The pathomechanisms of periodontal disease

Gingivitis and periodontitis are inflammatory diseases developing due to the protection and fight against plaque bacteria.
<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>AP PATIENTS (n=26)</th>
<th>HEALTHY SUBJECTS (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treponema sp.</td>
<td>29 (100)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>A.A.</td>
<td>26 (89.7)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>29 (100)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Fusobacterium sp.</td>
<td>29 (100)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>B. forsythus</td>
<td>28 (96.9)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>26 (89.7)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>P. micros</td>
<td>28 (96.6)</td>
<td>6 (30)</td>
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Host defense processes responsible for tissue destructions

Bacterial plaque is necessary but not sufficient for destructive periodontitis

Destructive periodontitis occurs in a small percentage of adult population

Week correlation between dental plaque and periodontal tissue destruction

Tween studies proved that genetic factors can be responsible for about half of the clinical manifestation of periodontitis
<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>AP PATIENTS diseased sites (n=116)</th>
<th>AP PATIENTS healthy sites (n=28)</th>
<th>HEALTHY SUBJECTS (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treponema sp.</td>
<td>114 (98.3)</td>
<td>13 (46.4)</td>
<td>22 (22)</td>
</tr>
<tr>
<td>A.A.</td>
<td>86 (74.1)</td>
<td>8 (28.6)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>113 (97.4)</td>
<td>14 (50)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>Fusobacterium sp.</td>
<td>116 (100)</td>
<td>20 (71.4)</td>
<td>58 (58)</td>
</tr>
<tr>
<td>B. forsythus</td>
<td>112 (96.6)</td>
<td>9 (32.1)</td>
<td>18 (18)</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>82 (70.7)</td>
<td>5 (17.9)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>P. micros</td>
<td>95 (81.9)</td>
<td>10 (35.7)</td>
<td>8 (8)</td>
</tr>
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</table>

Theoretically the absolutely healthy gingivia histologically shows no inflammatory reaction at all.

This can only be achieved by experimentally clean and plaque free circumstances.

The histomorphometry of the biopsies from this "super healthy" pristine gingiva shows 40% epithelial cells and 60% connective tissue.
Super-normal healthy gingiva
pristine gingiva

No cellular infiltrate

straight gingival capillaries
few emigrating PMN cells
No sulcus formation
Normal healthy gingiva

Under normal clinical conditions the histology of the healthy gingiva always shows some minimal inflammatory cellular infiltrate around the sulcular epithelia.

The gingival sulcus is filled by PMN leukocytes.

The cellular infiltrate comprises 5% of the total volume of the gingival connective tissue.

The cellular infiltrate is predominantly PMN cell macrophages and a few lymphocyte.
Normal healthy gingiva

max. cellular infiltrate 5% predominantly PMN cells T-B lymphocytes monocytes/macrophages

slight vascular proliferation capillary loops slight proliferation of junctional epithelium sulcus formation
Clinically healthy gingiva

defensive mechanisms:

a. local antibody production
b. PMN leukocytes and monocytes - phagocytosis in the crevice
c. sulcus complement system
d. sulcus epithelium continuous desquamation
e. intact epithelial barrier
f. sulcus fluid diluting effect
Controlling the EC Matrix physiological turnover

- MMPs, balanced with a group of Tissue Inhibitors of Metalloproteinases (TIMPs),
- to keep matrix remodeling highly regulated (Hannas et al., 2007).

- MMPs and TIMPs are regularly expressed in healthy periodontal tissues and maintains a homeostasis (Gonçalves et al., 2008).
Controlling alveolar bone turnover

- The major regulatory mechanism of osteoclast activity
  - receptor RANK (receptor activator of nuclear factor-κB), and its ligand RANKL,
  - and its soluble counterpart OPG (osteoprotegerin)

- The integrity of bone tissues depends on the maintenance of a delicate equilibrium between bone resorption by osteoclasts and bone formation by osteoblasts.

- Alveolar bone loss is a key event in PD.
**Generation of inflammatory stimuli:**

**How bacteria set up inflammatory responses in the gingiva?**

- The primary aetiologic factor of periodontal disease is the bacterial biofilm.
- Gram-positive and Gram-negative bacteria possess virulence factors
  - That may cause direct destruction to periodontal tissues
  - Or rather stimulates host cells to activate a wide range of inflammatory responses
- These responses are intended to eliminate the microbial challenge but may often cause further tissue damage.
Due to bacterial irritation the gingival mast cells degranulate and liberate vasoactive substances: histamine, serotonin.

The earliest sign of inflammatory reaction is manifested in the vasculature: the capillary network expands, the capillaries forms loops.

Abundant number of PMN leukocyte, lymphocytes and monocytes gather around the sulcular epithelia.
Gingivitis

initial lesion, early lesion, established lesion, advanced lesion - periodontitis

histopathological examination by Schroeder and Page
Pathogen-Associated Molecular Patterns (PAMPs),

- are recognized by a relatively small number of host cells’ receptors - called **pattern recognition receptors (PRRs)**.
- the same PRR may recognize the same bacterial component from different species and sometimes, different bacterial components.
The Toll-like receptors

- The innate host response
- recognition of microbial components as “danger signals” by host cells
- subsequent production of inflammatory mediators
The Toll-like receptors (TLRs) are expressed

- resident cells – epithelial cells
- leukocytes
- Activate the innate immune response by binding to various bacterial components:
  - lipopolysaccharide [LPS],
  - bacterial DNA,
  - diacyl lipopeptides,
  - peptidoglycan,
  - (Mahanonda and Pichyangkul, 2007).
After TLR activation, an intracellular signaling cascade is stimulated, leading to:

- the activation of transcription factors
- subsequent inflammatory cytokine expression,
- leukocyte migration,
- osteoclastogenesis

(Nakamura et al., 2008; Ukai et al., 2008; Gelani et al., 2009; Lima et al., 2010).
initial lesion

Aerobes and anaerobic bacteria accumulating in periodontal pocket produce great amount of substances with the capability to directly evoke characteristic vascular changes in the marginal gingiva.
Initial lesion - vascular changes

PLAQUE

JUNCTIONAL EPITHELIUM

PLAQUE

mast cell

PGE LTN4

IL-8 +

histamine, serotonin

bradykinin, plasmin

platelet

serotonin, thromboxan, platelet derived growth factor PGF
Initial lesion - vascular changes
- initial reactions

IgG, IgM, IgA

Increased crevicular fluid

PMN extravasation

Complement

Plaque

Junctional epithelium
Initial lesion - cellular reactions

- PLAQUE
- LYMPHOCYTE
- PMN
- MONOCYTE
- ADHESION
- extravasation
- cellular infiltrate 5%
Early gingival lesion

cellular infiltrate 15%
predominantly PMN cells
T lymphocytes
monocytes/macrophages
few plasma cells

increased PMN emigration
vascular proliferation
loss of collagen, fibroblast degeneration
proliferation of junctional epithelium
Accantotic sulcus epithelium

PLAQUE

PMN CELLS
T/B CELLS
MONOCYTES
PLASMA CELLS
COLLAGEN LOSS
PLAQUE

JUNCTIONAL EPITHELIUM

PMN

PMN

plasma cells

Th1 - Th2 - Th0 LYMPHOCYTA

extravasatio

ADHESION

cellular infiltrate 15%

15%

5-7 days

aerly laesion - cellular reaction
Established gingival lesion

cellular infiltrate 30-60%
predominantly T- B lymphocytes
monocytes/ macrophages
plasma cells 10-40%
greatly increased PMN emigration
vascular proliferation
severe loss of collagen,
fibroblast degeneration
severe proliferation of junctional epithelium
accantotic sulcus epithelium
deepening sulcus
established lesion - cellular reaction
7-21 days

PLAQUE

Th 1 - Th2 - Th0
LYMPHOCYTES

PMN
extravasatio
plasma cells

JUNCTIONAL EPITHELIUM

MONOCYTES

ADHESION

cellular infiltrate
30-60%
plasma cells 10-30%
COMPOSITION OF GINGIVA AT DAY 0 AND DAY 28 IN EXPERIMENTAL GINGIVITIS STUDY ON DOGS

baseline | Day 28
---|---
non infiltrated tissue | infiltrated conn tiss
oral epithelium | oral epithelium
junctional epithelium | junctional epithelium
non infiltrated tissue | non infiltrated tissue

0% | 100%
COMPOSITION OF GINGIVA AT DAY 0 AND DAY 28 IN EXPERIMENTAL GINGIVITIS STUDY ON DOGS

The diagram shows the composition of gingiva at baseline and Day 28 in an experimental gingivitis study on dogs. The composition includes:

- Residual tissue
- Leukocyte
- Vasculature
- Fibroblast
- Collagen

The percentage of each component is indicated on the y-axis, ranging from 0% to 100%. The baseline and Day 28 compositions are compared visually.
ALTERATION IN NUMBER OF CREVICULAR LEUKOCYTES AND IN GINGIVAL FLUID IN EXPERIMENTAL GINGIVITIS
PERIODONTITIS

cellular infiltrate >60% PMN cells
few T - B lymphocytes
macrophages
plasm cells dominance > 50%

severe PMN emigration
increased collagen loss
apical migration of the junctional epithelium
POCKET FORMATION
BONE LOSS
PERIODONTITIS

cellular infiltrate >60%
PMN cells
few T - B lymphocytes
macrophages
plasm cells dominance > 50%

severe PMN emigration
increased collagen loss
apical migration of the junctional epithelium
POCKET FORMATION
BONE LOSS
NUMEROUS PLASMA CELLS
Host defense processes responsible for tissue destructions

There are four distinct levels of protection against oral bacteria:

- saliva
- gingival crevice
- gingival tissue
- systemic immunity
O - PROTECION SALIVA

FIRST PROTECTIVE BARRIER – GINGIVAL SULCUS

SECOND PROTECTIVE BARRIER
GINGIVAL CONNECTIVE TISSUE

THIRD PROTECTIVE BARRIER SYSTEMIC IMMUNITY
Host defense processes responsible for tissue destructions

0 barrier level

Saliva contains several antibacterial factors that can control bacterial growth and spreading

- mucine
- salivary lactoferrin
- lysosome
- secretory IgA
- Whole saliva - IgG and IgM molecules
complement cascade

Ag+Ab

C1 \rightarrow Cl

C4+C2 \rightarrow C42 + C4a

C3 \rightarrow C3b + C3a

LPS

C5 \rightarrow C5b + C5a

C6 + C7 \rightarrow C5b67

C8 + C9 \rightarrow C5b6789

opsonization of bacteria

mast cell activation

chemotaxis for white blood cells

cell lysis
Host defense processes responsible for tissue destructions

1st protective barrier gingival sulcus

Many sophisticated and effective antibacterial mechanisms to keep bacteria out of tissues

Sulcus epithelium
Secretes cytokines and chemokines (IL-8)
Antibacterial peptides (α-defensin, β-defensin)
The Langerhans cells' membrane receptors play crucial role in innate protection
Host defense processes responsible for tissue destructions

1st protective barrier gingival sulcus

A layer of PMN leukocytes separated bacterial plaque from gingival epithelium

Crevicular PMN cells phagocytose bacteria
The majority of catabolic enzymes from PMN cells get into the crevicular fluid and will not cause tissue damage.
Monocytes in the sulcus can phagocytose PMN cells and bacterial debris clearing the waste products
The functional aberrations of sulcus leukocytes can lead to severe periodontal destructions
SULCUS BLEEDING
A. Chemotaxis

Bacterial pathogen → C5a

B. Phagocytosis

CR4 → iC3b → CD14

LPS → LBP → Septin

C. Killing

Defensins, lysozyme, some neutral serine proteases

NADPH oxidase, myeloperoxidase, nitric oxide synthase
Host defense processes responsible for tissue destructions

1st protective barrier gingival sulcus

Humoral factors

The crevicular complement system is one of the earliest reactions. Bacteria in the sulcus can activate complement by the classic and alternative pathways.

C3b complement is an opsonine

Abundant crevicular IgG and IgA molecules. Bacteria can directly stimulate B lymphocytes as a mytogen.
The complement cascade involves a series of reactions involving complement proteins and antigen-antibody (Ag+Ab) complexes. The process begins with the activation of C1 to C11, which leads to the formation of the membrane attack complex (MAC) C5b6789, resulting in cell lysis. Intermediate steps include the conversion of C4 to C42 + C4a, C3 to C3b + C3a, C5 to C5b + C5a, and C6 + C7 to C5b67. The reaction of C8 + C9 with C5b67 forms C5b6789, which inserts into the cell membrane, initiating lysis.

- **Opsonization of bacteria**: This occurs when C3b binds to bacteria, enhancing recognition by phagocytes.
- **Mast cell activation**: C5a, a potent mast cell activator, is released during the complement cascade.
- **Chemotaxis for white blood cells**: The release of C5a attracts white blood cells to sites of infection.

The diagram also highlights the role of LPS in activating the complement cascade via the classical pathway.
Classic and alternative complement cascade

**C3 convertases**

**CLASSIC CRP**
- AG + IgG, IgM
- C1q
- C4 + C2
- C4bC2b

**ALTERNÁTIVE C3**
- C3 + H2O
- C3b
- Factor B
- C3bBb

**amplification**
- C3b
- C3a

**regulation**
- C5a
- C5b

**regulation +**
- CR1-3 + C3bi

**chemotaxis**
- lysis
- C6-C9

**opsonisation**
- mast cells
Host defense processes responsible for tissue destructions

2nd protective barrier gingival connective tissue

If plaque bacteria exceed a certain limit that the crevicular protective barriers can cope with clinically manifest inflammation occurs

The number of lymphocytes is increasing

The reactions shift toward specific (adaptive) immunity

Cellular and humoral immune responses - T-cell, B-cell

Monocytes and macrophages
Host defense processes responsible for tissue destructions

3rd protective barrier  systemic immune response

Most healthy adults carries specific serum (IgM, IgG and IgA) against oral periodontopathogenic bacteria

In young healthy individuals the serum antibody titer is significantly lower than in healthy adults.
Mechanisms responsible for periodontal tissue destructions

Direct bacterial factors

The major cause is bacterial plaque. Bacteria can directly damage periodontal tissues but this is only a non significant factor in tissue destruction

- soluble proteolytic enzymes
- low molecular weight waste products (urea, sulfides etc)
- endotoxin (lipopolysaccharide- LPS)
- exotoxin - i.e.- leukotoxin
Mechanisms responsible for periodontal tissue destructions

The role of the host in the periodontal tissue destructions

Many different immune processes play role in the pathomechanisms of periodontatis . These are the decisive factors

innate immunity
"adaptíve reactions" acquired immunity
innate immunity

Humoral and cellular elements of innate immunity

Proteolytic enzymes
proteinazes
tissue collagenase - matrix metalloproteaz
MMP - produced by PMN cells and monocytes

Collagenases from PMN leukocytes and fibroblasts can digest type I, II and III collagen tripla-helix and cause extracellular matrix degradation
innate immunity

Polymorphonuclear leukocytes (PMN)

The number of PMN cells emigration into sulcus are increasing with the severity of gingival inflammation

PMN leukocytes are attracted to the site of inflammation from the capillaries. The chemotactic migration is determined by:

Endothelial cells
Adhesion molecules (receptors and its ligands)
The effector cell
**Basophil leukocytes, Mast cells**

Granules contain histamine, leukotrienes, heparin, serotonin, and other biologically active substances.

IgG and especially IgE bind to the Fc receptors.

These cause degranulation and liberation of biologically active substances.
Polymorphonuclear leukocytes (PMN)

Several adhesion molecules assist the extravasation and traversing of PMN leukocytes across gingival connective tissue and also emigration through sulcus epithelium.

- E-selectin
- Adhesin
- Endothelial Adhesion Molecules (ELAM)
- Intercellular Adhesion Molecules (ICAM)
SCHEMATIC ILLUSTRATION OF THE PROCESS WHEREBY NEUTROPHILS ARE ATTRACTIONED INTO THE JUNCTIONAL EPITHELIUM

- LPS PROTEASES fMet-Leu-Phe
- release of proinflammatory cytokines IL-1, TNF
- endothelial cell increase their adhesion molecule expression ICAM-1, ELAM-1
- chemotactic gradient of C5a, LTB4, IL-8, fMet-Leu-Phe
- PMN leukocytes migrate through the junctional epithelium into the crevice
- PMN binding and extravasation

PLAQUE

postcapillary venule
THE PROTECTIVE ROLE OF PMN CAN BE DIVIDED TO SIX STAGES.
The leukocyte contacts, rolls, sticks and extravasates out of the blood vessel prior to beginning its journey to the site of inflammation.
Molecules, cells and processes influencing the increased adherence of leukocytes to blood vessels so that they can extravasate to chemotact towards the microbes

**PMN rolling**

- **DIRECT**
  - LPS
  - Activation of endothelial cells
  - CAM-1
  - Selectin
- **INDIRECT**
  - LPS
  - IL-1β
  - TNF-α
  - PGE2
  - IL-8
  - MMP

**BACTERIA**

- Dental plaque bacteria
- Tooth surface
Polymorphonuclear leukocytes (PMN)

The main function of PMN leukocytes is phagocytosis. The precondition for phagocytosis is migration towards a chemotactic stimulus.

Chemotactic stimuli
- complement C5a,
- leukotriene B4,
- interleukin-8,
- bacterial metabolites
Polymorphonuclear leukocytes (PMN)

There are two chemotactic receptors with different affinities.

- High affinity receptor is responsible for chemotactic movements.
- Low affinity receptors will ignite the oxidative burst and degranulation and prepare the cells for phagocytosis.
Polymorphonuclear leukocytes (PMN)

The activity of PMN cells can lead to severe tissue destruction - periodontal abscess

Chemotactic stimuli can determine the character of the PMN cellular response - protective or destructive.

The most important chemotactic molecules are: C5a, LTB4 és az IL-8.
IL-8 is less potent in activation of phagocytosis, but more potent in enhancing MMP production.
Polymorphonuclear leukocytes (PMN)

Phagocytosis is an active energy consuming process.
There are three stages:

1. recognition and fixation of foreign particles,
2. engulfing foreign particles
3. degradation and digestion of foreign particles
Polymorphonuclear leukocytes (PMN)

Phagocytosis is an active energy consuming process. There are three stages:

1. recognition and fixation of foreign particles,
2. engulfing foreign particles
3. degradation and digestion of foreign particles
A. Chemotaxis

B. Initiate Phagocytosis

C. Oxygen Reduction

NADPH oxidase

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- + H^+ \leftrightarrow \text{HO}_2^- \]

\[ \text{O}_2^- + \text{HO}_2 + H^+ \rightarrow \text{H}_2\text{O}_2 \]

D. Killing

\[ \text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{HOCI} \]

Phagolysosome

Myeloperoxidase
Polymorphonuclear leukocytes (PMN)

Phagocytosis is an active energy consuming process. There are three stages:

1. recognition and fixation of foreign particles,

The recognition and fixation is promoted by opsonins (complement, immuno globulin).

Specific surface receptors on PMN cells:
for activated C3b complement (CR1 and CR3)
for immuno globulins Fcg.
Neutrophil iC3b receptor
Neutrophil Fcy receptor

Neutrophil iC3b receptor
Polymorphonuclear leukocytes (PMN) phagocytosis

- CR1, CR3 receptors
- FcI, FcII, FcIII receptors
- CD14 receptors
- LPS
- Opsonins
- Adherent bacteria
- Developing phagosome
- Phagosome
Polymorphonuclear leukocytes (PMN)

1. recognition and fixation of foreign particles,

Fc receptor binds immune globulins and also opsonised foreign particle
Any functional disturbances in Fc receptor can lead to severe periodontitis
The Ig subclasses have decisive effects on the quality of opsonisation

IgG1 and IgG3 subclasses are strong opsonines
IgG2 and IgG4 subclasses are weak opsonines
**Polymorphonuclear leukocytes (PMN)**

**Fc receptor**

Fc receptor is a very critical coupling factor between non-specific innate reactions and humoral immunity. _On the cell membrane of Neutrophils there are many receptors, with different affinity_

**FcI high affinity receptor**
**FcII and FcIII low affinity receptors**

FcII receptor binds all Ig subclasses
FcIII binds only IgG1 and IgG3 subclasses
FcIII receptor cannot ignite oxidative burst
FcII receptor can activate the whole process of phagocytosis
Polymorphonuclear leukocytes (PMN)

Fc receptor

In localized juvenile periodontitis, the expression of FcII and FcIII receptors is down regulated in crevicular PMN cells.

The circulating PMN cells show normal values.
Polymorphonuclear leukocytes (PMN)

CD14 receptor

A PMN leukocytes can opsonise bacteria without Ig and complements
  CD14 receptors on PMN cell membranes can bind LPS

PMN cells can phagocytose bacteria if complement or specific Ig molecules are destroyed by bacterial virulence factors
Polymorphonuclear leukocytes (PMN) phagocytosis

- CR1, CR3 receptors
- FcI, FcII, FcIII receptors
- CD14 receptors
- Opsonins
- Adherent bacteria
- Developing phagosome
- Phagosome
Polymorphonuclear leukocytes (PMN)

Phagocytosis is an active energy consuming process. There are three stages:

1. recognition and fixation of foreign particles,
2. engulfing foreign particles
3. degradation and digestion of foreign particles
**Polymorphonuclear leukocytes (PMN)**

There are different cytoplasmatic granules

**Three type of granules exist:**
- primary or azurophil granules
- secondary or specific granules
- terciary or C granules

**Primary granules** is identified by its peroxidase content
- myeloperoxidase, lysozyme és proteinase enzymes.

**Secondary granules are peroxidase negative** - Containing:
- lactoferin, B12 binding protein, fibronectin receptors, laminin receptors
  - **Secondary granules are released chiefly extracellularly while primarily granules serving the intracellular digestion.**
A. Chemotaxis

Bacterial pathogen

C5a

B. Initiate Phagocytosis

iC3b
CR3

C. Oxygen Reduction

NADPH oxidase

\[ O_2 + e^- \rightarrow O_2^- + H^+ \rightarrow HO_2^- \]

\[ O_2^- + HO_2 + H^+ \rightarrow H_2O_2 \]

D. Killing

\[ H_2O_2 + Cl^- \rightarrow HOCl \]

Myeloperoxidase

IC3b
CR3
Phagolysosome
Polymorphonuclear leukocytes (PMN) phagocytosis

- GLYCOLYSIS
- LYSOSOMES
- BACTERIA
- ACID
- MYELOPEROXIDASE, LACTOFERIN, LYSOZYME, PROTEASES
- oxydative burst
- NADPH -NADP
- O2
- H2O2
- OH−
- O2−

extracellular secretion of PMN mediators
Polymorphonuclear leukocytes (PMN)

PMN leukocytes make up the first defensive line with the complement system and Ig molecules

neutrophil/complement/antibody axis

Deficiencies in PMN functions - greater risk for destructive periodontal disease

Leukocyte Adhesion Deficiency
Papillon-LeFevre syndrome
Chediak-Higashi syndromes
Cyclic neutropenia
Leukemia
Localized juvenile periodontitis
LEUKOCYTE
ADHESION
DEFICIENCY, (LAD)

SCHEMATIC ILLUSTRATION OF THE PROCESS WHEREBY
NEUTROPHILS ARE ATTRACTIONED INTO THE JUNCTIONAL
EPITHELIUM

- Selectin
- CD-15
- Integrin
- CD-11 a,b,c
- CD-18
As integrin molecules are also responsible for binding the opsinized antigens, in this disease not only the diapedesis but also the phagocytosis is hampered.
Papillon LeFevre syndrome
Gene mutation on 11 chromosome (11q14-q21)
cathepsin C gene

HART TC, HART PS, BOWDEN DW és mts. Mutation of the cathepsin C gene are responsible for Papillon-Lefevre syndrome J Med Genet 1999; 36:881-888

Papillon LeFevre syndrome
Gene mutation on 11 chromosome (11q14-q21) cathepsin C gene
Chronic granulomatous disease

PMN leukocytes  NADPH oxydaze enzyme disfunction

The disease is inherited by recessive inheritance

The PMN cells are not able to produce reactive free oxygen radicals and is not capable of killing phagocytosed bacteria
Mononuclear cells, monocytes and macrophages

Langerhans cells and gingival macrophages, macrophages make up 2% of the cellular elements of the gingival sulcus fluid. Plays important role in the production of inflammatory and regenerative cytokines (IL-1, IL-6, TNF, TGF, PGE2, LTB4).
Mononuclear cells, monocytes, and macrophages

The reactivity and cytokine production by monocytes determine the course of the inflammatory periodontal disease and the severity of tissue destruction.

The life span of the monocytes is much longer than that of the PMN leukocytes. After phagocytosis, the cells survive.
Monocytes from different individuals secrete different amount of proinflammatory cytokines and PGE in response to endotoxin stimulation.

Those individual differences are genetically inherited and determined by chromosomal gene polymorphisms.

MOLVIG J, BAEK L, CHRISTEN P. et al.:
A nucleotid base change on the locus of IL-1B $^{+3953}$ of the homologue chromosome will lead to a four fold increase in IL-1 production by monocytes.

The destructive and protective host response

- A “maximum” intensity of host immune inflammatory reaction leads to excessive tissue damage without enhancing the control of infection
  - (Trombone et al., 2009).

- Patients presenting hyper-inflammatory genetic variants present the same frequency and load of red-complex periodontopathogens and *A. actinomycetemcomitans*
  - as patients genetically not prone to develop exacerbated responses
  - (Ferreira et al., 2008);
Th1 – Th 2 differentiations
Natural killer lymphocytes (NK cells)

Non committed lymphocytes
They simply bind to target cells and kill them without previous sensitization

Provide immediate non specific reaction

High affinity Fc receptors for IgG which enables them to play an important role in antibody dependent cell mediated cytolysis
inflammatory cytokines

secondary inflammatory mediator production

PAF, histamine, bradykinin, PGE2
Cytokines are soluble proteins produced by cells and act on other cells from the same cell line or on different cell lines.

- Autocrine regulation
- Paracrine regulation
Cytokines

Common features

1. locally produced and acting and degrading locally
2. several cytokines have similar biologic effects
3. Most inflammatory cytokine pre coded in mRNA - and a signal can trigger the translation and secretion
4. Very strong hormones, acting on high affinity membrane receptors in very low concentration $10^{-9}$ - $10^{-8}$ Mol
5. Several cells have different membrane cytokin receptors and can give opposing answers
6. Cells have membrane receptors with different affinity
Interleukin 1 (IL-1)

IL-1 plays a crucial role in the pathogenesis of periodontal destruction.

Disease activity in periodontal pockets leads immediately to increased (3-4 fold) IL-1β concentration.
Interleukin 1 (IL-1)

One of the earliest cytokines
Osteoclast stimulating factor
LPS stimulated lymphocytes in cell cultures produced a substance which increased bone resorption in bone organ cultures

IL-1 exists in two forms IL-1a and b.
Interleukin 1 (IL-1)

Locally acts to up-regulate adhesion molecules on fibroblasts, endothelial cells, lymphocytes, PMN leukocytes and monocytes

Bacterial LPS stimulates sulcus Langerhans cells to produce IL-1α and β-1t. They play important role in periodontal inflammation

Directly increase all catabolic processes in connective tissue
Interleukin 1 (IL-1)

Increases PGE2 secretion by fibroblasts and monocytes which in turn stimulates vasodilatation, oedema and bone resorption.

Stimulates PMN leukocytes and monocytes MMP production.

IL-1 is autostimulatory acts on other cells to produce more IL-1.
Interleukin 1 (IL-1)

There are IL-1 receptor antagonists (IL-1ra)

They can bind to IL-1 receptors and occupies IL-1 receptors

IL-1ra synthesis is enhanced by steroids and certain anti inflammatory cytokines  IL-4, IL-10.
Interleukin-6 (IL-6)

Produced by lymphocytes, fibroblasts and monocytes.

IL-6 promotes the proliferation and differentiation of B lymphocytes, T lymphocytes and the differentiation of monocytes into multinucleated osteoclasts.

IL-6 produced during inflammation plays essential role in periodontal bone resorption.
IL-6
Interleukin-6 (IL-6)

Estrogens and progesterons inhibit the secretion of IL-6 by mononuclear cells and osteoblasts.

Supposedly this plays an important role in the postmenopausal osteoporosis and the increased severity of periodontal bone loss after menopause.
Tumor necrosis factor alpha (TNFα)

Promotes matrix degradation and bone resorption
Less potent than IL-1.
The mechanisms of action on bone metabolism is different from that of IL-1.
It exerts a decoupling effect on bone, inhibiting bone formation and facilitating osteoclastic bone resorption

Chronic low levels of IL-1 and TNFα in the bloodstream can promote endothelial cell damage and atherosclerosis !!!!!
Tumor necrosis factor alfa (TNFα)

In low concentration protect against inflammation. In high concentration extremely toxic to the tissues

Causing abortion in pregnant animals

Triggers the release of histamine, serotonin Promotes matrix degradation and bone resorption
Less potent than IL-1.
INFLAMMATORY BONE RESORPTION - RANKL

- Inflammatory bone diseases enhance the local RANKL expression and the RANKL/OPG ratio is shifted
- Interleukin-1, IL-6 and TNF-α are strong bone resorbers and they increase the RANKL/OPG expression in osteoblasts and other stromal cells. These cells can locally control the extent of bone resorption
The role of osteoblasts in the osteoclastogenesis and modulation of bone resorption
The molecular communication factors between osteoblasts and osteoclasts

- *Macrophag Colony Stimulating Factor (M-CSF)*
- *Receptor Aktivator of Nuclera Factor K Ligand (RANKL).*

- The M-CSF binds to the membrane receptors of osteoclast precursors igniting their proliferation and ensures their survival
- **RANKL** is a trigger factor, that facilitates the differentiation of osteoclast precursor cells and stimulates the resorptive capacity of the matured osteoclasts

The role of osteoblasts in the osteoclastogenesis and modulation of bone resorption

- The effect of RANKL can be antagonized by osteoprotegerin (OPG) (Simonet és mtsai., 1997).
- OPG synthesized by osteoblasts and other stromal cells.
- OPG can bind to RANKL- and can block the RANKL/RANK coupling and the triggering of osteoclasts.
**PERIODONTAL BONE RESORPTION**

- **LPS**
- **MO**
- **T-sejt**
  - **INF-γ**
  - **OPG**: osteoprotegerin
  - **RANKL**: Receptor Activator of Nuclear Factor kB
  - **MCSF**
  - **B-sejt**
- **OSTEOCLASTOGENESIS**
- **OSTEOBLASTOK**
  - **BONE FORMATION**
  - **MATURE OSTEOCLAST**
  - **BONE RESORPTION**

**Key Terms**

- **OPG**: osteoprotegerin
- **RANK**: Receptor Activator of Nuclear Factor kB
- **RANKL**: Receptor Activator of Nuclear Factor kB - Ligand

**Equations**

- **OPG > RANK**
- **OPG < RANK**
Chemotactic cytokines

IL-8

Chemokines
Produced by epithelial cells, monocytes and endothelial cells stimulates by IL-1, LPS or TNFα.

IL-8 stimulates MMP secretion by PMN leukocytes
**IL-8**

In gingivitis the LTB4 concentration in the gingival sulcus is high

In destructive periodontitis, especially in periodontal abscess the IL-8 concentration is high

The IL-8 evoked PMN response is more destructive due to the elevated MMP production

PMN cell response attracted by LTB4 or C5a chemotactic factors is more protective due to the stimulated phagocytosis
Modulation of arachidonic acid metabolism

- Tissue damage
- Membrane phospholipids
- Arachidonic acid
- COX-1, COX-2
- Lipoxygenase
- PGE
- Prostaglandins
- Prostacyclins
- Thromboxane
- Leukotrienes
- Lipoxins

Cell-cell and enzyme interactions
Prostaglandin

Two isoforms of cyclooxygenase enzymes
COX-1
COX-2

COX-1 PG-s for homeostasis
COX-2 PG-s for inflammation
local inflammatory PG production

monocytes
macrophages
fibroblasts
PMN leukocytes
other white blood cells
endothelial cells
Prostaglandin

IL-1β or TNFα stimulus on monocytes immediately induce COX-2 gene transcription and de novo production of PGE2.

The same stimulus has no effect on the COX-1 enzyme system.

In the periodontal tissues the main source of PGE2 is monocytes and fibroblasts.
Genetically determined variance in host immune response to bacterial infections has been identified in individuals with aggressive periodontitis.
Monocytes from aggressive periodontitis patients showed increased PGE production upon stimulation with LPS

Shapira L et al. The secretion of PGE, IL-1β and TNFα by adherent mononuclear cells from early onset periodontitis patients  J. Periodontol 1994;65:139-146
Prostaglandin

PGE increases vascular permeability, vasodilatation and edema.
Stimulate MMP production by monocytes and fibroblasts and consequently promotes connective tissue matrix catabolism

Directly and indirectly stimulates bone resorption
Synergistically enhances biologic effect of TNFα and IL-1
Prostaglandin

In gingivitis the PGE2 concentration of the crevicular fluid is significantly increased.

In gingivitis there is a three fold increase

In periodontitis there is an additional 3-5 fold increase
PG is a potent bone resorber

periodontitis
periapical periodontitis
cyst
orthodontic tooth movement
traumatic occlusion
malignant tumors
### NONSTEROID ANTI-INFLAMMATORY DRUGS

- ASPIRIN
- APRANAX
- CATAFLAM
- DICLOFENAC
- DONALGIN
- FLUGALIN
- HOTEemin
- INDOMETACINUM
- NAPROSYN
- PROFÉNID
- SURGAM
- TILCOTIL
- VOLTAREN
Reparative and anabolic cytokines

During wound healing and tissue repair monocytes, platelets, fibroblasts and other cells produce factors with anabolic biological effects.

- Platelet Derived Growth Factor (PDGF)
- Fibroblastic Growth Factor (FGF)
- Insulin-like Growth Factor (IGF)
- Transforming Growth Factor (TGF)
- Bone Morphogenic Proteins (BMPs).
Reparative and anabolic cytokines

1. These are chemotactic for fibroblasts, periodontal ligament mesenchymal cells, and osteoprogenitor cells
2. Many, as a growth factor induce cellular differentiation of connective tissue mesenchymal cells into matured matrix-secreting cells
3. Many of the growth factors are incorporated into the newly formed matrix. I.e. Bone contains large amount of BMP, TGF and IGF.
Reparative and anabolic cytokines

- Matrix degradation immediately triggers the compensatory anabolic cytokine production.

- If bacterial stimuli persist, the pro-inflammatory cytokines will suppress the regenerative processes.

- After the bacterial factors having been eliminated, the regenerative processes will dominate the tissue reactions, and the connective tissue regenerates.
Reparative and anabolic cytokines

- In periodontal inflammation the majority of the pro-inflammatory cytokines are produced by monocytes. The monocyte reaction plays a decisive role in the determination of the severity of inflammatory reaction and tissue damage.

- The healing is also promoted by anabolic cytokines produced by monocytes.
Established gingival lesion

cellular infiltrate 30-60%
predominantly T- B lymphocytes
monocytes/ macrophages
plasma cells 10-40%
greatly increased PMN emigration
vascular proliferation
severe loss of collagen,
fibroblast degeneration
severe proliferation of junctional
epithelium
accantotic sulcus epithelium
deepening sulcus
Specific - adaptive - immunity in the pathomechanism of periodontal disease

- In a relatively early stage of gingivitis T lymphocytes are present in the inflammatory cellular infiltrate
- They are primarily protective
- There are three different kind of T cells responding to different antigens
Specific immunity in the pathomechanism of periodontal disease

1- CD8+ cytotoxic lymphocytes,
2 - CD4+ lymphocytes
3 - natural killer T lymphocytes

CD4+ cells are helper T cells
They are divided into two sub groups
Th1 and Th2 helper cells
Th17 and Treg cells (regulatory)
CYTOKINES PRODUCED BY TYPE 1 (TH1) AND TYPE 2 (TH2) CD4+ HELPER CELLS

TH1
- IL-2
- INFγ
- TNF-β

TH2
- IL-4
- IL-5
- IL-6
- IL-10
- IL-13

ENHANCE CELL MEDIATED RESPONSE
ENHANCE HUMORAL IMMUNE RESPONSE
Specific immunity in the pathomechanism of periodontal disease

- Th1 and Th2 cells play different role in periodontitis

- Th1 cells primarily associated with active inflammation and restricted to a relatively limited area

- Th2 cells are widely spread all over the tissue and rather associated with chronic inflammation
BONE RESORPTION BY ADAPTIVE IMMUNE RESPONSE

- It has been reported that T-cells are involved in bone destruction via IL-17 production, which in turn is described as an inducer of RANKL production

- (Kotake et al., 1999; Sato et al., 2006)
The destructive host response from the tissue damage perspective Th2

- Th2 cells’ commitment and action are primarily dependent on IL-4, the prototypical Th2 cytokine,
- which also acts as a B-cell stimulatory Factor

(Murphy and Reiner,
The destructive host response from the tissue damage perspective

- **IL-6** contributes to B-cell differentiation and antibody production
- **B-cells produce RANKL in response to periodontal pathogen stimulation**
- **The majority of B-cells in periodontal lesions are RANKL+**
  - (Kawai *et al.*, Han *et al.*, 2009),
The destructive host response from the tissue damage perspective

- In a later stage B-cells outnumber T-cells in periodontal lesions,
- the predominance of a Th2-type response in periodontal lesions
- It potentially leads to the accumulation of RANKL-producing cells and, consequently, to tissue destruction and bone loss
  - (Gemmell et al., 2002b)
THE SPREAD OF INFLAMMATION IS DETERMINED BY THE CHARACTER OF THE IMMUNE RESPONSE AND THE COMPOSITION OF CYTOKINES
The inflammatory cytokines and other local factors – like PGE - will upset the balance of the normal coupled bone remodeling and shifts this towards net bone loss

- Partly by inhibiting bone formation
- Partly by promoting osteoclastic bone resorption,
LOCAL REGULATION IN INFLAMMATION

LPS

IL-1, TNF-a, PGE2, RANKL

MO

IL-1
IL-6
LYMPHOTOXIN

LYMPHOCYTA

FIBROBLASTS

OSTEOBLAST
OSTEOCLAST
BONE

OSTEOBLASTS

PREOSTEOBLASTS

OSTEOCLASTS

MO

LPS

IL-1, TNF-α, PGE2, RANKL

LYMPHOCYTA

FIBROBLASTS

OSTEOBLAST

OSTEOCLAST
LOCAL REGULATION
BONE RESORPTION

IL-1

POTENT BONE RESORBING CYTOKINE

• DIRECT STIMULATION ON OSTEOCLAST PRECURSORS
• INDIRECT STIMULATION ON MATURERED OSTEOCLATS
• LOCALLY STIMULATES OSTEOBLASTIC PGE SYNTHESIS WHICH IN TURN STIMULATES BONE RESORPTION
• SPECIFIC RECEPTOR ON OSTEOCLASTS FOR IL-1
• ITS PERMANENT PRESENCE INHIBITS BONE FORMATION
• AT EARLY STAGE STIMULATES OSTEBLAST PRECURSORS TO PROLIFERATE BUT THE MATURERED OSTEOBLASTS ARE BLOCKED
LOCAL REGULATION
BONE RESORPTION

IL-6

- NO DIRECT EFFECT ON BONE RESORPTION
- DIRECT STIMULATION ON OSTECLAST PRECURSORS
- INDIRECT STIMULATION OF BONE RESORPTION
LOCAL REGULATION
BONE RESORPTION

TNF - LYMPHOTOXIN

• INDIRECTLY STIMULATES OSTEOCLASTIC
BONE RESORPTION

• LOCALLY ENHANCES PGE PRODUCTION

• ITS PERMANENT PRESENCE INHIBITS
MATURE OSTEOBLASTS BUT ALSO
STIMULATES PRECURSOR CELLS
REPLICATION AND DIFFERENTIATION
The key between T cells and osteoclastic activations is RANKL

- Receptor Activator of Nuclear Factor kB (RANK)
- ITS RANKL LIGAND CAN BE FOUND IN OSTEOBLAST, T AND B CELLS
PERIODONTAL BONE RESORPTION

**LPS**

**MO**

**T-sejt**

**B-sejt**

**ACTIVATED T-CELL**

**OSTEOCLAST PRECURSOR**

**ACTIVATED B-CELLS**

**INF-γ**

**TNF, IL-1, IL-6**

**TNF, IL-11, IL-17**

**OPG**

**RANK**

**RANKL**

**OPG: osteoprotegerin**

**RANK : Receptor Activator of Nuclear Factor κB**

**RANKL: Receptor Activator of Nuclear Factor κB-Ligand**

**OSTEOCLASTOGENESIS**

**OSTEOBLASTOK**

**MATURED OC**

Bone formation

OPG > RANK

Bone resorption

OPG < RANK
The role of osteoblasts in the osteoclastogenesis and modulation of bone resorption
The molecular communication factors between osteoblasts and osteoclasts

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- The M-CSF binds to the membrane receptors of osteoclast precursors igniting their proliferation and ensures their survival.
- RANKL is a trigger factor, that facilitates the differentiation of osteoclast precursor cells and stimulates the resorptive capacity of the matured osteoclasts.

The role of osteoblasts in the osteoclastogenesis and modulation of bone resorption

• After RANKL having been bound to the membrane receptors (RANK) of osteoclast precursor cells previously activated by M-CSF significant changes are taking place in the cell and will be able to rezzorb and digest bone matrix

• (Takayanagi és mtsai., 2002).
LOCAL REGULATION
BONE RESORPTION

PROSTAGLANDIN GROUP

• STIMULATES OC PRECURSOR DIFFERENTIATION
• STIMULATES MATURED OSTEOCLAST ACTIVITY
• MEDIATES SEVERAL OTHER LOCAL FACTORS’ EFFECT ON BONE – EGF, IGF, TGF
• IT IS LOCALLY PRODUCED IN A LARGE QUANTITY BY OSTEOBLASTS AND THIS HAS MAJOR EFFECT ON COUPLED BONE REMODELING
• IN VITRO LOW DOSES STIMULATES BONE FORMATION WHILE LARGE DOSES ENAHNCE BONE RESOPRTION
• IN VIVO ITS MAJOR EFFECT ON BONE IS TO STIMULATE PERIOSTEAL BONE FORMATION
The regulation of periodontal bone resorption and formation

- PDL and gingival fibroblasts play a key role in the local regulation of RANKL and osteoprotegerin (OPG).
- PDL fibroblasts can synthesize both RANKL and OPG.
- The decrease in OPG by PDL fibroblasts will enhance alveolar bone resorption.
- (Hasegawa és mtsai., 2002)
SYSTEMIC REGULATION
PARATHYROID HORMONE - PTH
CALCITONIN
1,25 DIHYDROXY VITAMIN D₃
STEROID HORMONES
GROWTH HORMONES
THYROID HORMONES
TFGβ MINT COUPLING FACTOR

- hormone PTH
- plasminogen Activator
- matrix képzés
ACTIVE OSTEOCLAST

nucleus

Golgi

lysosomes

Ca sensor

HCIO3

CI

Ca ++

H^+

protease

Ruffled Border

bone resorption

bone matrix
OSTEOCLASTS

STRONG WELL DEVELOPED CYTOSKELETON SERVING ACTIVE MOTILITY

SPECIAL MEMBRANE RECEPTORS
OSTEOCLASTS

ENZYME SYSTEM
ACID PHOSPHATASE
β-GLUCORONIDASE
CYSTEIN PROTEASE – CATHEPSIN B
TISSUE PLASMINOGEN ACTIVATOR
MATRIX METALLOPROTEINASES COLLAGENASE
LYSOZYME
SOUND ATTACHMENT APPARATUS

SEVERE ATTACHMENT LOSS AND BONE LOSS
poor oral hygiene → normal flora → pathogenic flora

Antibody response

PMN clearance: Yes → gingivitis
PMN clearance: No → parodontitis

bacterial penetration

inflammation tissue destruction

cytokines PGE2 mediators

monocyte lymphocyte axis

pocketing bone loss

Systemic exposure
poor oral hygiene → normal flora → pathogenic flora → PMN clearance → Antibody response → gingivitis

→ pocketing → bone loss

→ inflammation → tissue destruction

→ cytokines PGE2 mediators

→ monocyte lymphocyte axis

→ bacterial penetration

→ systemic exposure

→ host stress

Yes → No
poor oral hygiene → normal flora → pathogenic flora → antibody response

Risk factors: genetic traits, diabetes, smoking

PMN clearance: Yes → gingivitis

PMN clearance: No → pocketing bone loss, inflammation, tissue destruction

Risk factors: genetic traits, diabetes, smoking

Cytokines, PGE2 mediators, monocyte lymphocyte axis → bacterial penetration

Systemic exposure: Yes

Systemic exposure: No
Poor oral hygiene and normal flora can lead to pathogenic flora, which further results in gingivitis. Antibody response can lead to PMN clearance, which can prevent pocketing and bone loss. Debridement can help in the treatment cycle. Inflammation and tissue destruction can be mediated by cytokines, PGE2, and mediators. Monocyte lymphocyte axis, bacterial penetration, and growth factors can contribute to healing, repair, and regeneration. Systemic exposure can also play a role.