

الرحمن الرحيم

Pathogenesis of Periodontal Disease



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Innate (Natural) immunity

Adaptive (Acquired) immunity



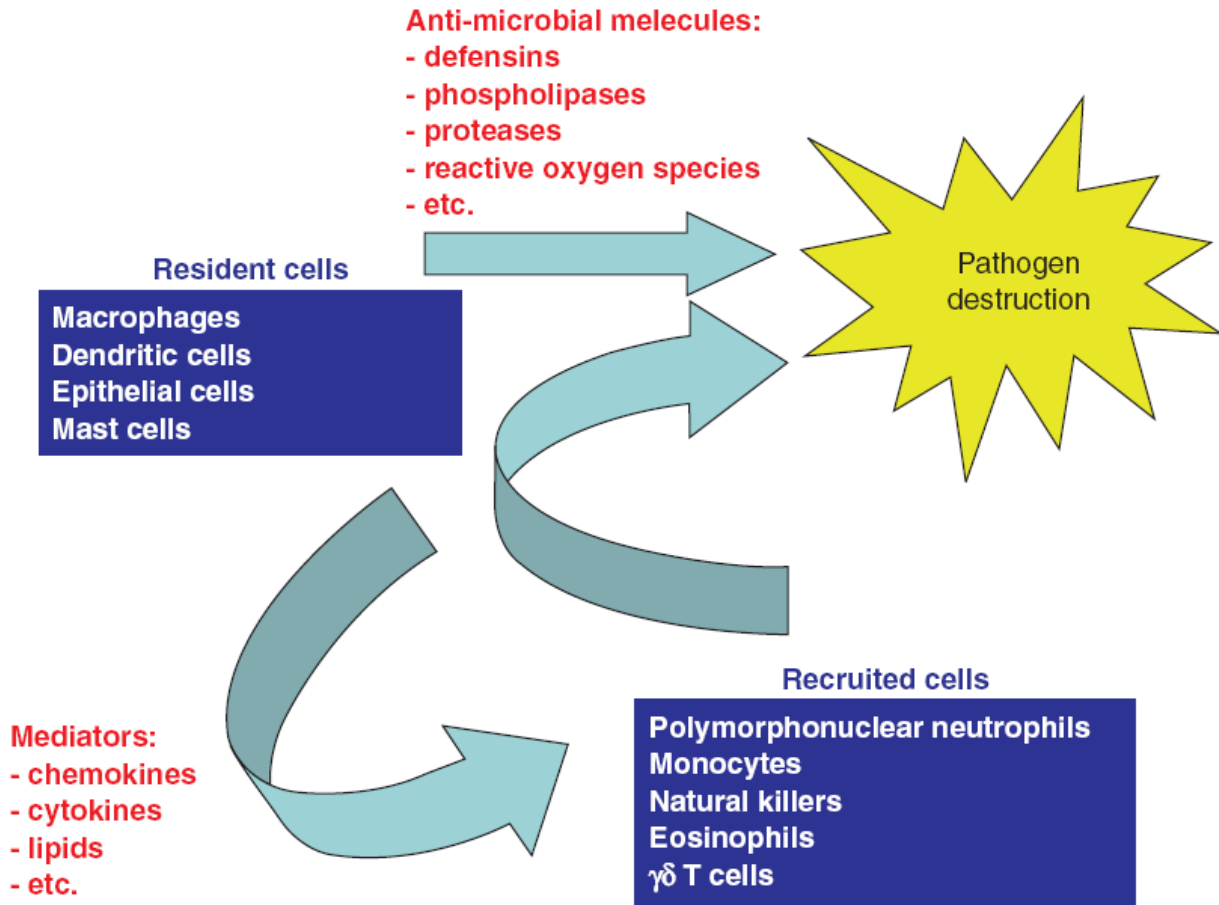
- Innate immune responses are mediated by the release of inflammatory cytokines and chemokines, and by phagocytic or killer cells.
- Adaptive immune responses are mediated by the generation of antigen-specific T and B cells. Antigen-primed T cells induce clonal expansion and differentiate into effector T cells that produce various cytokines or elicit cytotoxicity to eliminate target cells. B cells secrete immunoglobulins, which are responsible for eliminating extracellular microorganisms.
- Innate responses are generated at the periphery of sites of microbial penetration, whereas
- Adaptive immune responses are generated at secondary lymphoid tissues, such as lymph nodes and the spleen



Property	Innate immune system	Adaptive immune system
Cells involved	Phagocytes (macrophages, neutrophils, DCs), NK cells	T cells, B cells
Recognition	Fixed in genome	Encoded in gene segments
Receptors	Rearrangement not necessary Limited diversity	Rearrangement necessary High diversity
Ligand/antigens	Conserved molecular patterns(components of microorganisms)	Details of molecular structure (peptides, proteins, carbohydrates)
Development	Nonclonal Selected over evolutionary time	Clonal Selected in individual
Response time	Immediate (0–4h)	Delayed (~72h)
Site of response	Local, periphery	Secondary lymphoid tissues
Response	Production of inflammatory cytokines (IL-1, IL-6, TNF- α) and chemokines (IL-8); Induction of costimulatory molecules (CD86, CD40)	Clonal expansion or anergy; production of effector cytokines (IFN- γ , IL-4)

DC dendritic cell; *IFN- γ* interferon- γ ; *IL* interleukin; *NK* natural killer; *TNF- α* tumor necrosis factor- α

Fig. 3.1 Cells of the innate immune system. Resident cells directly kill pathogens (phagocytosis, release of anti-microbial molecules, etc.) and also synthesize mediators (chemoattractants, etc.) that trigger the recruitment of other cells participating in the pathogen clearance (modified from Si-Tahar et al. 2009) (with permission from Wiley-Blackwell)



Main Receptors and Signaling Pathways



- Toll-like receptors (TLRs)
- CD14
- Nucleotide-binding oligomerization domain proteins (Nod)
- G-protein-coupled receptors, including formyl-methionyl peptide receptors and protease-activated receptors.
 - Of the above-mentioned bacterial and host molecules, evidence from experimental animal studies implicate TLRs and CD14 in periodontal tissue or alveolar bone destruction

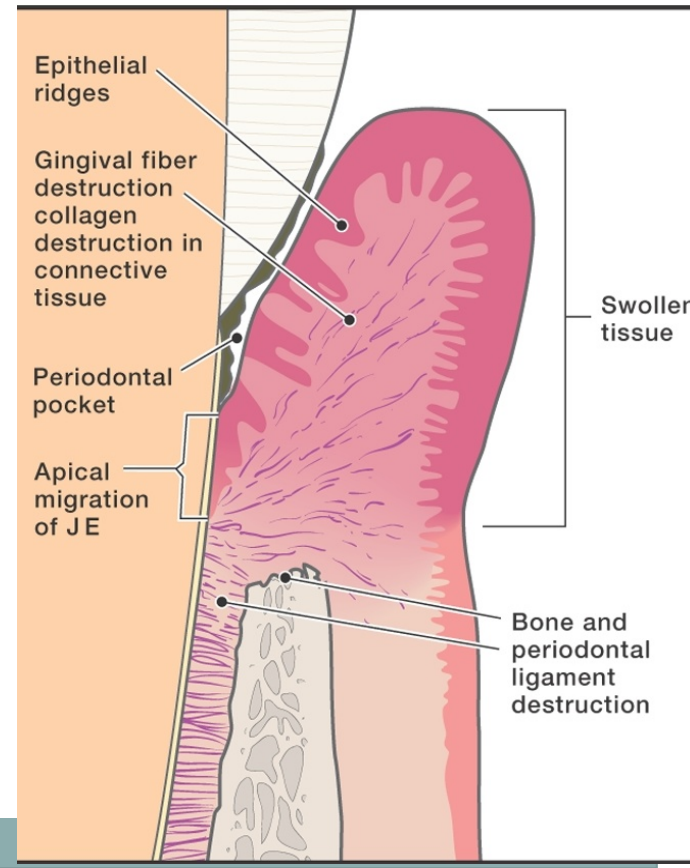
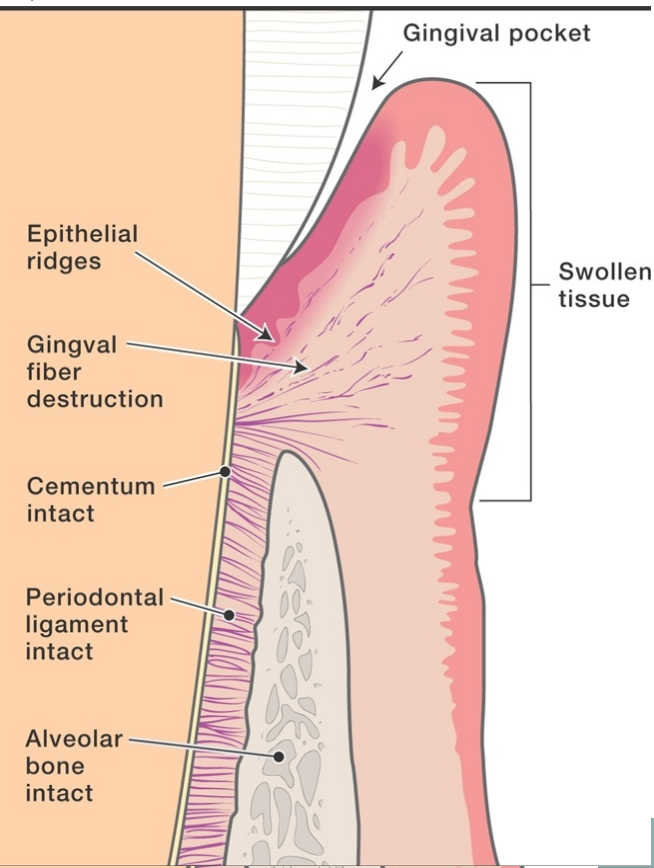
Complement System



- Complement proteins are present in gingival fluid at levels as high as 85% of that reported for serum
- complement is more active in saliva than in serum

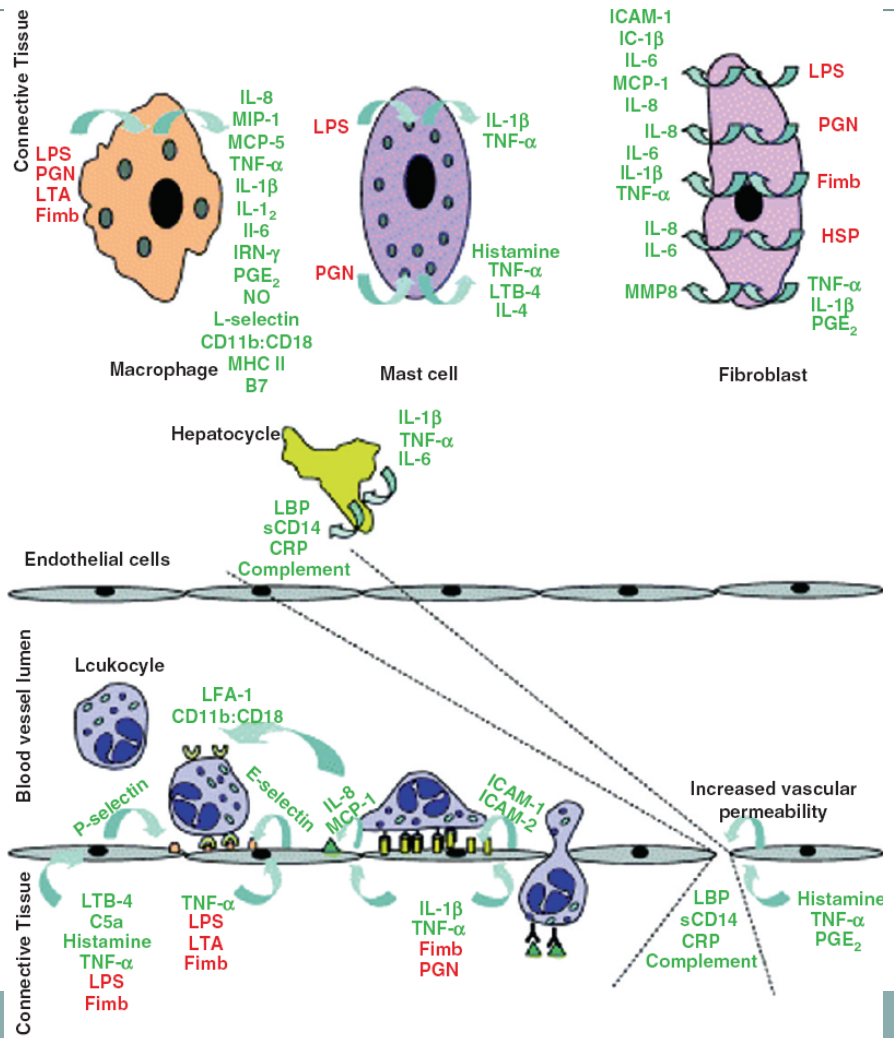
Initiation of Inflammation in Periodontal Tissues





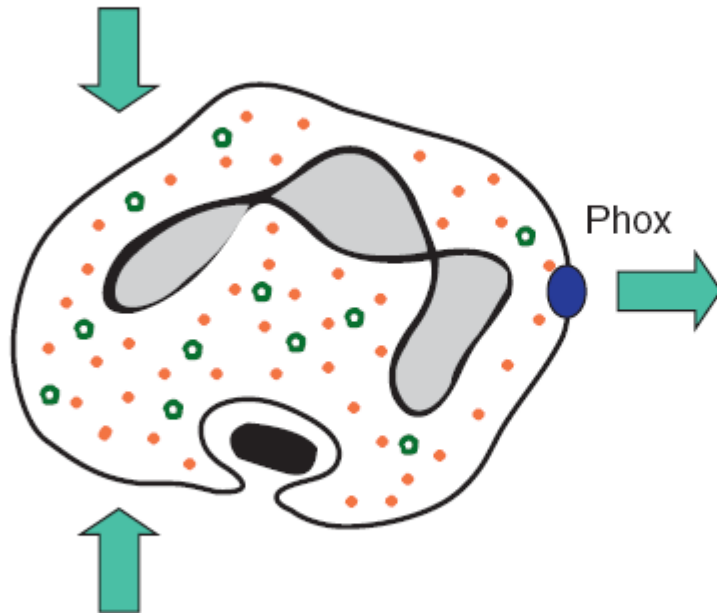
Cells of the Innate Immune Response



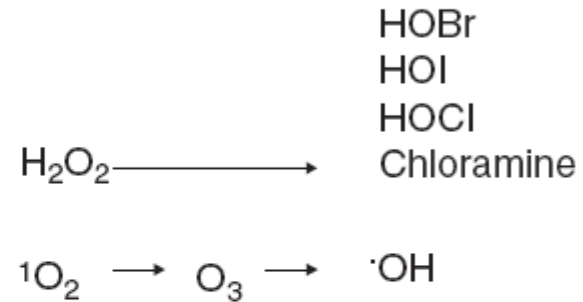


Azurophilic (also known as primary) granules:
elastase, cathepsin G, urokinase, myeloperoxidase, lysozyme and mannosidase, cathepsin B, cathepsin D and β -glucuronidase, defensins

Neutrophils in Periodontal Lesions



Specific and tertiary granules:
Lactoferrin, neutrophil collagenase [matrix metalloproteinase-8 (MMP-8)], lysozyme, gelatinolytic MMP-9 and MMP25



Mechanisms of bacterial elimination by neutrophils

Oxygen-independent	Oxygen-dependent
Myeloperoxidase	Myeloperoxidase-mediated
Defensins	Hypochlorous acid
Bactericidal or permeability-inducing protein	Chloramines
Cathepsin G	Myeloperoxidase-independent
Elastase	Hydrogen peroxide
Proteinase 3	Superoxide anion
Azurocidin	Hydroxyl anion
Lysozyme	Hydroxyl radicals
Lactoferrin	Singlet oxygen

Systemic Conditions with Associated Neutrophil Deficits



PAPILLON-LEFEVRE SYNDROME

DOWN SYNDROME

LEUKOCYTE ADHESION DEFICIENCY (LAD)

CHÉDIAK-HIGASHI SYNDROME

LAZY LEUKOCYTE SYNDROME

CHRONIC GRANULOMATOUS DISEASE (CGD)

NEUTROPENIA

Papillon-Lefevre syndrome



- Decreased neutrophil chemotaxis,
- Reduced random neutrophil migration,
- Impaired neutrophil phagocytosis,
- Reduced myeloperoxidase activity, and
- Increased superoxide radical neutrophil production, associated with a decreased lymphocyte response to pathogens

Down syndrome



- Defects of the chemotaxis and intracellular killing of PMN and other phagocytes, which explains the high incidence of pocketing and marginal bone loss

Leukocyte adhesion deficiency (LAD)



- Individuals with LAD defect in innate host defense display a severe form of periodontitis that does not require specific periodontal pathogens due to entrapment of neutrophils within the blood vessel

Chédiak-Higashi syndrome



- A rare autosomal recessive disease associated with impaired function of cytoplasmic microtubules or microtubule assembly in PMNs
- Severe gingivitis and rapid loss of attachment, leading to exfoliation of the teeth

Lazy leukocyte syndrome



- ✦ An extremely rare disorder that manifests in both quantitative and qualitative neutrophil defects
- ✦ Oral stomatitis, recurrent ulcerations of the buccal mucosa and tongue, severe gingivitis and periodontitis with advanced alveolar bone loss and tooth loss

Chronic granulomatous disease (CGD)



- *An extremely rare inherited disease*
- The defect in this disease is in the ability of phagocytic cells, both PMN leukocyte and monocytes, to perform killing by the oxidative pathway
- No specific periodontal condition has been attributed to this disease. But a generalized prepubertal periodontitis case associated with CGD was reported in a 5-year old male patient.

Neutropenia

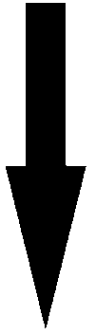


- *Neutropenia is defined as an absolute neutrophil count <1500 cells/mm³*



Health

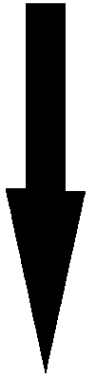
Plaque



Hormones
(Pregnancy, Puberty)
Drugs
(ion channel
blockers, smoking)

Gingivitis

Plaque



Host/site
Susceptibility

Periodontitis



Macrophages in Periodontal Lesions



- Macrophage numbers do not increase and there is little evidence of macrophage activation in advanced periodontitis compared with minimally inflamed tissues
- In the progression from gingivitis to periodontitis, there is a decrease in the macrophage/B-cell ratio

Macrophages in Periodontal Lesions



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- in the progression from gingivitis to periodontitis, there is a decrease in the macrophage/B-cell ratio
- Prostaglandin E² is a bone-resorbing inflammatory lipid PGE₂
- Non-selective cyclooxygenase-1 (COX-1) inhibitors :such as aspirin, flurbiprofen, ibuprofen, naproxen and piroxicam.
- Selective COX-2 inhibitors: termed “coxibs” include meloxicam, nimesulide, etodolac, and celecoxib

Mast cells in Periodontal Lesions



- The “antennas” of the immune response
- In human periodontal disease that there is an increase in the number of mast cells that may be participating either in the destructive events or in the defense mechanism of periodontal disease via secretion of cytokines, including perpetuation of the Th2 response, and cellular migration and healing processes
- The strategic location of mast cells in the body and their diversity of receptors and cytokines indicate an important role for mast cells in regulating innate and adaptive immunity

	Neutrophil	Macrophage	Mast cell	Eosinophil	Dendritic cell
Cytokines	Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , -6	IL-1 β , -6, transforming growth factor (TGF)- β , TNF- α	IL-1, -3, -4, -5, -6, -13, -16, VEGF, TNF- α , TGF- β	IL-2, -3, -4, -5, -6, -10, -12, -13, INF- γ , TNF- α , GM-CSF, SCF, TGF- α	IL-1 β , TNF- α , IL-5, IL-6, IL-12, IL-18, IFN- γ
Chemokines	IL-8		IL-8, MIP-1 α , MCP-1	RANTES, eotaxin, ENA-78/CXL5, GRO- α	MIP-1 α , MCP-1, eotaxin
Proteases and anti-proteases	PR3, elastase, cathepsin G, Matrix metalloproteinases (MMPs), TIMPs	MMPs, TIMPs			
Anti-microbial factors	α -Defensin, elafin, SLPI, lactoferrin, myelo-peroxidase		LL-37		
Adhesion molecules	CD11(a,b,c,d)/CD18; α 4 β 1	Intracellular adhesion molecule-1			CD11c/CD18, CD86, CD40, major histocompatibility complex/HLA class II
Receptors	fMLP-R, LTB4-R, PAF-R, TLR-1, -2, -4, -6		TLR-1, -2, -4, -6, Fc ϵ R		
Others	ROS, RNS	ROS, RNS	LCT4, PAF, prostaglandin D ₂		

Dendritic Cells in Periodontitis Lesions



- Conductors of the immune response
- Immature cells, dendritic cells are scattered throughout the body in nonlymphoid organs
- Activated dendritic cells migrate to draining lymph nodes
- Dendritic cells, including Langerhans cells and dermal dendritic cells, are found in gingival tissue, and mature CD83+ dendritic cells are present in tissues from patients with periodontitis

Eosinophils in Periodontitis Lesions



- Effector cells of allergic responses and also function in elimination of parasites
- During allergic inflammation, eosinophils release granule contents, as well as inflammatory mediators including lipid mediators such as leukotriene C4 and platelet-activating factor, which may cause dysfunction and injury to other cells
- Presence of activated eosinophils and high IgE titers in GCF of patients with adult periodontitis was revealed

Natural Killer Cells (NKT Cells) in Periodontitis Lesions



- capacity to rapidly produce a variety of cytokines upon T cell receptors (TCR) engagement

Adaptive Host Response in Periodontitis



Parts of the adaptive host response in periodontitis



- (1) The nature of the lymphocyte type (T and B cells),
- (2) Antigen recognition by TCRs,
- (3) Cytokine profiles of T helper (Th) cells, and
- (4) Autoimmune reactions that may influence the adaptive host response in periodontitis

Antigen Presenting Cells (APCs) in Periodontitis Lesions



- **T-cells in Periodontitis Lesions**
- **B Cells in Periodontitis Lesions**

professional APCs



- Langerhans cells
- Macrophages
- Dendritic cells are



- Plasma cells are the most common cell type and represent about 50% of cells, while B cells comprise about 18%.
- The proportion of B cells is larger than that of all T cells,
- Th cells occur in larger numbers than cytotoxic T cells.
- PMN cells and macrophages are found in fractions of <5% of all cells

T-cells in Periodontitis Lesions



- T cells are functionally divided into helper T, regulatory T, and cytotoxic T cells
- After activation by antigen-presenting cells, helper T cells begin to secrete a range of cytokines
- classified as T helper type 1 or type 2 cells based on the cytokine profile they secrete
- T helper type 1 cells secrete mainly IL-2, interferon- γ (IFN- γ), and lymphotoxin- α
- T helper type 2 cells secrete mainly IL-4, IL-5, IL-10, and IL-13

B Cells in Periodontitis Lesions



- the proportions of plasma cells and B cells appear to be larger in lesions obtained from sites of severe periodontitis than in lesions from areas with moderate or mild periodontitis.
- The fact that plasma cells develop from B cells and that these two groups of cells dominate in periodontitis lesions indicates that specific attention should be given to the role of B cells in periodontitis



- *B-1 cells, or unconventional B-lineage cells, are divided into B-1a and B-1b. The B-1a cells express the surface marker CD5, while B-1b cells do not*
- *B-2 cells, i.e., conventional B cells, are the traditional and representative B cells of the adaptive immune system*
- Elevated levels of B-1a cells have been demonstrated in both periodontitis lesions and peripheral blood of subjects with severe forms of periodontitis

Tissue Destruction



- Plasma cells also produce cytokines such as TNF- α , IL-6, IL-10, and transforming growth factor (TGF)- β .
- TNF- α regulates the turnover of extracellular matrix by inducing the expression of MMPs, while TGF- β down-regulates the synthesis and secretion of these MMPs and promotes the production of their inhibitors (tissue inhibitors of MMPs or TIMPs).
- Plasma cells located in close proximity to blood vessels express the vascular endothelial growth factor, which in turn stimulates angiogenesis and MMP activation

Connective Tissue Breakdown in Periodontal Disease



MMP



- Metalloproteases, are endopeptidases that contain an active site Zn^{2+} (hence, the prefix “metallo”) and are divided into subfamilies or classes based on evolutionary relationships and structure of the catalytic domain
- MMPs comprise a family of currently 25 related



- The evidence for the role of MMPs in periodontal destruction is strong and has been supported over many years by a number of findings, including the production of elevated levels of collagenase by diseased gingival tissues in culture and the presence of MMP messenger RNA in cells of the periodontal lesion, such as periodontal ligament and gingival fibroblasts, as well as keratinocytes, endothelial cells, osteoblasts and even osteoclasts
- Most of the findings supporting the production of MMPs by these cells have been obtained using cell culture systems



- Inflammatory cells, particularly neutrophils, are thought to play a particularly important role in the MMP-mediated periodontal destructive lesion
- MMP protein in periodontal lesions and GCF of patients with different periodontal conditions (Similar results were reported in patients with peri-implantitis)



It has been suggested that periodontal destruction is an outcome of the imbalance between MMPs and their inhibitors

- The imbalance between MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) is considered to trigger the degradation of extracellular matrix, basement membrane, and alveolar bone



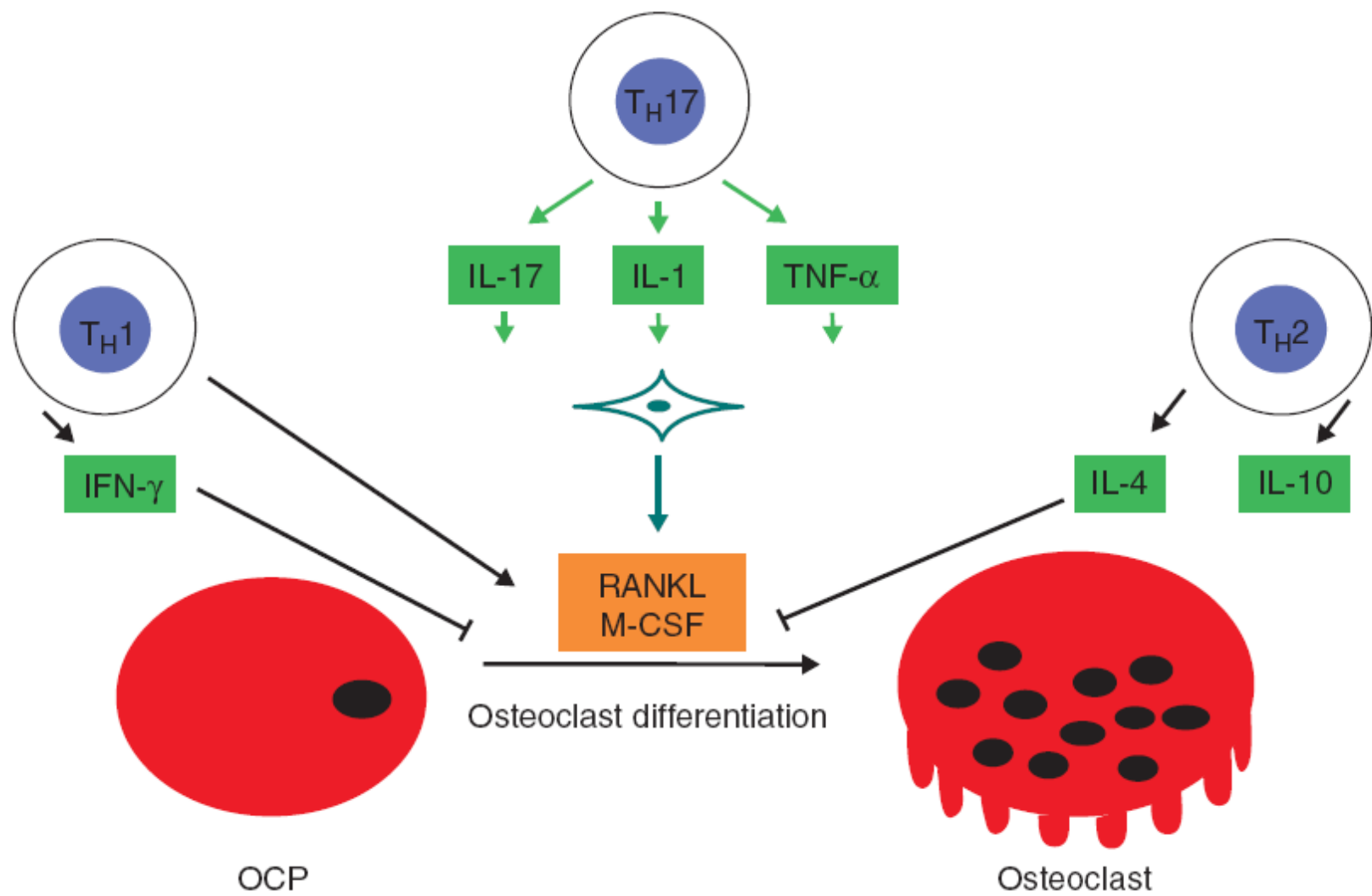
- non-antimicrobial activities of low-dose tetracycline and tetracycline analogs via the inhibition of MMP-8 and -13 protease mechanisms

Designation ^a	Common name	Other name(s)
MMP-1	Collagenase-1	Fibroblast collagenase, interstitial collagenase
	Mcol-A, Mcol-B	
MMP-2	Gelatinase-A	72-kD gelatinase, 72-kD type IV collagenase
MMP-3	Stromelysin-1	Transin-1
MMP-7	Matrilysin	PUMP
MMP-8	Collagenase-2	Neutrophil collagenase
MMP-9	Gelatinase- B92-kD	Gelatinase, 92-kD type IV collagenase
MMP-10	Stromelysin-2	Transin-2
MMP-11	Stromelysin-3	
MMP-12	Metalloelastase	
MMP-13	Collagenase-3	Rat collagenase

MMP-14	MT1-MMP	Membrane-type MMP
MMP-15	MT2-MMP	
MMP-16	MT3-MMP	
MMP-17	MT4-MMP	
MMP-18	RASI-1	
MMP-19	Enamelysin	
MMP-20		
MMP-21		
MMP-22		
MMP-23	CA-MMP	
MMP-24	MT5-MMP	
MMP-25	Leukolysin	MT6-MMP
MMP-26	Endometase	Matrilysin-2
MMP-27		
MMP-28	Epilysin	

Mechanisms of Alveolar Bone Destruction





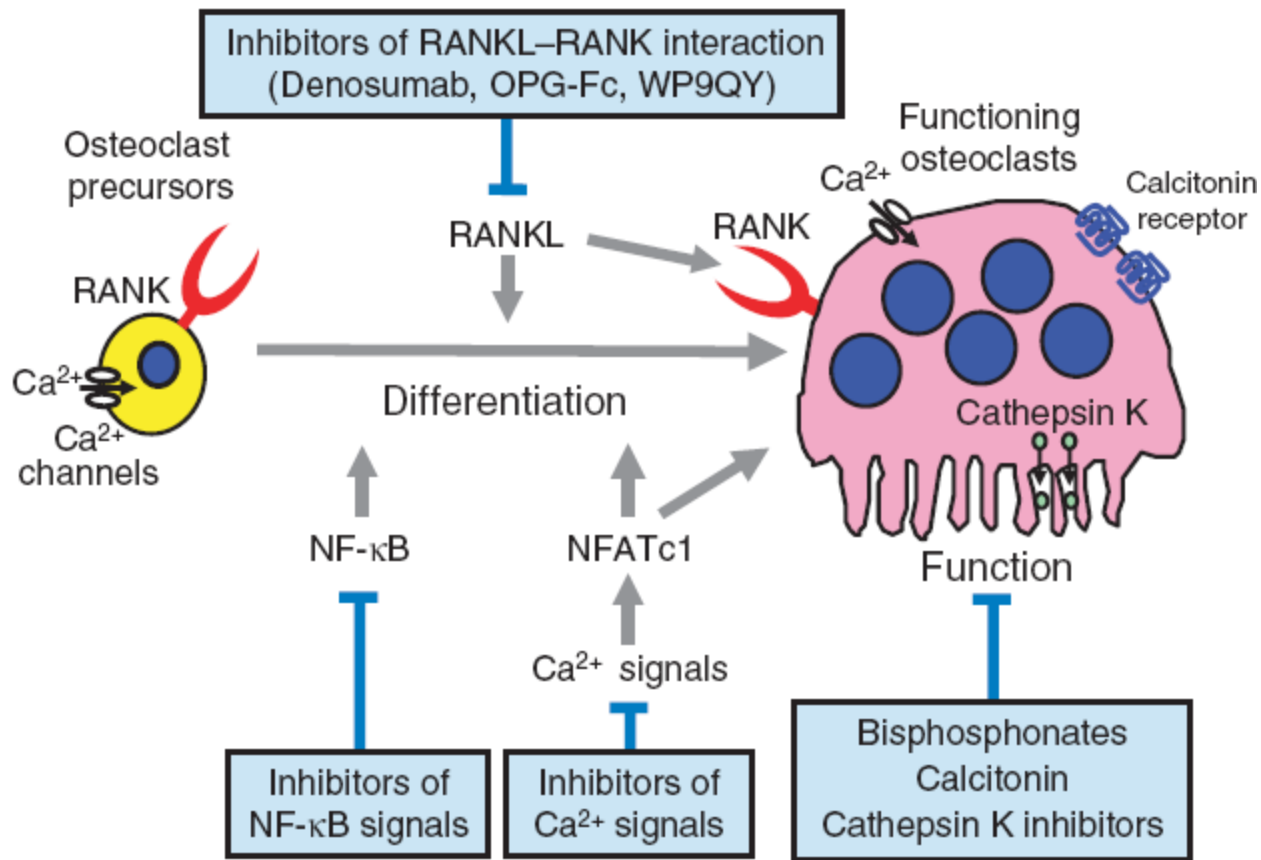


Table I. Immune factors promoting bone resorption.

<i>Factor</i>	<i>In inflamed gingiva</i>	<i>Secreted by</i>	<i>Action on bone</i>	<i>Action on immunity</i>
1,25 dihydroxy vitamin D ₃	Not done	Monocytes, macrophages	Promotes fusion of osteoclast progenitors, osteoclast differentiation	Suppresses monocytes; decreases T cell help for B cells
Reactive oxygen intermediates (ROI)	Transient	Neutrophils, macrophages, osteoclasts	Formation and activation of osteoclasts	Neutrophil oxidative bactericidal activity
Nitric oxide (NO)	Transient	Macrophages with LPS, IL-1, TNF or IFG	High amounts: stimulates resorption and inhibits osteoblast proliferation	Potent antimicrobial; levels enhanced by IFG
IL-8 (chemokine)	Yes	Macrophages	Chemotactic for neutrophils; promotes neutrophil secretion of IL-1 β and TNF α	Chemotactic for neutrophils
IL-3	Not done	CD4 ⁺ T cells (Th1 and Th2), NK cells, mast cells	Promotes bone marrow stem cell differentiation to macrophage lineage, then CSF-1 can induce osteoclasts	Supports growth of hematopoietic cell growth; stimulates mast cell growth and histamine secretion
RANK-ligand*	Not done	T cells, osteoblasts	Promotes osteoclast differentiation	Activates mature dendritic cells, enhancing antigen presentation
IFG	Yes	Th1 cells, T _C cells, NK cells	In vivo promotes resorption; enhances effects on IL-1 or TNF inhibition of matrix formation	Enhances macrophage activity, increases MHC expression; induces class switch to IgG2a; inhibits Th2 cells

Cytokine group	Function	Examples
Pro-inflammatory cytokines	Primary innate immune responses and activation of inflammation	IL-1 family; TNF- α ; IL-12; IL-23; IL-32
gp130 signaling cytokines	Leukocyte differentiation and growth, acute-phase reactions	IL-6; IL-11; LIF; oncostatin M
T-cell regulatory cytokines	Balance of T-cell subsets, regulation of adaptive immunity, enhancement of inflammatory responses	TH ₁ cytokines: IFN γ ; IL-12; IL-15; IL-18 TH ₂ cytokines: IL-4; IL-5; IL-25; IL-33 TH ₁₇ cytokines: IL-17; IL-21; IL-23; IFN- γ ; IL-6 T _{reg} cytokines: IL-2; TGF- β , IL-10

Cytokine group	Function	Examples
Anti-inflammatory cytokines	Down-regulation of immune responses and inflammation	IL-10, IL-13, TGF- β , IL-1Ra, IL-1F5
Chemokines	Activation of neutrophil chemotaxis	CXCL8 (IL-8); MCP-1; MIP-1 α
Type 1 interferons	Antiviral immune responses	IFN- α ; IFN- β
Bone cell activators	Bone cell development and function	RANKL
Growth factors	Regulation of tissue function and turnover, fibrosis and repair	TGF- β superfamily (inc. BMP), EGF, FGF, PDGF, VEGF, HGF
Colony stimulating factors	Hematopoiesis, localized immune cell differentiation	G-CSF; GM-CSF, IL-3, IL-7
Adipokines	Metabolic regulation, immune regulation	Leptin, adiponectin, visfatin, TNF- α , IL-6

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IL-1	Yes	Macrophages, all cells except T lymphocytes and erythrocytes	High concentration: increase number of osteoclasts by precursor fusion; inhibit collagen synthesis	Costimulates CD4 ⁺ T cells; clonal expansion of B cells; attracts neutrophils and macrophages; increases adhesion molecule expression on endothelial cells
TNF α	Yes	Macrophages, T cells, NK cells	Blocks bone formation: inhibits collagen synthesis; stimulates resorption by inducing osteoblast release of PGE ₂ and M-CSF	Induces cytokine secretion; weight loss of chronic inflammation
TNF β (lymphotoxin)	Not done	Lymphocytes	Blocks bone formation: inhibits collagen synthesis ; with PGE ₂ stimulates resorption: calcium release, increases effect of IL-1	Enhances neutrophil and macrophage phagocytic activity
IL-6	Yes	T cells, gingival fibroblasts, osteoblasts if stimulated with IL-1 or TNF	Increases osteoclast number	Promotes B cells differentiation into plasma cells; stimulates antibody secretion
PGE ₂	Yes	Monocytes, macrophages; osteoblasts if stimulated with IL-1, TNF, TGF α or β	Promotes osteoclast fusion; blocks bone formation	Mast cell degranulation; increases vascular permeability in inflammation

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IFG	Yes	Th1 cells, T _C cells, NK cells	In vivo promotes resorption; enhances effects on IL-1 or TNF inhibition of matrix formation	Enhances macrophage activity, increases MHC expression; induces class switch to IgG2a; inhibits Th2 cells

Table II. Immune factors inhibiting bone resorption and/or promoting bone formation*.

<i>Factor</i>	<i>In inflamed gingiva</i>	<i>Secreted by</i>	<i>Effects on bone</i>	<i>Effects on immune system</i>
IL-1-Ra	Yes	Macrophages	Blocks resorption induced by IL-1 or TNF but not by vitamin D	Blocks T-cell proliferation
IL-1	Yes	Macrophages, all cells except T lymphocytes and erythrocytes	Low concentrations: stimulates osteoblast proliferation and collagen synthesis	Co-stimulates CD4 ⁺ T cells; clonal expansion of B cells; attracts neutrophils and macrophages; increases adhesion molecule expression on endothelial cells
PDGF	Not done	Macrophages	Promotes bone matrix synthesis; proliferation of connective tissue cells	Differentiation of glial cells (immune derived cells that support neurons); Indirect: wound healing
TGF β	Not done	Macrophages, Th cells, mast cells	Inhibits formation and differentiation of osteoclast precursors; inhibits osteoclast activity; stimulates bone matrix synthesis	Activates blood monocytes but suppresses tissue macrophage IFG, ROI and NO, increases IL-1-Ra; T cell development, activation; induces B cell class switch to IgA; anti-inflammatory; promotes wound healing and oral tolerance
IL-4	No	Th2 cells, NK cells, mast cells	Forces bone marrow precursors to differentiate as macrophages not osteoclasts	Co-stimulates B cells and class switch to IgG1 and IgE; upregulates B cell and macrophage MHC II; increases macrophage phagocytosis
IL-10	Not done	Th2 cells	Decreases resorption by inhibiting neutrophil and macrophage IL-1 and TNF gene expression and IL-1 and IL-6 secretion	Antiinflammatory; acts on macrophages to down-regulate MHC II, stop their activation of Th1 cells, stop NO, IL-1, IL-6. and TNF α

Table III. Bone regulators induced by responses of immune cells to periodontal bacteria*.

<i>Species</i>	<i>Bacterial component</i>	<i>Effect on bone</i>	<i>Cell acted on</i>	<i>Regulators induced</i>
<i>P. gingivalis</i>	Lipopolysaccharide	Resorption; stimulates collagenase; increases numbers of osteoclasts	Monocytes/macrophages	IL-1 β , TNF- α , IL-6, IL-8, GM-CSF, IFG, PGE ₂
	Polysaccharide Fimbriae	Resorption	Neutrophils	IL-1 β , TNF- α , IL-8
			Monocytes/macrophages	IL-1 β
			Monocytes/macrophages	IL-1 α , IL-1 β , TNF- α , IL-6, IL-8
	75-kDa surface protein 24-kDa outer membrane protein 12-kDa protein	Resorption; stimulates osteoclast formation	Neutrophils	IL-6
			Mononuclear cells	IL-6
			T cells	TNF- α , IFG
<i>A. actinomycetem-comitans</i>	Lipopolysaccharide	Resorption or inhibition resorption; increases osteoclast number, or blocks their increase	Monocytes/macrophages	IL-1 β
			Murine macrophages	IL-1-Ra, IL-1 α , PGE ₂
	Capsular polysaccharide	Resorption; stimulates osteoclast formation		Prostaglandins
	GroEL chaperone protein	Resorption	CD4 ⁺ T cells	IL-6
	14 kDa protein		CD4 ⁺ T cells	Inhibits release of IL-2, IL-4, IL-5 and IFG

