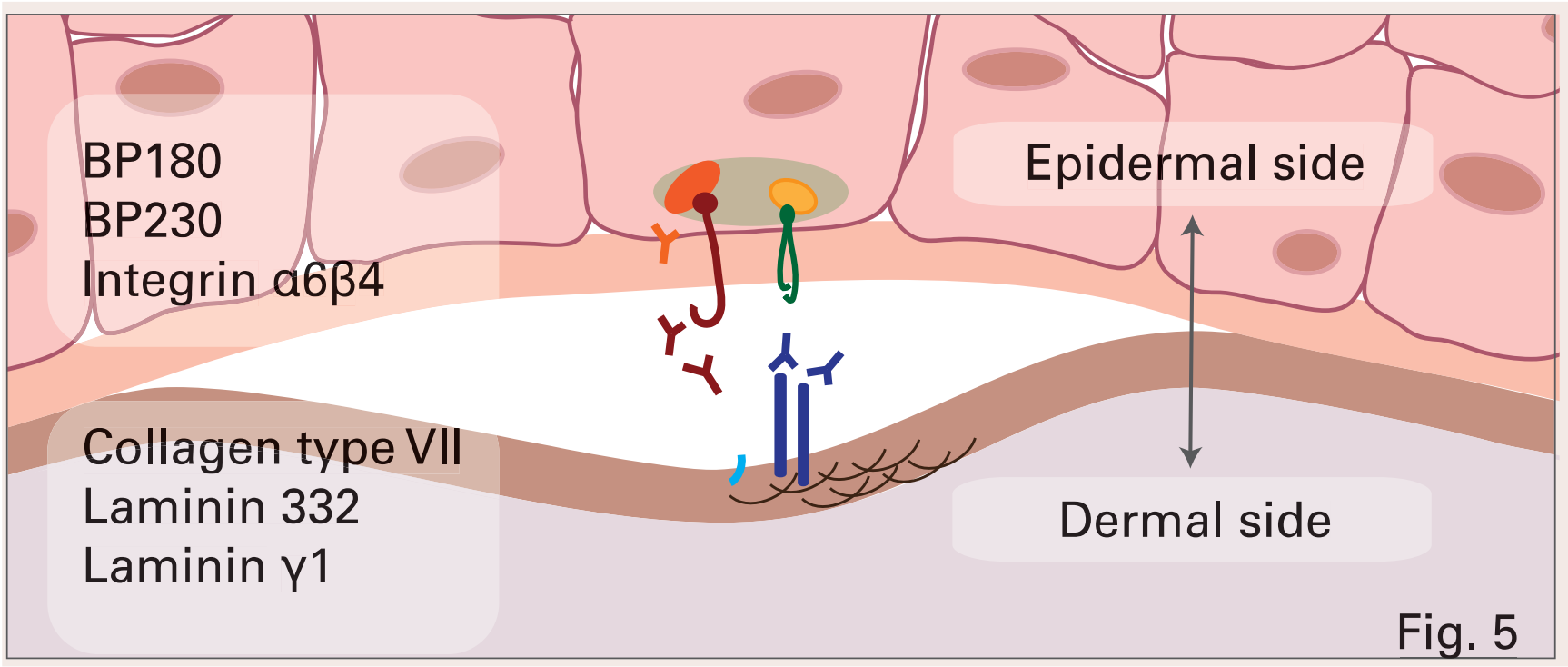
Laminins: non-collagenous glycoproteins

laminin 5, laminin 6

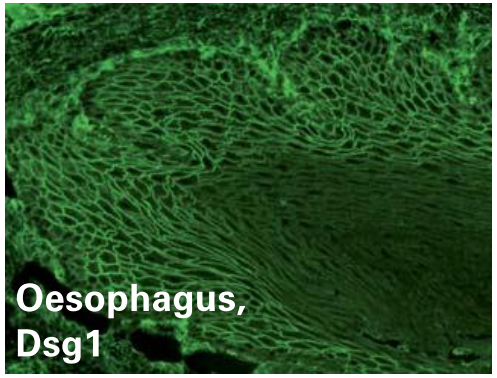
Bullous pemphigoid

BP180: transmembrane molecule

BP230 (=*BPAG1, Bullous pemphigoid antigen 1*): hemidesmosome inner plate



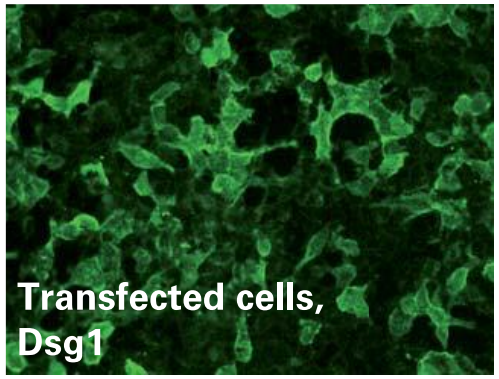
Diagnosis

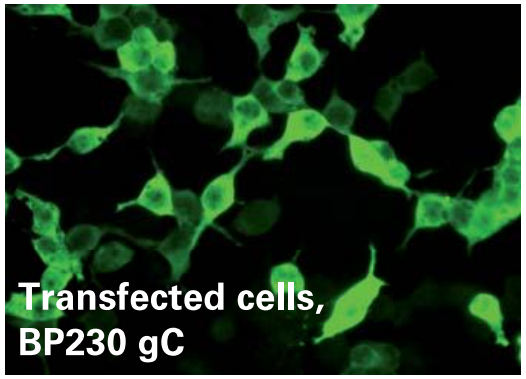


Oesophagus: detection of antibodies against **prickle-cell desmosomes** (pemphigus) and **basal lamina** (pemphigoid).



Salt-split skin: differentiation of autoantibodies against antigens of the epidermal (**BP180, BP230**) and dermal (**collagen type VII, laminin 332, p200**) sides of the skin.





Transfected cells: Monospecific detection of antibodies against **Dsg1**, **Dsg3** (pemphigus), **BP230 gC** (pemphigoid), and **collagen type VII** (EBA).

Recurrent aphthous stomatitis (RAS)



Characterized by oral ulcers

Heals spontaneously in 7-21 days

Prevalence: ~10%

Genetics:

~90% concordance in identical twins

Possible association with HLA-A2 and HLA-B12

Cause: ~unknown

(Definition: recurrent oral ulceration in the absence of known systemic factors…)

Hypothesis:

Unknown trigger (chemical or infective agent) → decrease in normal suppression → autoimmune response to oral mucosa

Recurrent aphthous stomatitis (RAS)

Findings:

- Autoantibodies against epithelial cells (leading to cell death)
- Cytotoxic T cells sensitized to oral mucosa

Trigger agent:

- Possibly cross-reacting with oral mucosa
- Candidate: heat-shock protein (HSP) 60kDa
- Microbial HSP → stimulate mucosal Langerhans cells → generation of T-cells that recognize microbial HSP + homologous human HSP

Several other types of (non-aphthous) oral ulcers with underlying causes

- (*Hematological diseases, gastrointestinal enteropathies, dermatological conditions etc…)*

Differential diagnosis is important!

Oral candidiasis

Candida species: present in ~40% of population

Oral candidiasis: usually with underlying causes
- Immunosuppression: therapy, HIV
- Other oral diseases present
- Xerostomia

Main types:
- Acute pseudomembranous candidiasis (very young or elderly)
- Acute atrophic candidiasis (antibiotics)
- Chronic atrophic candidiasis (prosthesis)
- Chronic hyperplastic candidiasis (risk of malignant transformation)
- Erythematous candidiasis (HIV infection)



Mucosal immune response to Candida

Innate immune response: **polymorphonuclear** cells found in biopsies

Oral candidiasis present in 40% of HIV+, 75% of AIDS patients → role of **T cells**

T_H1: elevated IL-12, IFNγ observed in patients

T_H17: elevated IL-17 and IL-23 associated with protection

T_H17-deficient patients are susceptible to oral candidiasis

IgA-deficiency: increased prevalence of oral candidiasis → role of **B cells**

Secreted aspartyl protease 2 (SAP2): important Candida antigen

Immunization agatinst SAP2 → secretory IgA-type antibodies → protection in mouse model

Herpes simplex



Usually caused by Herpes simplex virus 1 (HSV1)

Prevalence: 58% between ages 14-49

Primary infection: *herpetic gingivostomatitis*

- Children or young adults
- Pathogenesis: lytic replication of the virus in epithelial cells → lysis of keratinocytes
- Immune response: inflammation + adaptive (neutralizing antibodies + CD8+ T_C)
- Self-limiting in immunocompetent patients

- Characteristic clinical appearance: ulceration of oral mucosa + malaise, fever

- Therapy: acyclovir only at beginning of infection + symptomatic treatment

Herpes simplex

HSV1: Rapid transmission to peripheral sensory nerve fibers of n. trigeminus

Retrograde transport of the virus to trigeminal ganglion

Before appearance of neutralizing antibodies!!

Stays latent for years

Reactivation: in 15-40% of seropositive patients; appears as herpes simples labialis

Trigger factors: UV, stress, illness, immunocompromised conditions

Recurrence: usually in same spot

Herpes simplex labialis

Virus migration from neural cell body to periphery

- infects and replicates within keratinocytes
- keratinocyte death → inflammation → papule formation → vesicle formation

Resolve spontaneously in 7-10 days

- appearance of neutralizing antibodies
- T_H: produce IFNγ and IL-12
- T_C: cytotoxicity (keratinocyte lysis!)