Genetics in dentistry 4

Caries

- Multifactorial disease with endogenous and exogenous factors

- predisposing factors:

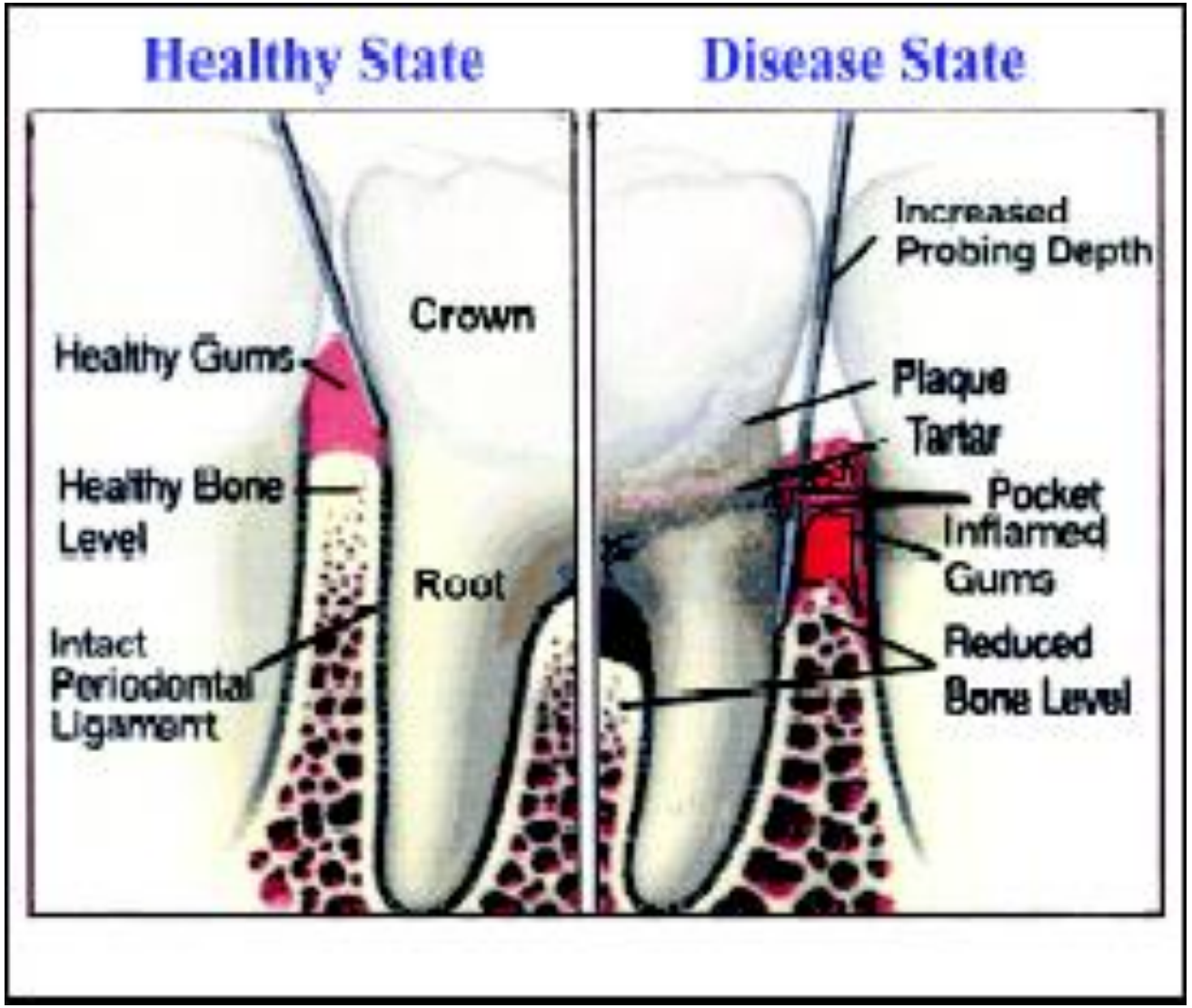
  - inheritance- relation to caries or caries resistance
  - diet rich in carbohydrates
  - fluorine content in drinking water-resistant dental caries have higher fluorine content in hard tissues
  - personal hygiene-enhanced plaque formation by a lack of hygiene

  - tooth- shaped teeth with fissures – more caries

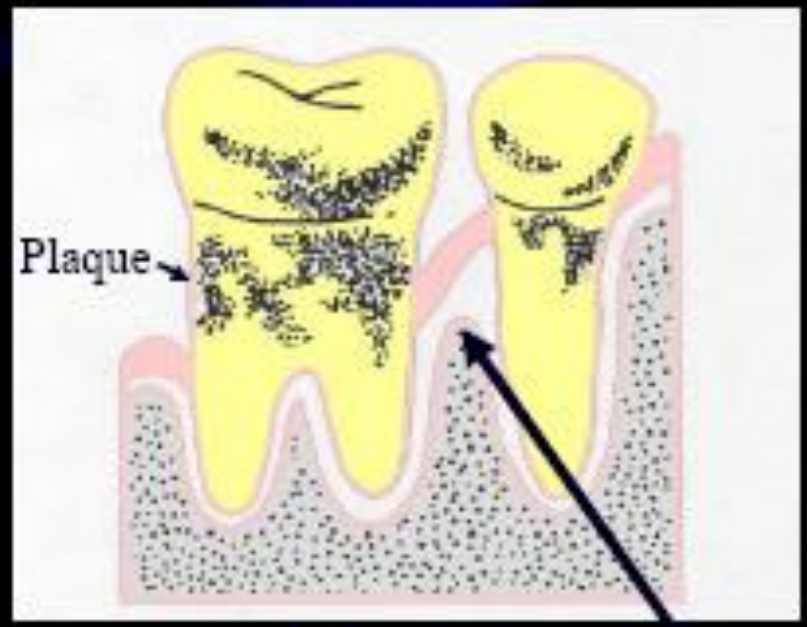
Caries (caries dentium)

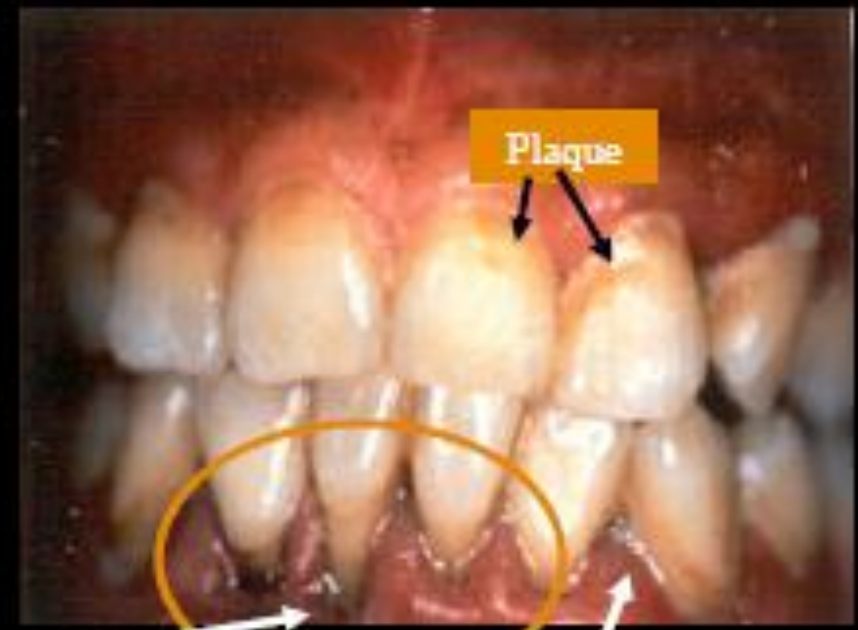
- dekalcification of hard dental tissues
- bacteria-Lactobacillus acidophilus and Streptococcus that break down sugars, food debris, forming organic acids causing decalcification-proteolytic enzymes break down organic ingredients
- plaque-thin coating of bacteria, mucus and desquamated epithelial cells
- other effects-type food composition of saliva, pH
- Decalcification of enamel-whitish chalk stain, softening, a cavity filled with food residue and bacteria-spreading further decay process affects dentin, spreads thrue the dentin tubules until marrow - occurs pulpitis
- **Mutations in the genes that cause structural failure of enamel formation (AMLEX, Enam, KAL4 and MMP20) lead to the formation of the enamel more susceptible to tooth decay.**

Periodontal disease



Periodontal Disease





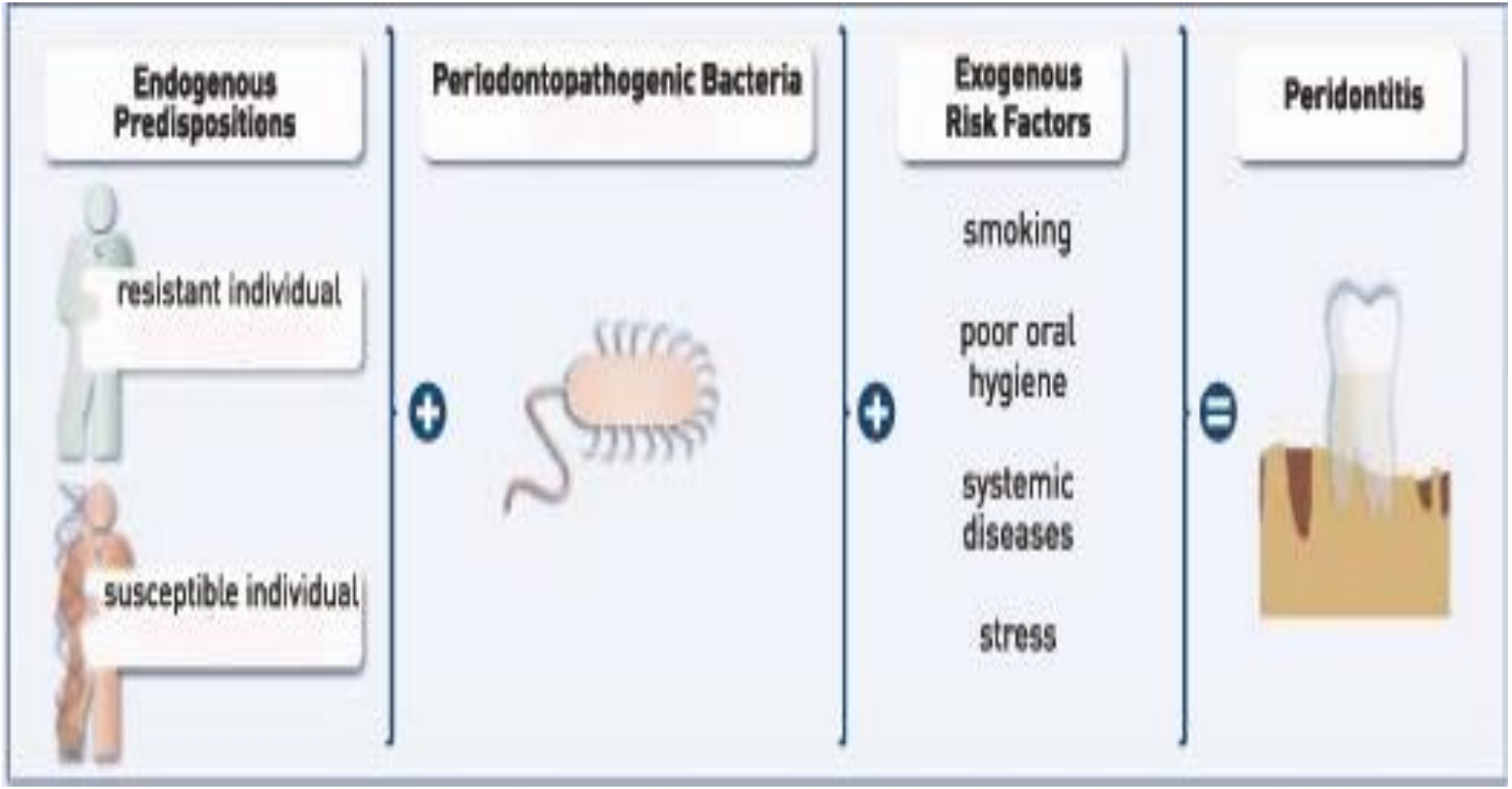
Periodontitis

- Advanced gum inflammation
- Bone loss
- Destruction of ligaments

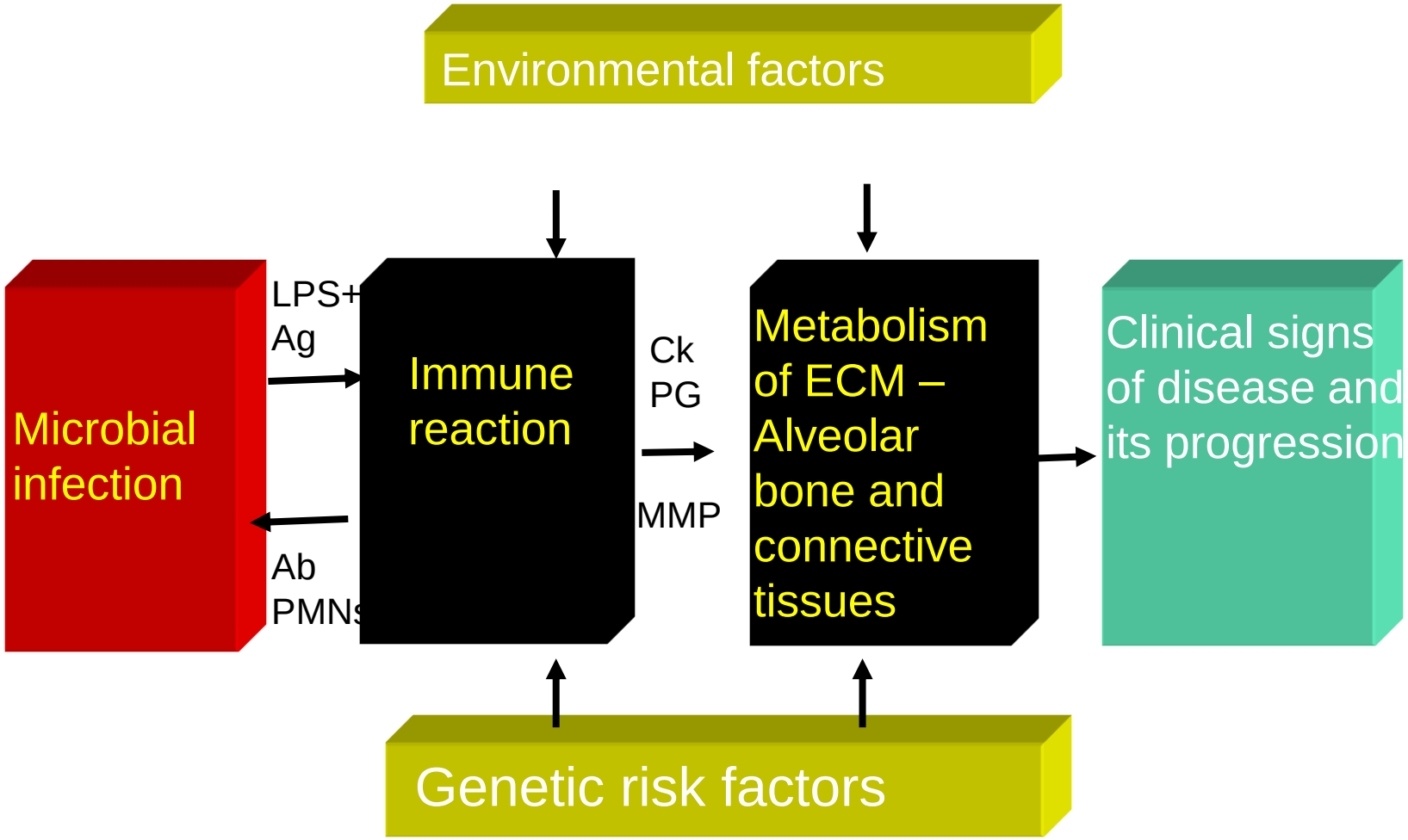
Gingivitis

- Inflamed Gums

Multifactorial disorder



Conception of periodontal disease



Page & Kornman

Syndromic Forms of Periodontitis

- Severe periodontitis presents as part of the clinical manisfestations of several monogenetic syndromes.

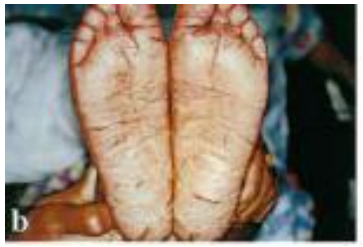
- Significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis.

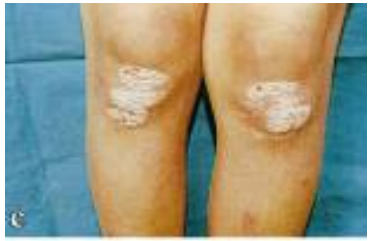
TABLE 2

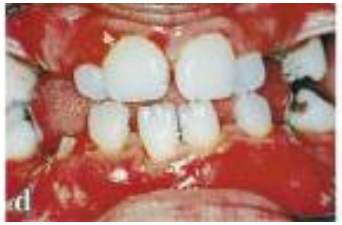
Examples of Syndromic Forms of Periodontitis in Which Inheritance is Mendelian and Due to a Genetic Alteration at a Single Gene Locus

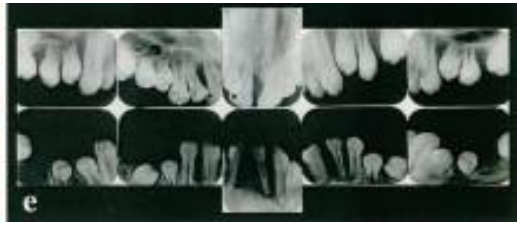
Condition	Biochemical/Tissue Defect	Inheritance	OMIM
Papillon-Lefèvre syndrome	Cathepsin C	AR	245000
Haim-Munk syndrome	Cathepsin C	AR	245100
Ehlers-Danlos syndrome type 4	Collagen	AD	130050
Ehlers-Danlos syndrome 8	Collagen	AD	130080
Cyclic neutropenia	neutrophil elastase	AD	162800
Chronic familial neutropenia	Defect unknown	AD	162700
Chediak-Higashi syndrome	lysosomal trafficking regulator gene	AR	214500
Congenital disorder of glycosylation type IIc	GDP-fucose transporter-1	AR	266265
Leukocyte adhesion deficiency type 1	Leukocyte chain adhesion molecule CD18	AR	116920

Papillon LeFevre Syndrome









- Clinically characterized by:
  - Palmoplantar hyperkeratosis
  - **Severe early onset periodontitis** that results in premature loss of the primary and secondary dentition (distinguishes PLS from other plamoplantar keratoderma)
- Prevalence 1/ 4million
- No gender or racial predilection

CTSC gene encodes for Cathepsin C protease

- **CTSC gene lies on chromosone 11q14-q21; seven exons encoding for lysosomal protease cathepsin C.**

- **It is expressed at high levels in a variety of immune cells including polymorphonuclear leucocytes, macrophages, and in epithelial regions commonly affected by PLS, including the palms, soles, knees, and oral keratinized gingiva (RT-PCR) (Hart et al., 1999).**

- **Cathepsin C is a protease enzyme that processes and activates a number of granule serine proteases critical to immune and inflammatory responses of myeloid and lymphoid white blood cells**

Mutations in CTSC gene

- **Mutations in Cathepsin C (CTSC) gene are implicated for PLS**

- **For example:**
  - **One exon 1 nonsense mutation (856C→T): introduces a premature stop codon at amino acid 286.**
  - **Three exon 2 mutations:**
    - **single nucleotide deletion (2692delA) of codon 349: introduces a frameshift and premature termination codon,**
    - **2 bp deletion (2673-2674delCT): introduces a stop codon at amino acid 343, and**
    - **G→A substitution in codon 429 (2931G→A): introduces a premature termination codon.**

- **Truncated or altered conformation of the protein may not be transported to the organelle and may not be able to activate protein kinases**

- **In other words, Cathepsin C activity in these patients is nearly absent**

Polymorphism Studies on Periodontitis

- Host response is predominantly influenced by genetic make-up.
- Several features of host's innate immune response may contribute to susceptibility to AgP and include epithelial, connective tissue, fibroblast, and PMN defects.
- Aspects of the host inflammatory response namely cytokines are crucial variants influencing host respone in periodontitis.

Immunological Polymorphisms

- MHC or HLA genes determine our response to particular antigens.
- Japanese study of AgP pts found a significant association for pts with atypical BamH1 restriction site in the HLA.DQB gene (Takashiba et al. 1994).
- Hodge & Kinane (1999) found no assoc. in caucasian AgP pts and this restriction site.

IL-1 Gene Polymorphisms

- In 1997 Kornman et al found an association between polymorphisms in genes enconding for IL-1a(-889) and IL-1B(+3953) and an increased severity of periodontitis.
- The specific genotype of the polymorphic IL-1 cluster (called PST-periodontitis susceptibility trait) was associated with severity of PD in only non-smokers, and distinguished individuals with severe periodontitis from those with mild disease.

Genetic control of IL-1: Genes and Locus of SNPs associated with controlling IL-1 biological activity

Genes	Polymorphism Locus	Current Locus assessed with test	Controlled product
IL-1A	Allele 2 -889	Allele 2 IL-1A +4845	IL-1 alpha
IL-1B	Allele 2 +3953	Allele 2 IL-1B +3954	IL-1 beta
IL-1RN			Protein receptor antagonist (impedes IL-1 alpha and beta)

Genetic Susceptibility Test for periodontitis: tests for the presence of at least one copy of allele 2 at the IL-1A +4845 loci and at least one copy of allele 2 at the IL-1B +3954 locus.

**IL-1A +4845 is being used because it is easier to identify than IL-1A -889 and it is essentially concordant with it.*

*** IL-1B +3953 has been now renumbered as IL-1B +3954 because the current convention indicates that the numbering of the transcription should begin at +1 instead of zero.*

Interleukin 1

- A proinflammatory multifunctional cytokine.
- Enables ingress of inflammatory cells into sites of infection
- Promotes bone resoroption
- Stimulates eicosanoid (PGE2) release by monocytes and fibroblasts
- Stimulates release of MMP’s that degrades proteins of the ECM.
- Forms IL-1α and IL-1B

IL-1 as modulator for Periodontitis

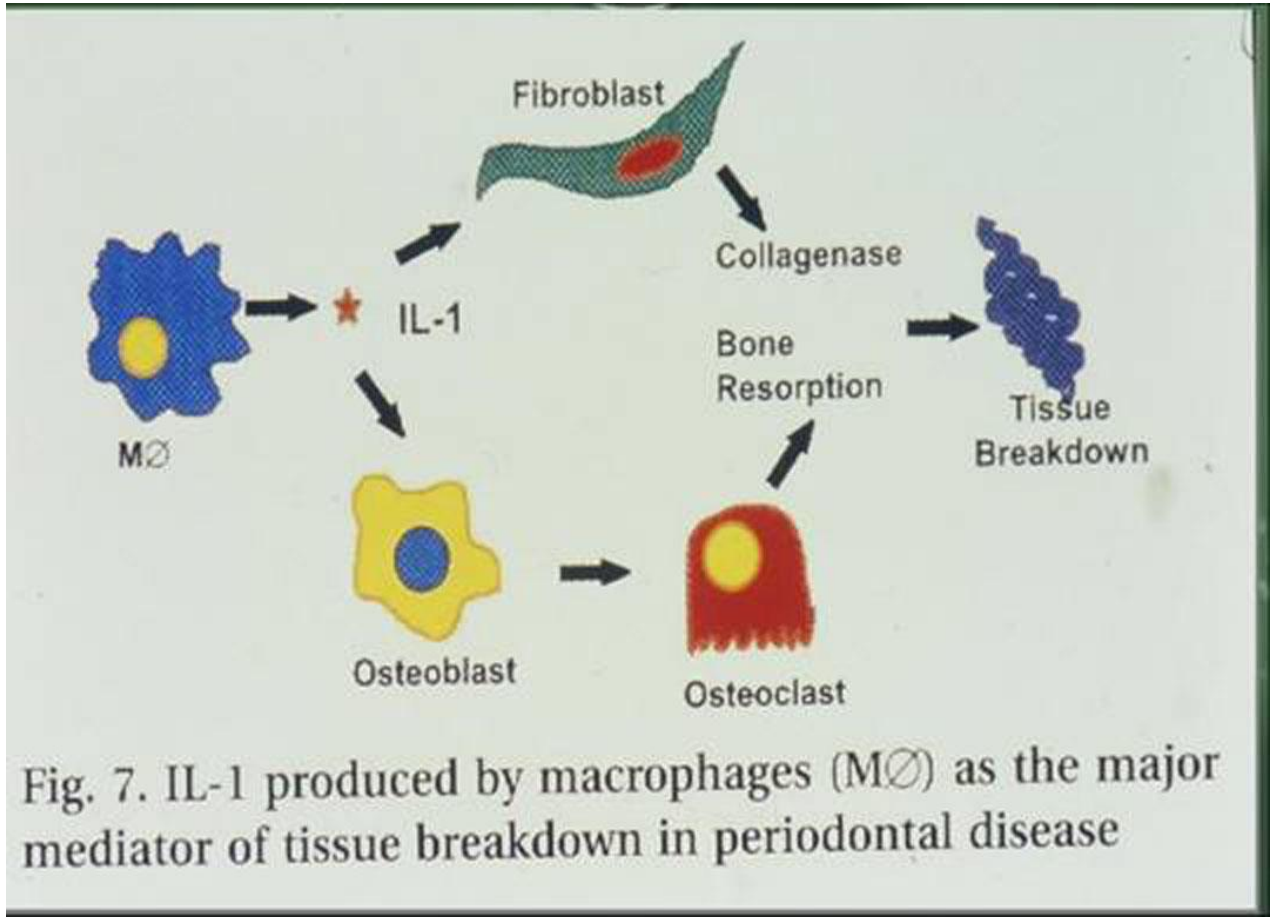


Fig. 7. IL-1 produced by macrophages (MØ) as the major mediator of tissue breakdown in periodontal disease

- **Kornman et al. (1997)**
  - Genotype-positive non-smokers → 18.9 times more likely to have severe periodontitis (when compared with genotype-negative non smokers)
  - No significant association between periodontal status and genotype detected when smokers were included in the statistical analysis.
  - 86% of the severe periodontitis patients were accounted for by either smoking or IL-1 genotype
  - Presence of allele 2 at IL-1A -889 or IL-1B +3953 did not significantly increase the risk of periodontitis among smokers and non-smokers.

+IL-1 genotype and IL-1 protein

- The specific periodontitis-associated IL-1 genotype consists of a variant in the IL-B gene that is associated with high levels of IL-1 production.(Poiciot et al 1992)

- Patients positive for composite IL-1A (+4845) and IL-1B (+3954) periodontitis-associated genotype has higher level of IL-1B in GCF, but not in gingival tissue before and after treatment (Kornmann et al 1999)

- Carriage of allele 2 in the (-889) locus resulted in an almost four fold increase of IL-1 protein levels in chronic periodontitis patients (Shirodaria et al., 2000)

- **Attachment loss**

  Kornman et al. (1997)

  - Reported 18.9 times greater risk (OR=18.9) of finding severe periodonitis among non-smokers genotype-positive.

  McDevitt et al. (2000)

  - Among non-smokers or former smokers, genotype positive individuals had 3.75 greater odds of having moderate to advanced periodontitis than genotype-negative.

- **Genotype and tooth loss**

  McGuire and Nunn (1999)

  - Genotype-positive individuals had a 2.7 greater chance than genotype-negative patients of losing a tooth.

  - Combined effect of being genotype-positive and heavy smoker increased the odds of tooth loss to 7.7 compared with genotype-negative non-smokers.

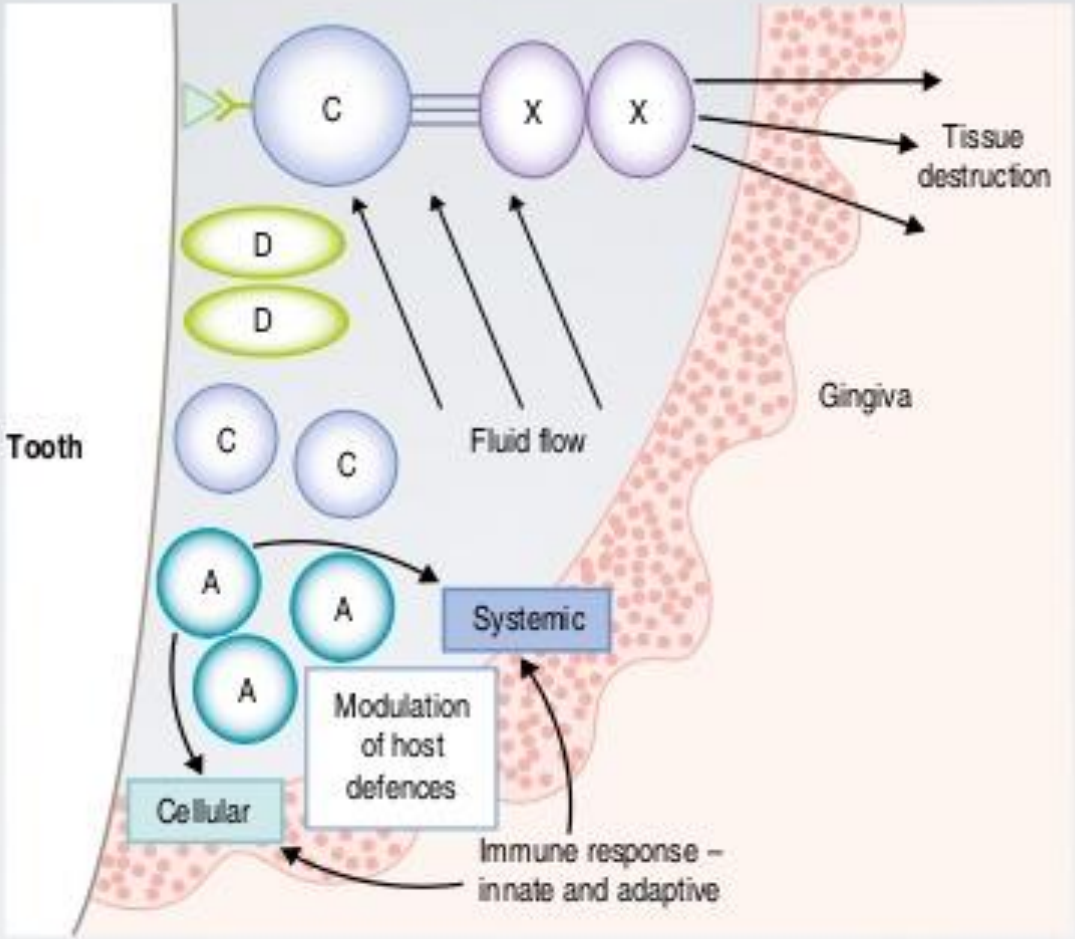
  - PST can be helpful in treatment planning.

Prevalence of genotype positive individuals in different ethnic groups

- Frequency of many genetic alleles varies between ethnic groups, therefore, it is necessary to establish allele frequencies in populations before genetic test can be evaluated and used.

  - **Caucasions:**
    - 29% of northern european caucasions were genotype positive (Kornman et al., 1997)
  - **African Americans:**
    - 14.5% of non-diseased individuals and 8% of patients with localized form of aggressive periodontitis were genotype-positive. (Walker et al., 2000)
  - **Chinese:**
    - 2.3% of sample of 132 mod-severe periodontitis cases were genotype-positive (Armitage et al., 2000)
  - **Hispanics:**
    - 26% of hispanic individuals with peridontitis were genotype-positive (Lopez et al., 2005)

Take home message: Dissimilarity in the prevalence of genotypes in different ethnic groups precludes extrapolating data from one group to another.



Pathogenic synergy in the aetiology of periodontal diseases. Bacteria capable of causing tissue damage directly (e.g. species X) may be dependent on the presence of other cells (e.g. organisms C and D) for essential nutrients or attachment sites so that they can grow and resist the removal forces provided by the increased flow of GCF. Similarly, both of these groups of bacteria may be reliant for their survival on other organisms (e.g. A and C) to modulate the host defences. Individual bacteria may have more than one role (e.g. organism C) in the aetiology of disease.

Microbial factors

- *Porphyromonas gingivalis*
- *Bacteroides forsythus*, newly *Tanarella forsythensis*

- *Actinobacillus Actinomycemcomitans*
- …….

BUT

- The bacteria themselves are not able to cause disease:
  - A wide range of host susceptibility
  - Differences in prevalence and extent between the teeth

Dental plaque biofilm infection

- Ecological point of view
  - Ecological community evolved for survival as a whole
  - Complex community of more than 400 bacterial species
- Dynamic equilibrium between bacteria and a host defense
  - Adopted survival strategies favoring growth in plaque
  - “Selection” of “pathogenic” bacteria among microbial community
  - Selection pressure coupled to environmental changes
  - Disturbed equilibrium leading to pathology
  - Opportunistic infectio

Reaction of the organism to bacterial infection

- Gate input - usually mucosal surfaces (violation of integrity)
- The fate of the host depends on:
  - Immunity (largely genetically determined)
  - Pathogenicity of bacteria (invasive ability, production of toxins, the ability to resist the defense mechanisms of the host)

non-invasive - multiply the point of entry into the body
threatening in case of toxin production
(defense - only neutralizing Pt)

Invasive - penetrate into the organism (x extracellular intracellular)
(defense are Pt, complement, phagocytosis vs. macrophages)

  - The size of the infectious dose

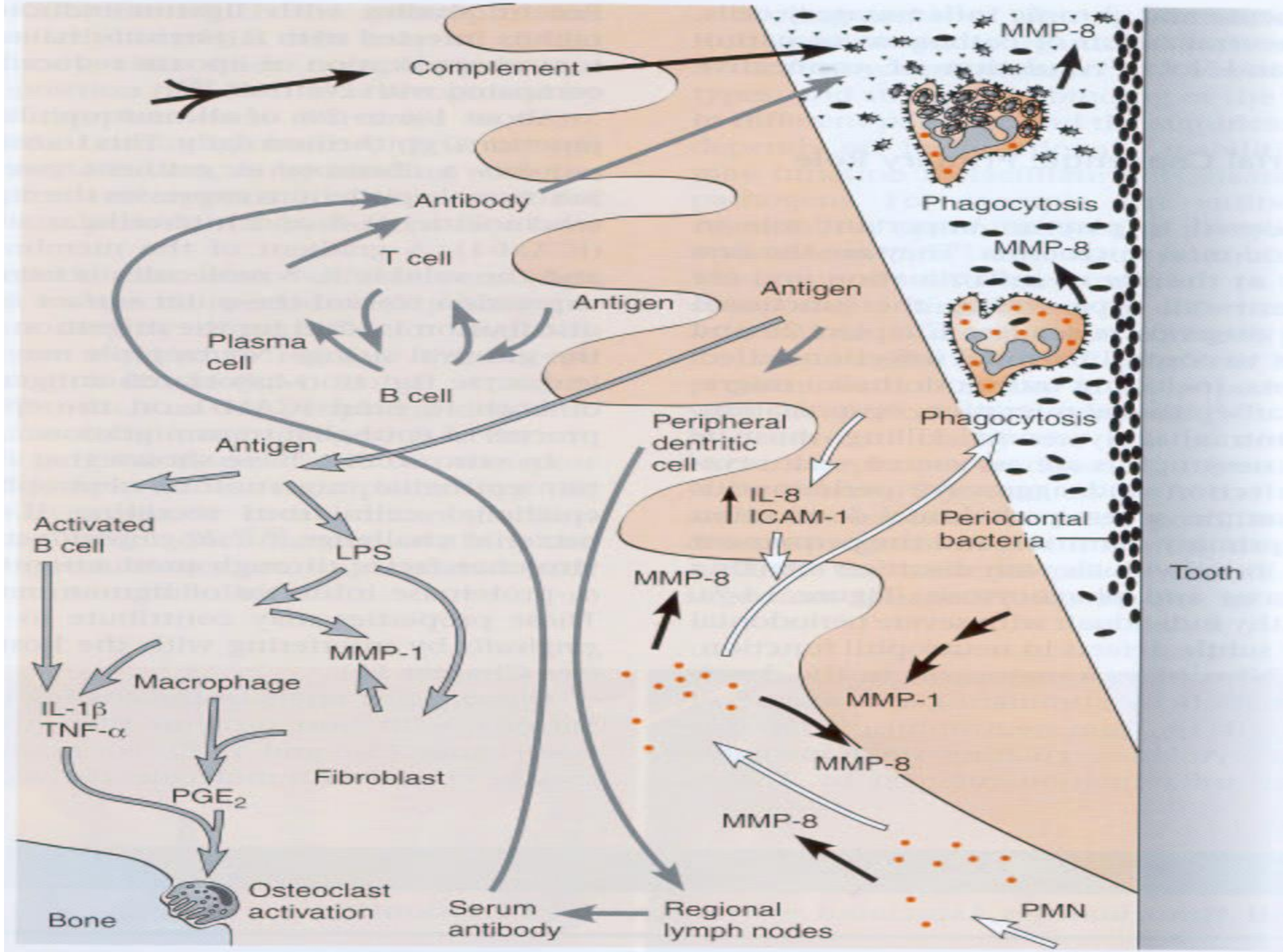
Identifying virulence factors

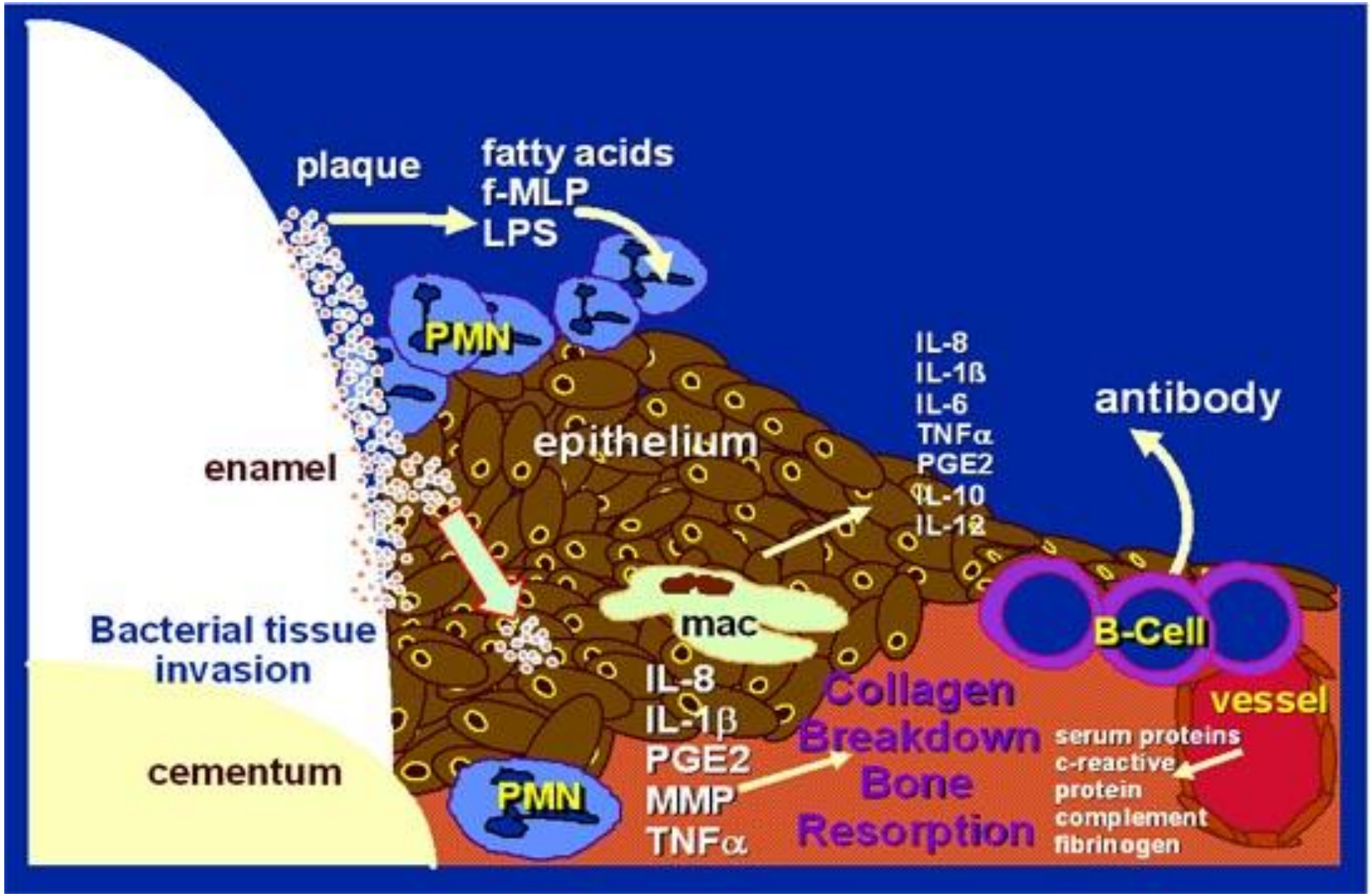
- Microbiological and biochemical studies
    - *In vitro isolation and characterization*
    - *In vivo systems*
- Genetic studies
    - Study of genes involved in virulence
    - Genetic transmission system
    - Recombinant DNA technology
        - Isogenic mutants
        - Molecular form of Koch’s postulates (Falkow)

Koch’s postulates

- A Molecular form of Koch’s postulates
  - The phenotype should be associated with pathogenic species (strains)
  - Specific inactivation of genes associated with virulence should lead to a decrease in virulence
  - Complementing inactivated genes with the wildtype genes should restore full virulence

Falkow, 1988


Complement
MMP-8
Antibody
Phagocytosis
T cell
MMP-8
Antigen
Antigen
Plasma cell
B cell
Phagocytosis
Peripheral dentritic cell
Antigen
IL-8
ICAM-1
Periodontal bacteria
Activated B cell
LPS
Tooth
MMP-8
MMP-1
Macrophage
MMP-1
IL-1β
TNF-α
MMP-8
Fibroblast
PGE2
MMP-8
Osteoclast activation
Bone
Serum antibody
Regional lymph nodes
PMN


plaque
fatty acids
f-MLP
LPS
PMN
epithelium
enamel
IL-8
IL-1ß
IL-6
TNFα
PGE2
IL-10
IL-12
antibody
Bacterial tissue
invasion
mac
B-Cell
IL-8
IL-1β
PGE2
MMP
TNFα
Collagen
Breakdown
Bone
Resorption
vessel
serum proteins
c-reactive
protein
complement
fibrinogen
cementum
PMN

TLRs

- First described as a gene for type I transmembrane receptor

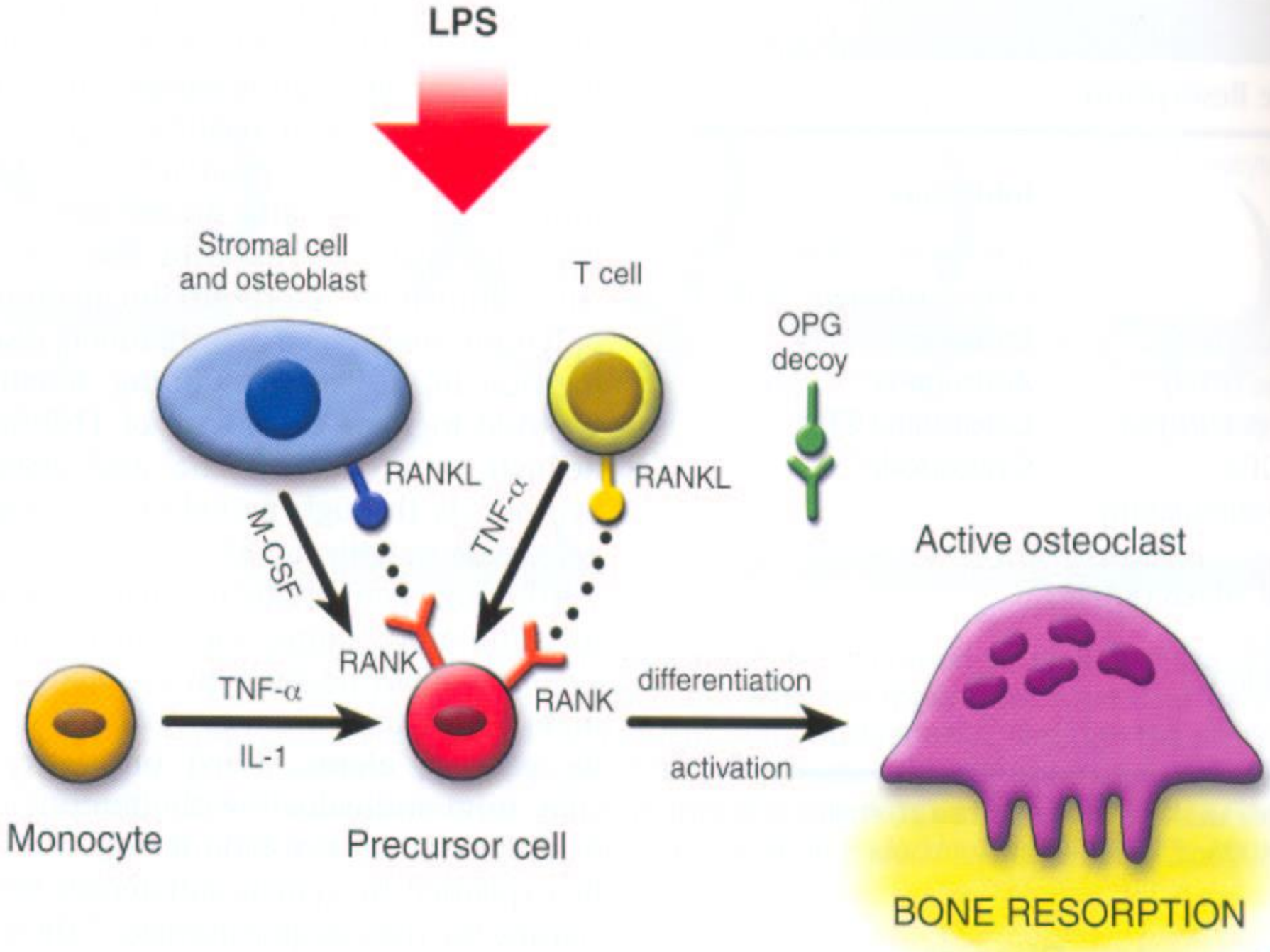
$\rightarrow$ important role in dorzoventral embryonic development of Drosophila

$\rightarrow$ absence of tolls has led to a serious brake-down of defense against fungi and bacteria G+

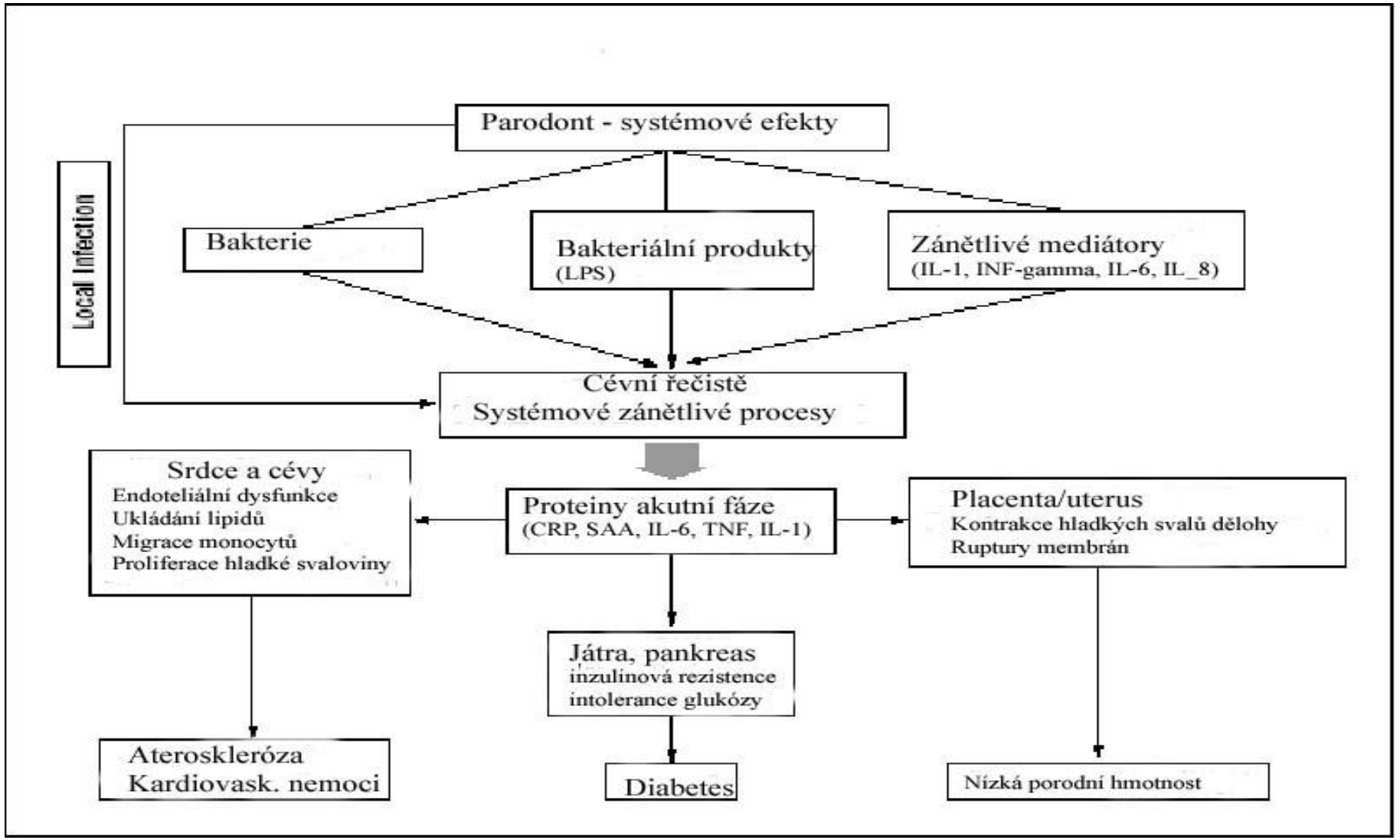
$\Rightarrow$Mammalian homologues - similar role??

TLRs receptors and ligands in periodontal disease

PRR	PAMP	Periodontal Pathogen
TLR-2	Lipoproteins	*Bacteroides forsythus**
	Atypical LPS	*P. gingivalis, C. ochracea*
	Outer membrane proteins	Oral treponemes
	Fimbriae	*P. gingivalis*
	Nonendotoxic glycoprotein	*P. intermedia*
TLR-4	HSP-60 (GroEL)	*P. gingivalis*
	LPS	*A. actinomycetemcomitans, F. nucleatum*
TLR-9	CpG-containing DNA	*A. actinomycetemcomitans, P. gingivalis, P. micros*


LPS
Stromal cell
and osteoblast
T cell
OPG
decoy
RANKL
RANKL
M-CSF
TNF-α
RANK
RANK
TNF-α
IL-1
differentiation
activation
Active osteoclast
Monocyte
Precursor cell
BONE RESORPTION

Local problem?



Periodontitis

- follows the loss of pulp vitality - the dead tooth

- Infection is usually odontogenic
- Trauma - one-time or repetitive microtrauma
- Acute periodontitis (apical)
- Hyperemia and serous exudation
- Suppuration, osteoclastic bone remodeling

- Strong pain at all stages, swelling in the later stages

- **Relationship of polymorphisms in genes IL1B, IL1RN, FcγRIIIb, VDR and TLR4 with an aggressive type of periodontitis.**

Periodontitis

Chronic periodontitis (periapical)

- Secondarily from acute periodontitis
  Primarily chronic (more frequent)
- Forms:
  Granulomatous
  Granulomatous progressive - fistula (mucosal and cutaneous)
  Diffusion - dismantled and alveolar bone

- granulation tissue
  macrophages
  Possibility of creating radicular cysts

- The type of chronic periodontitis is associated with polymorphisms in genes for IL1B, IL1RN, IL6, IL10, VDR, CD14, TLR4 and MMP1.

- Meta-analysis of published data have associated variants ofpolymorphisms IL1A-889, IL1B 3954, IL1B-511, TNFA-308 and IL6-174 to aggressive and chronic periodontitis.