Etiopathogenesis -2 Host responses to oral biofilms

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Reading Resources

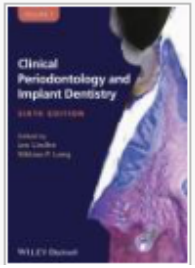
BOOK

Periodontology at a Glance

Clerehugh, Valerie. ; Tugnait, Aradhna. ; Genco, Robert J. ;
Somerset : Wiley; 2013

Available Online >

▼ Clinical Periodontology and Implant Dentistry, 2 Volume Set



by Niklaus P. Lang, , Jan Lindhe, , and Niklaus P Lang

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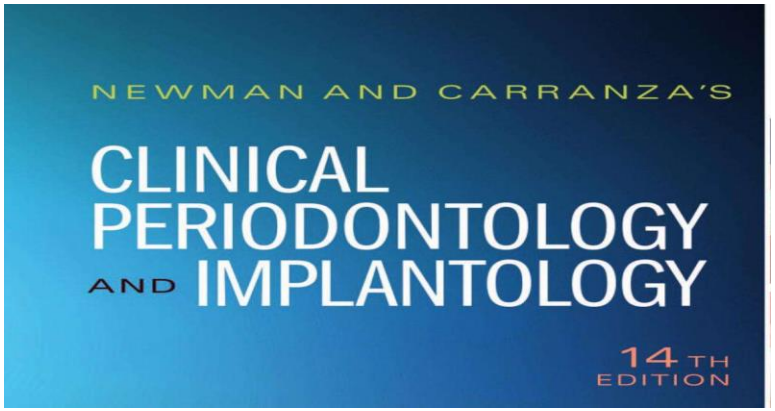


Learning outcomes

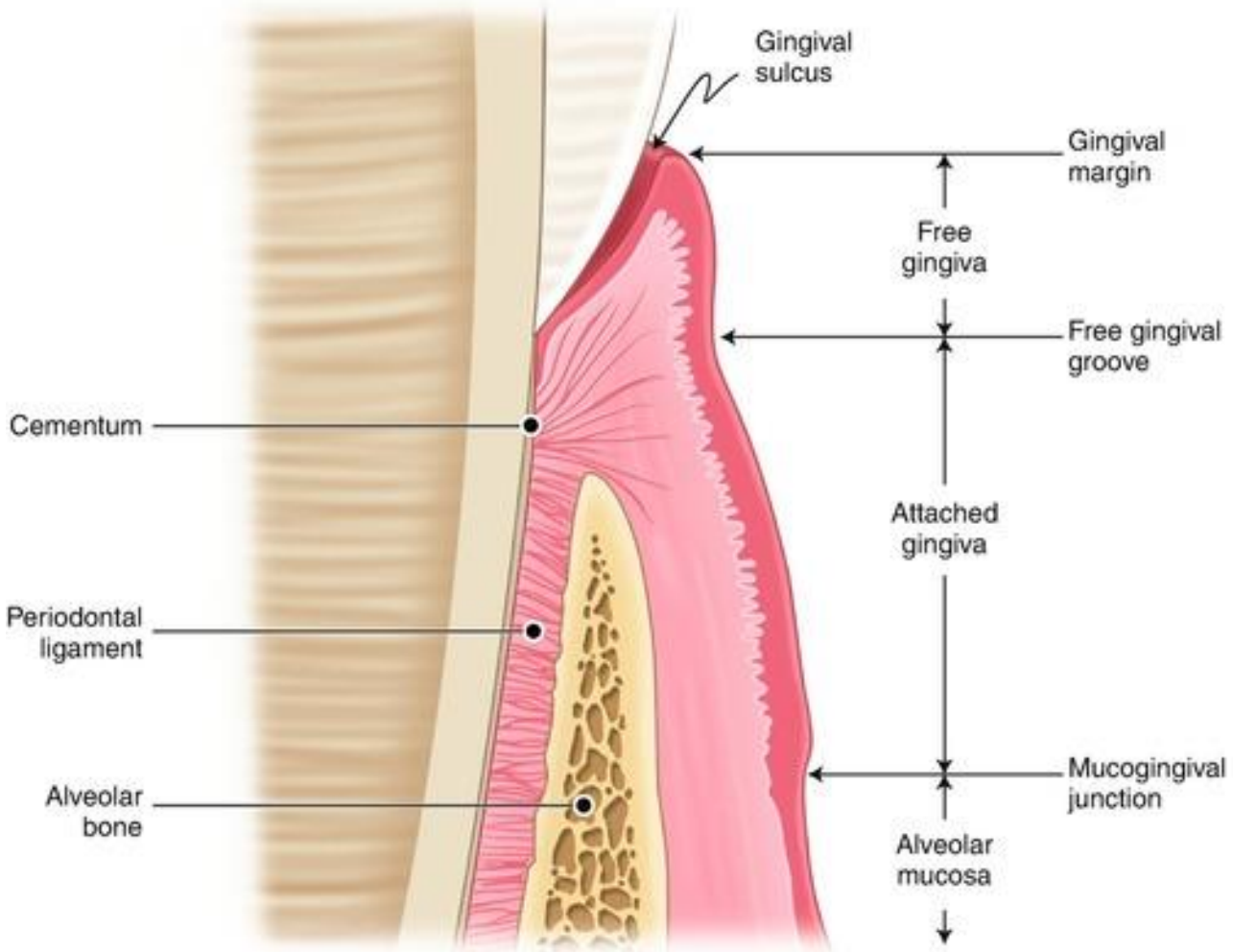
Recognize periodontal health and disease;

Understand the peculiarities of the periodontal niche;

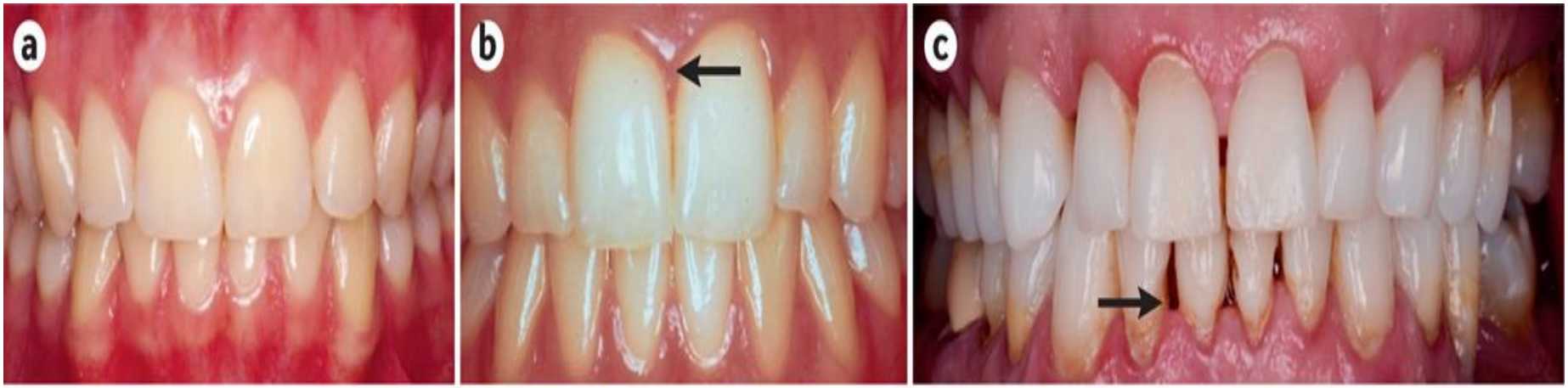
Identify the key components of the host response in the dento-gingival area;

Understand the histopathology of periodontal diseases.

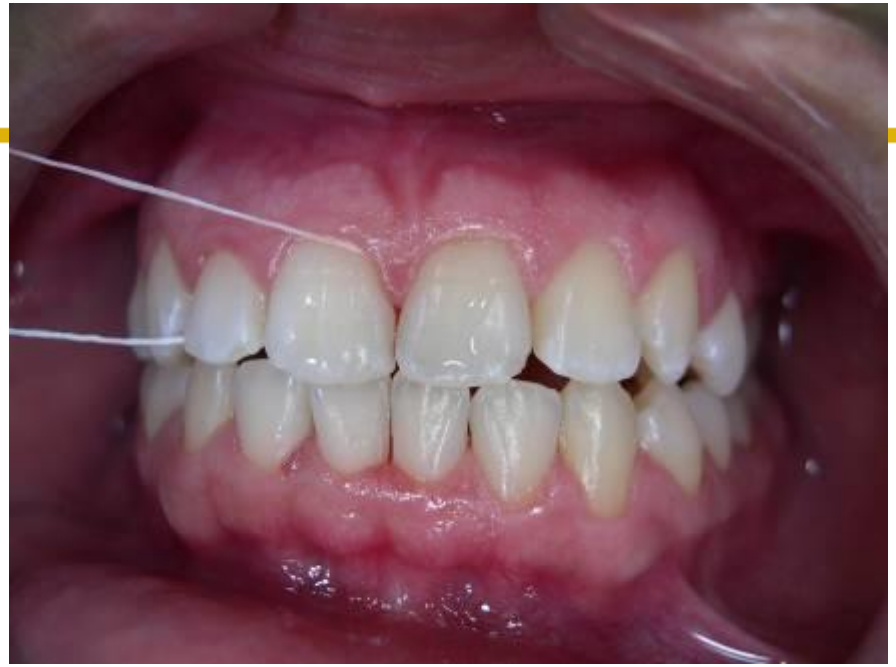
Periodontium

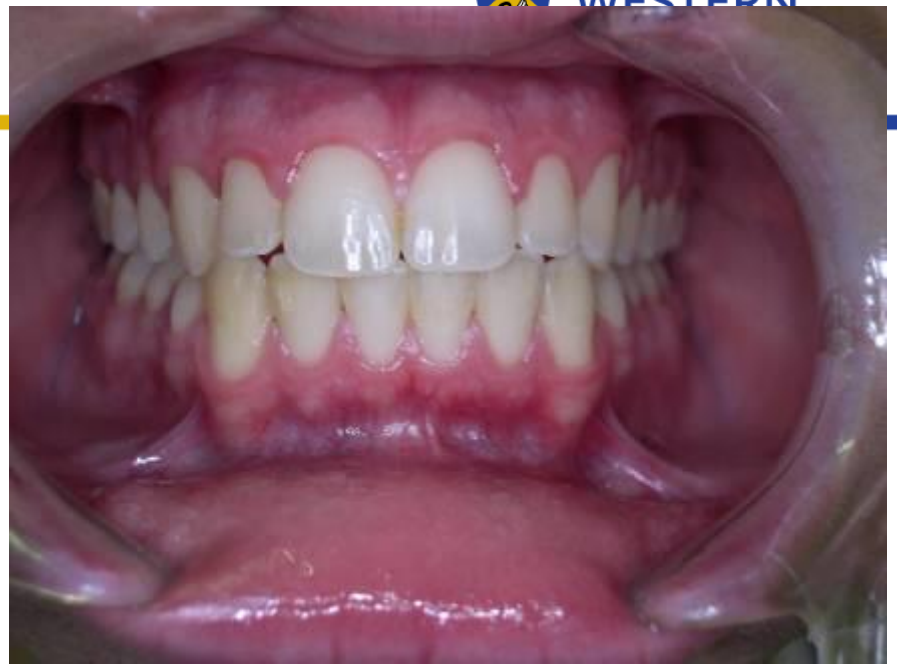


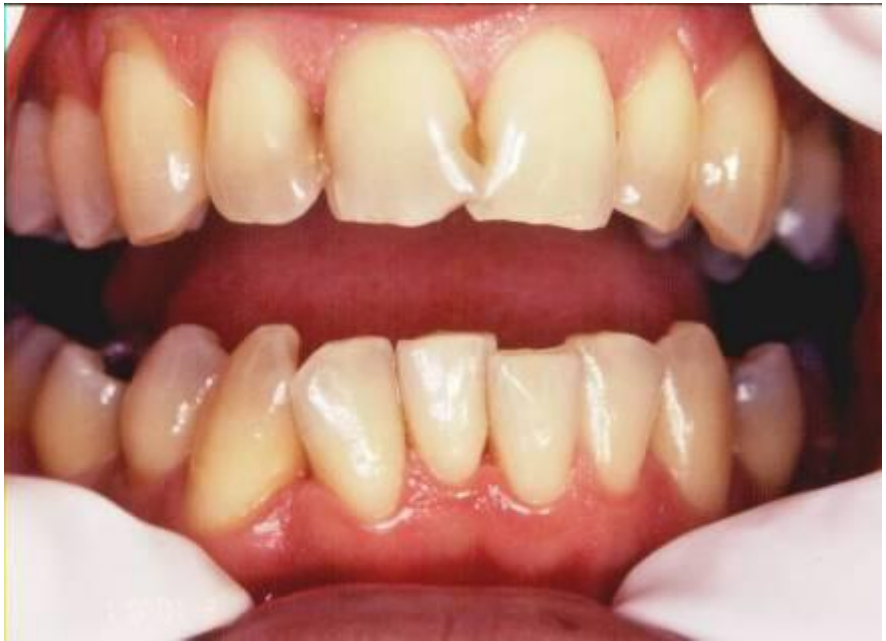
Periodontal health and diseases

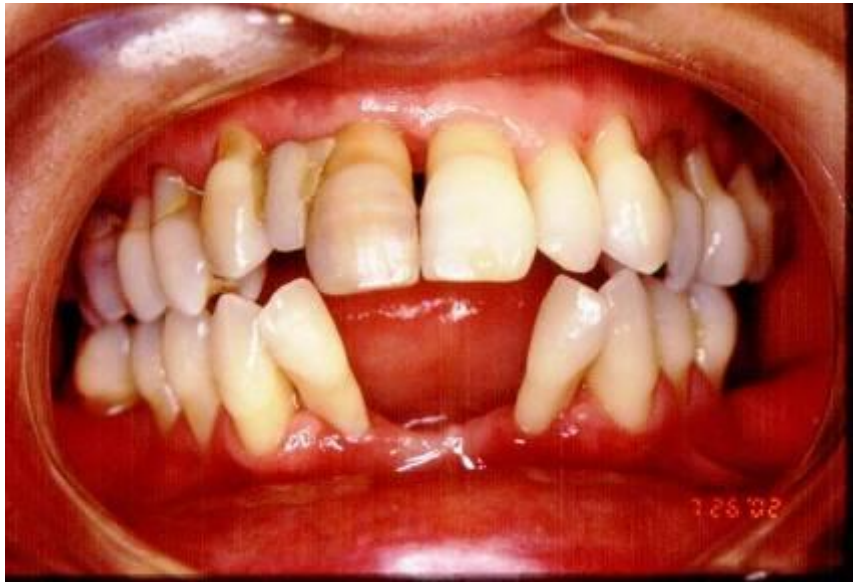


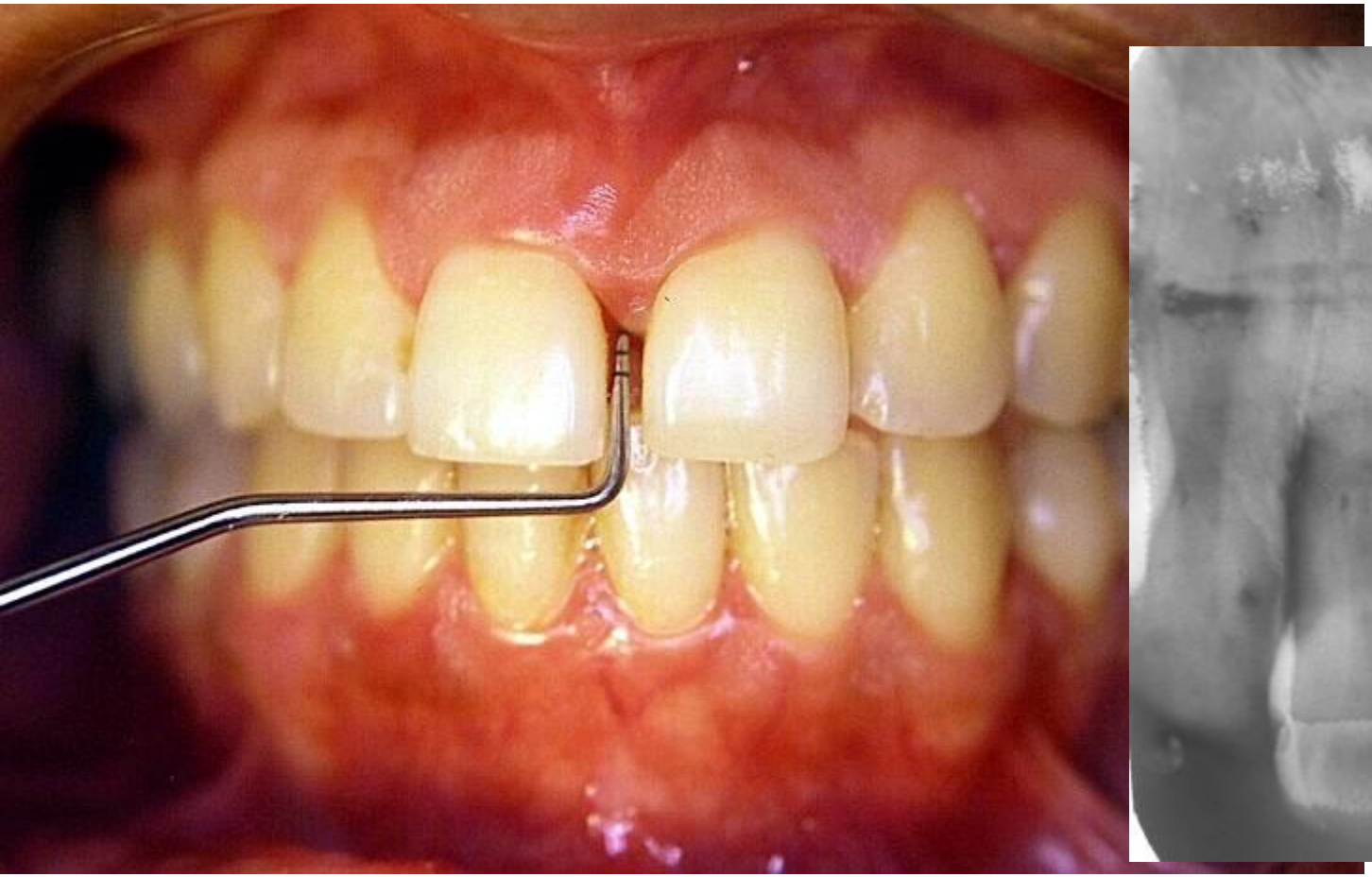
Nature Reviews | Disease Primers

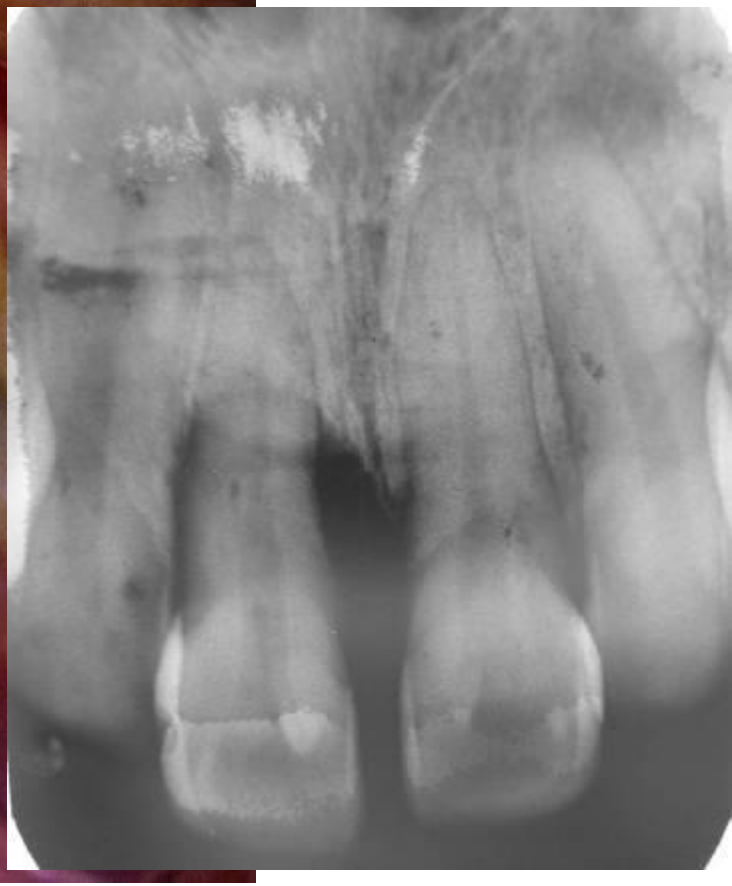






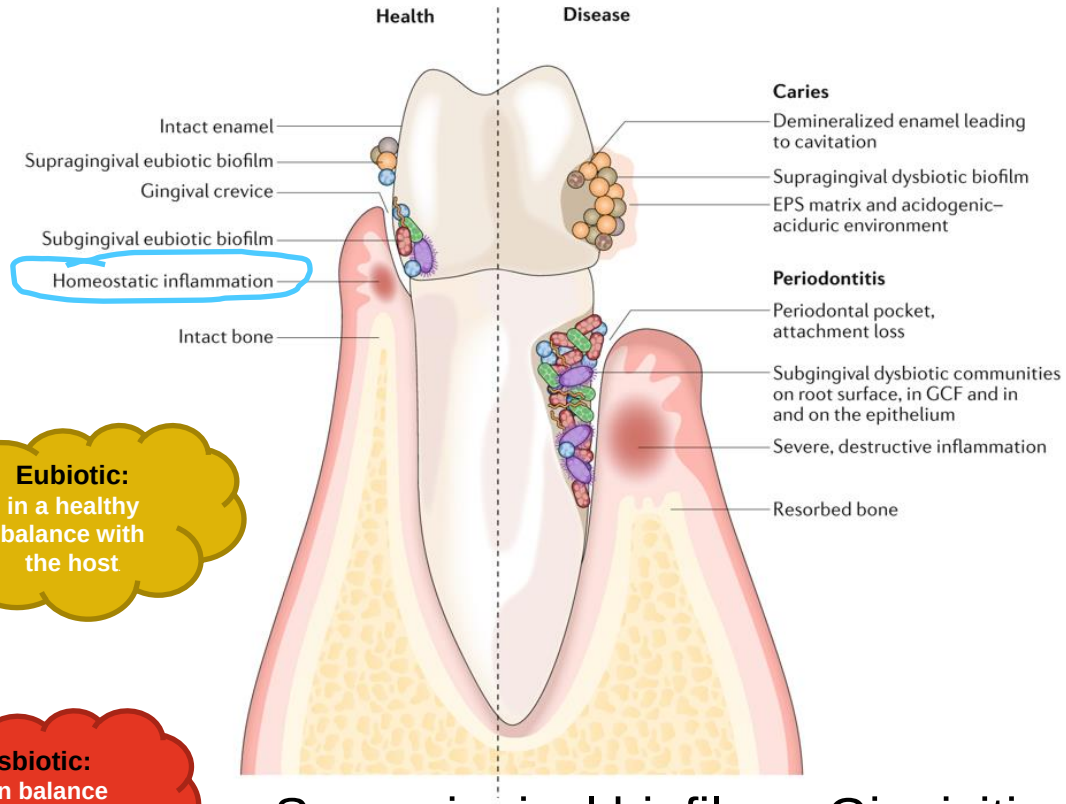






Periodontal area peculiarities

Biofilms always present



Eubiotic: in a healthy balance with the host

Dysbiotic: When balance with the host. Is disrupted

Supragingival biofilm = Gingivitis

Subgingival biofilm = Periodontitis

Periodontitis peculiarities

Tooth: non-shedding surface, partially outside the body (=oral cavity)

Bacterial load is located generally “outside” the body

Challenge for the immune-inflammatory response to take action

Endogenous/oportunistic infection

Dysbiosis

Plaque as a biofilm

Hajishengallis et al 2015

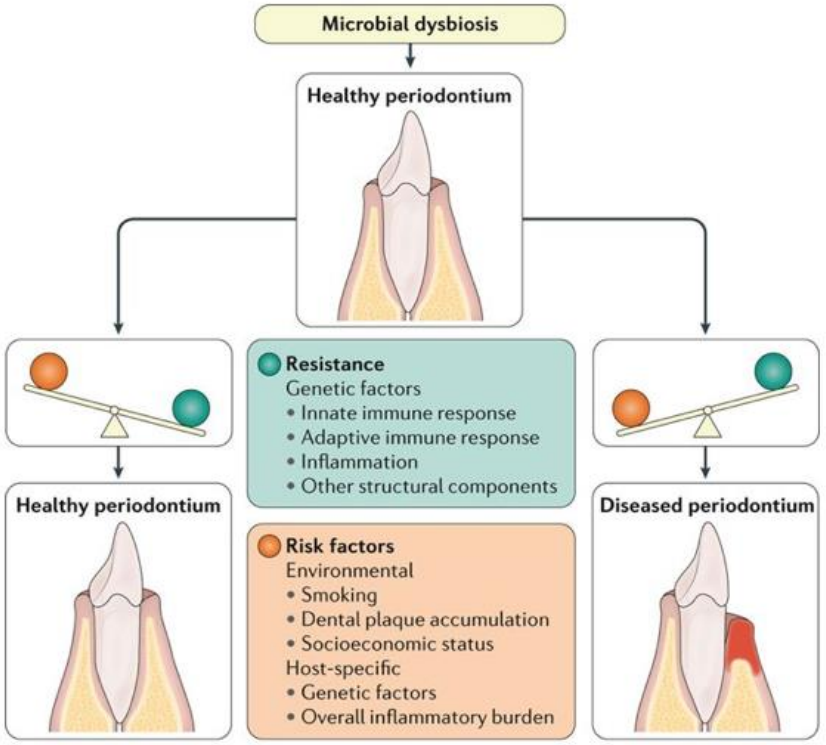
Periodontal health and diseases

Dysbiosis

Dysbiosis is any perturbation of the normal microbiome content that could disrupt the symbiotic relationship between the host and associated microbes, a disruption that can result in diseases, such as inflammatory bowel disease and other gastrointestinal (GI) disorders, including gastritis, peptic ulcer disease, irritable bowel syndrome, and even gastric and colon cancer [3–6].

From: The Microbiota in Gastrointestinal Pathophysiology, 2017

Figure 5: Susceptibility to periodontal diseases.



Disease progression depends on the extent and severity of the microbial biofilm challenge (microbial dysbiosis) and the host response, which is influenced by protective factors (resistance) and promoting factors (risk factors).

Kinane, D. F. *et al.* (2017) Periodontal diseases *Nat. Rev. Dis. Primers* doi:10.1038/nrdp.2017.38

7 Host defences

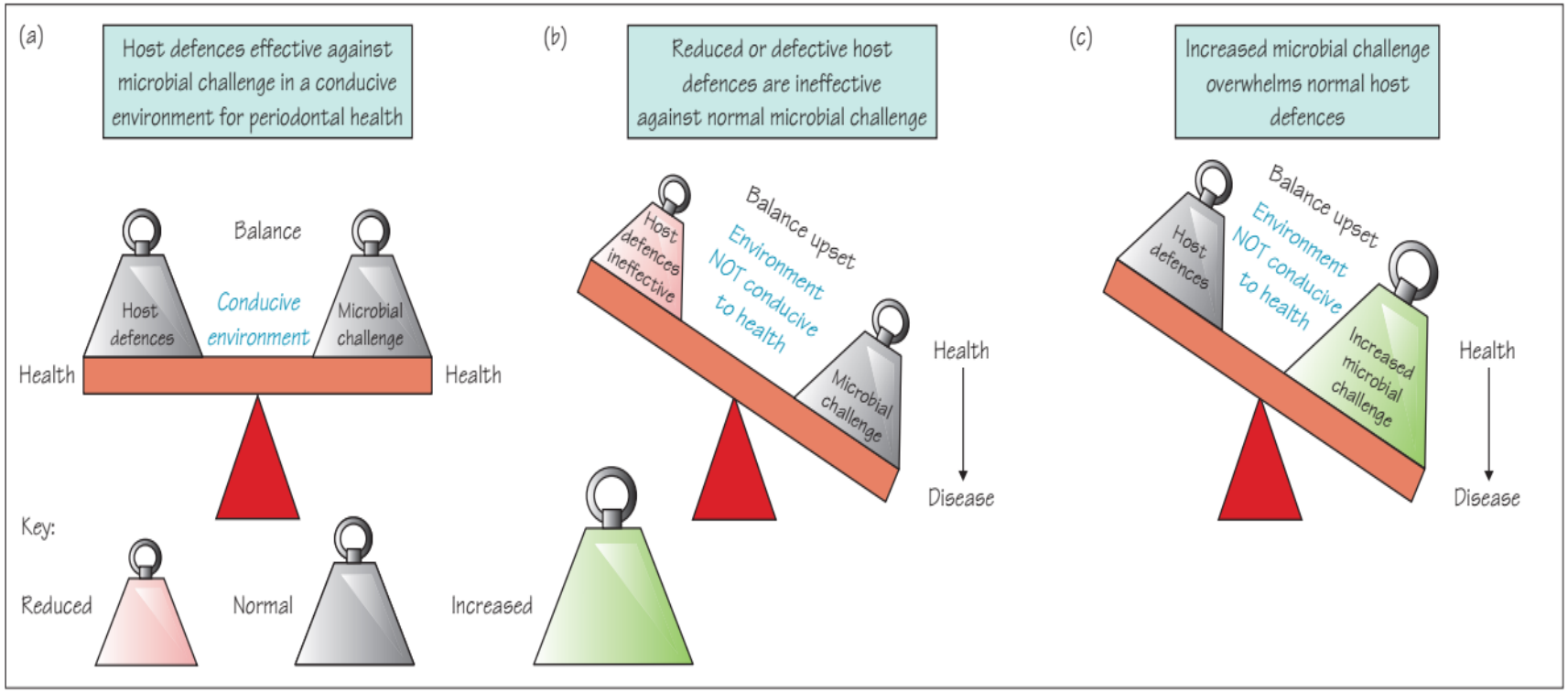


Figure 7.1 *Concept of balance between host defences, microbial challenge and environment. (a) Balance and periodontal health. (b) Host defences have a defect or are ineffective against microbial challenge, tipping the balance to periodontal destruction. (c) Microbial challenge overwhelms the host defences leading to an upset balance and periodontal destruction – this may relate to the environment not being conducive and/or changes in quality, quantity or virulence of microorganisms.*

Current PD etiopathogenesis model

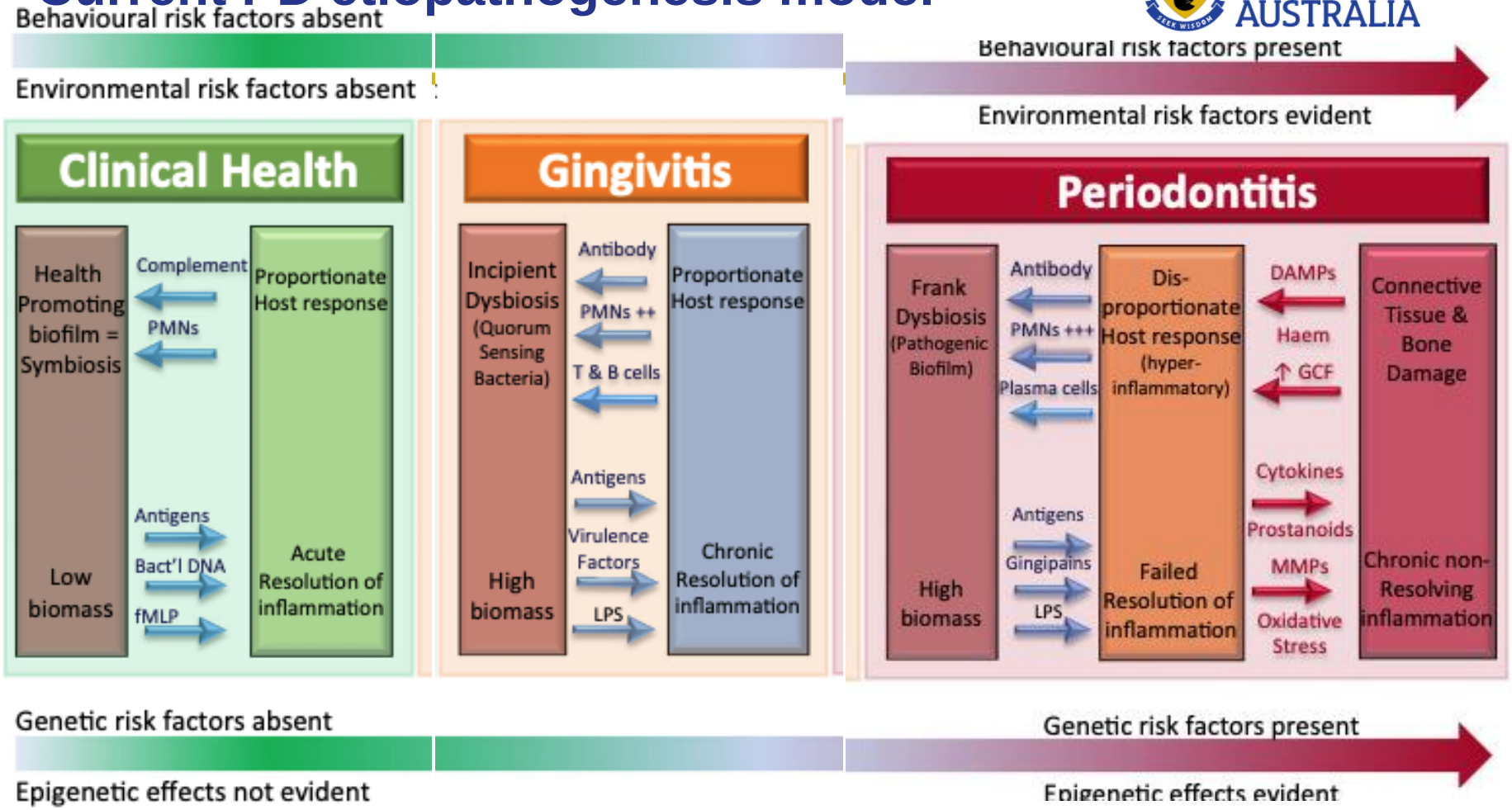


FIGURE 1 Contemporary model of host–microbe interactions in the pathogenesis of periodontitis, in which the host response drives an incipient dysbiosis (gingivitis). If the biofilm is not disrupted/removed, frank dysbiosis results and perpetuates a chronic nonresolving and destructive inflammation. DAMPs, damage-associated molecular patterns; fMLP, N- formylmethionyl- leucyl- phenylalanine; GCF, gingival crevicular fluid; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; PMNs, polymorphonuclear neutrophils. This figure is referred from ref. 106.

Murakami S, Mealey BL, Mariotti A,Chapple ILC. Dental plaque–induced gingival conditions.J Clin Periodontol. 2018;45(Suppl 20):S17–S2

Periodontal microbiota

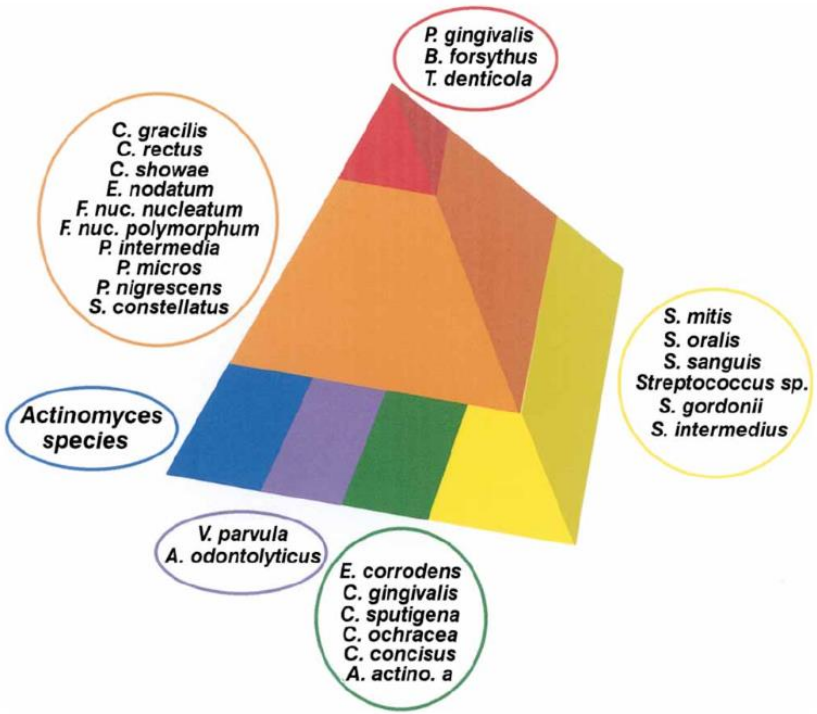


Fig. 1. Diagram of the association among subgingival species (adapted from Socransky et al. (174)). The data were derived from 13,321 subgingival plaque samples taken from the mesial aspect of each tooth in 185 adult subjects. Each sample was individually analyzed for the presence of 40 subgingival species using checkerboard DNA-DNA hybridization. Associations were sought among species using cluster analysis and community ordination techniques. The base of the pyramid is comprised of species thought to colonize the tooth surface and proliferate at an early stage. The orange complex becomes numerically more dominant later and is thought to bridge the early colonizers and the red complex species which become numerically more dominant at late stages in plaque development.

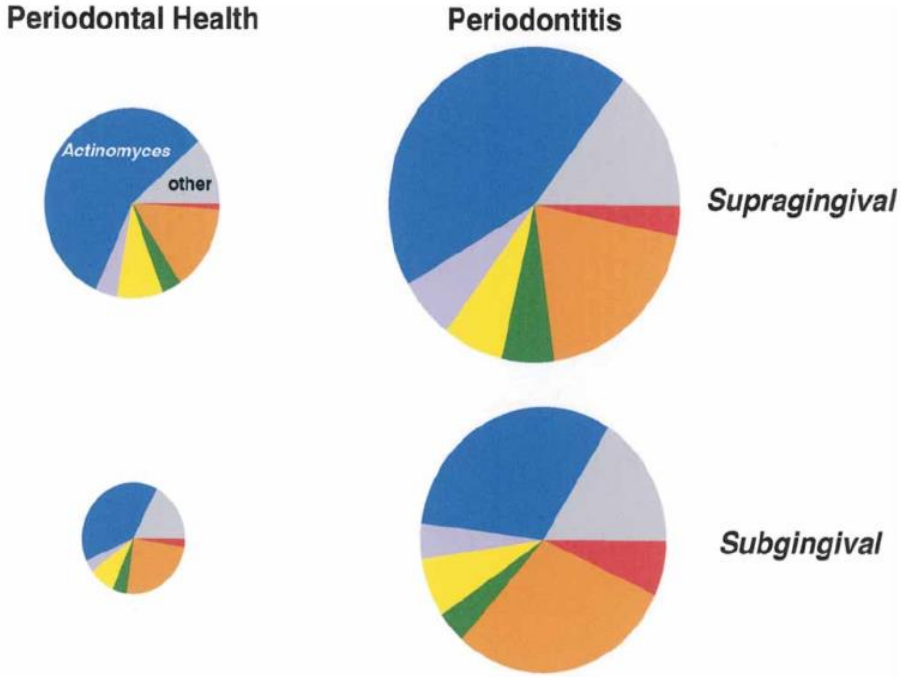


Fig. 10. Pie charts of the mean percentage DNA probe count of microbial groups in supragingival and subgingival plaque samples from 22 periodontally healthy and 23 periodontitis subjects. The species were grouped into seven microbial groups based on the description of Socransky et al. (174). The areas of the pies were adjusted to reflect the mean total counts at each of the sample locations. The significance of differences in mean percentages of the supragingival and subgingival complexes in health and disease was tested using the Kruskal-Wallis test. The "red", "orange" and *Actinomyces* species were significantly different at $P<0.001$, and the "green" complex species differed at $P<0.05$ after adjusting for 7 comparisons. The "other" category represents probes to species that did not fall into a complex as well as probes to new species whose relationships with other species have not yet been ascertained. Reprinted with permission of the *Journal of Clinical Periodontology* (Ximenez-Fyvie et al. (217)).

SOCRANSKY, S.S. and HAFFAJEE, A.D. (2002), Dental biofilms: difficult therapeutic targets. Periodontology 2000, 28: 12-55.

Virulence factors – perio microbiota

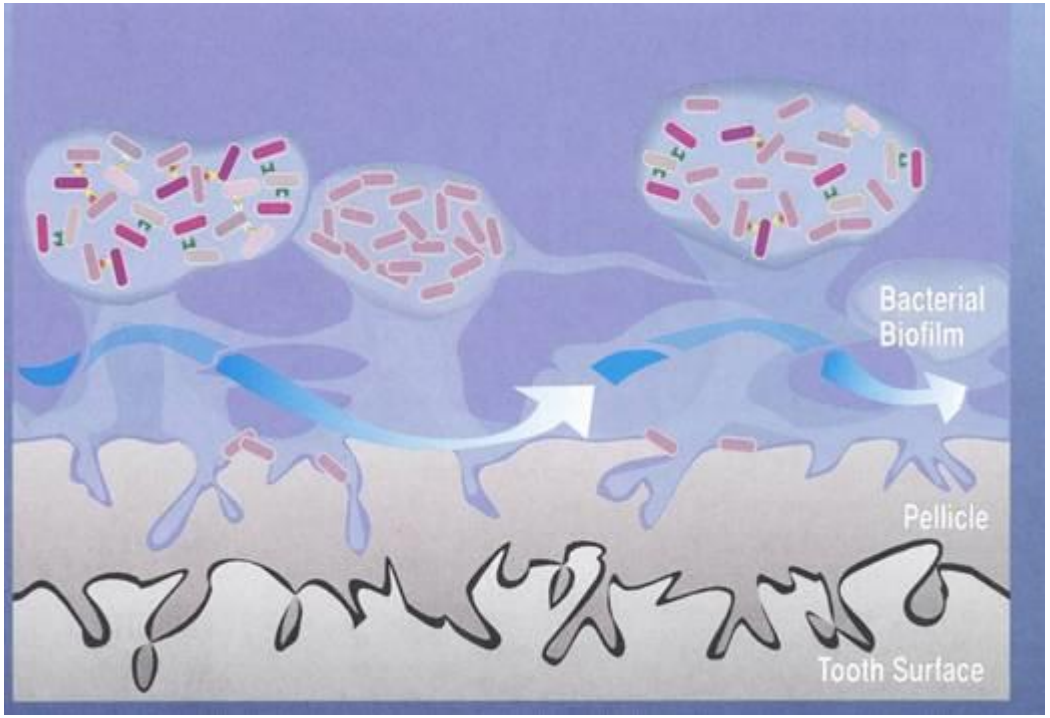
Enzymes

Waste products

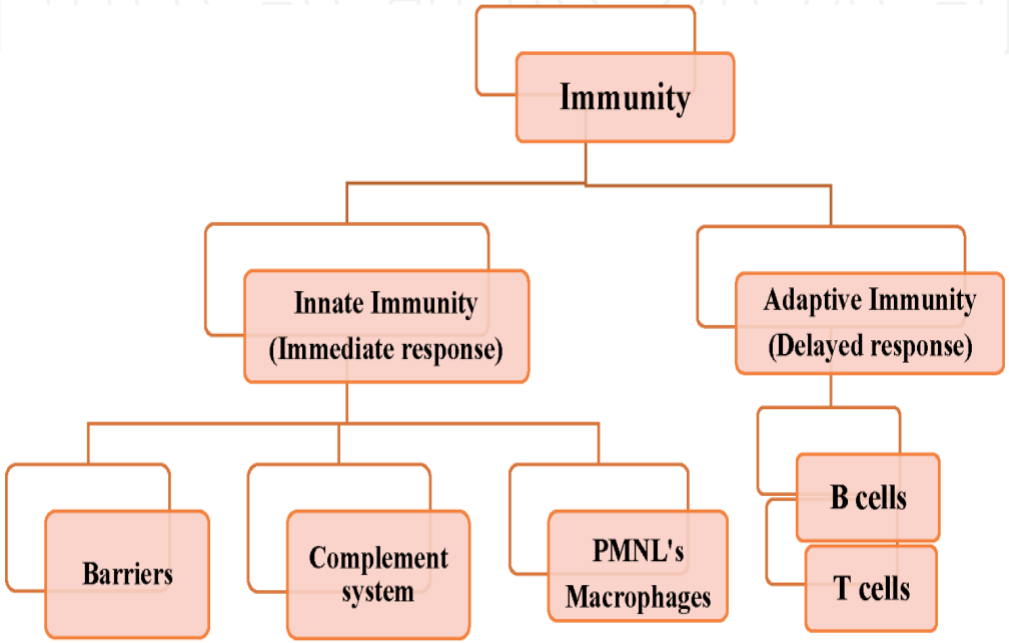
Proteinases

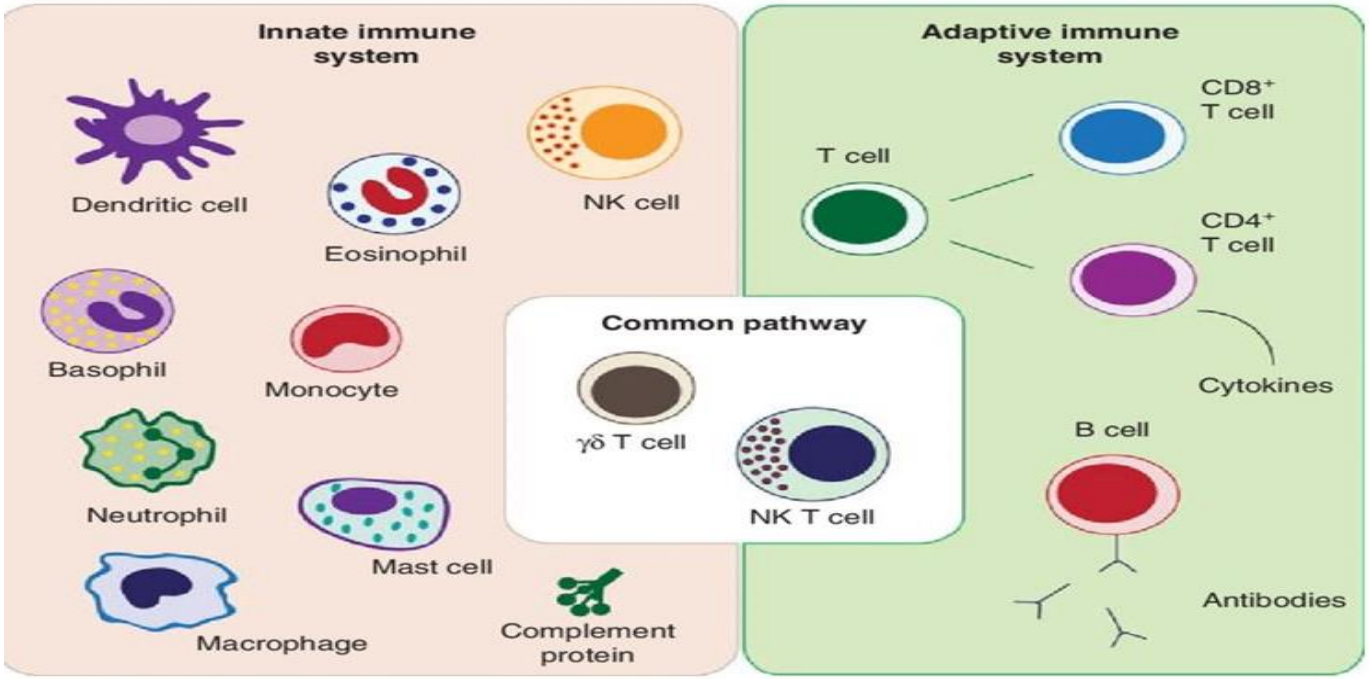
Leukotoxins

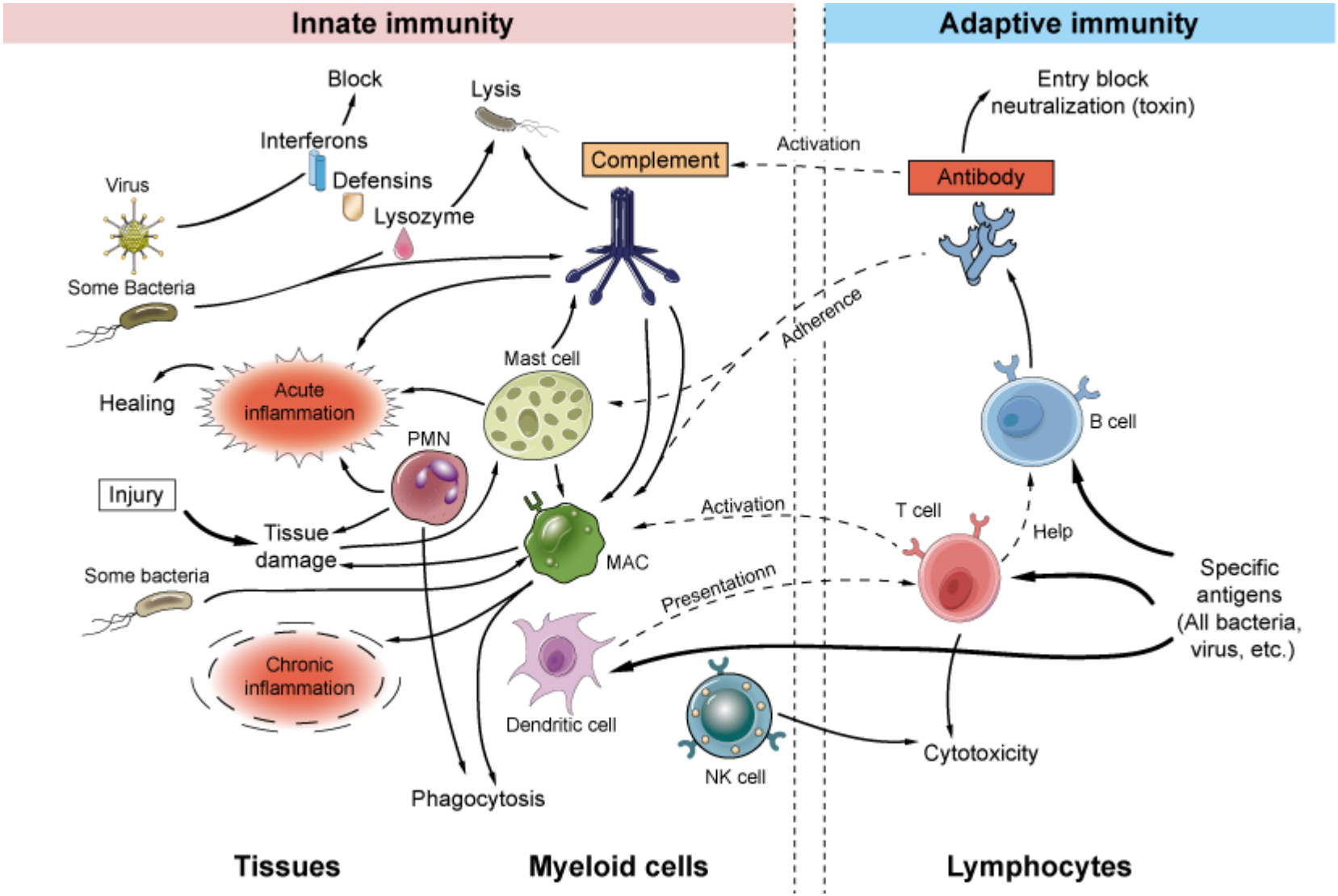
Lipopolysaccharides



EVADE HOST RESPONSES


Immunity
Innate Immunity
(Immediate response)
Adaptive Immunity
(Delayed response)
Barriers
Complement system
PMNL's
Macrophages
B cells
T cells


Innate immune system
Dendritic cell
Eosinophil
NK cell
Basophil
Monocyte
Neutrophil
Mast cell
Macrophage
Complement protein
Common pathway
γδ T cell
NK T cell
Adaptive immune system
T cell
CD8+ T cell
CD4+ T cell
Cytokines
B cell
Antibodies


Innate immunity
Adaptive immunity
Block
Lysis
Interferons
Defensins
Lysozyme
Virus
Some Bacteria
Complement
Activation
Entry block
neutralization (toxin)
Antibody
Adherence
Mast cell
Healing
Acute
inflammation
PMN
B cell
Injury
Tissue
damage
Activation
T cell
Help
MAC
Some bacteria
Presentationn
Specific
antigens
(All bacteria,
virus, etc.)
Chronic
inflammation
Dendritic cell
NK cell
Cytotoxicity
Phagocytosis
Tissues
Myeloid cells
Lymphocytes

Dento–gingival host defenses

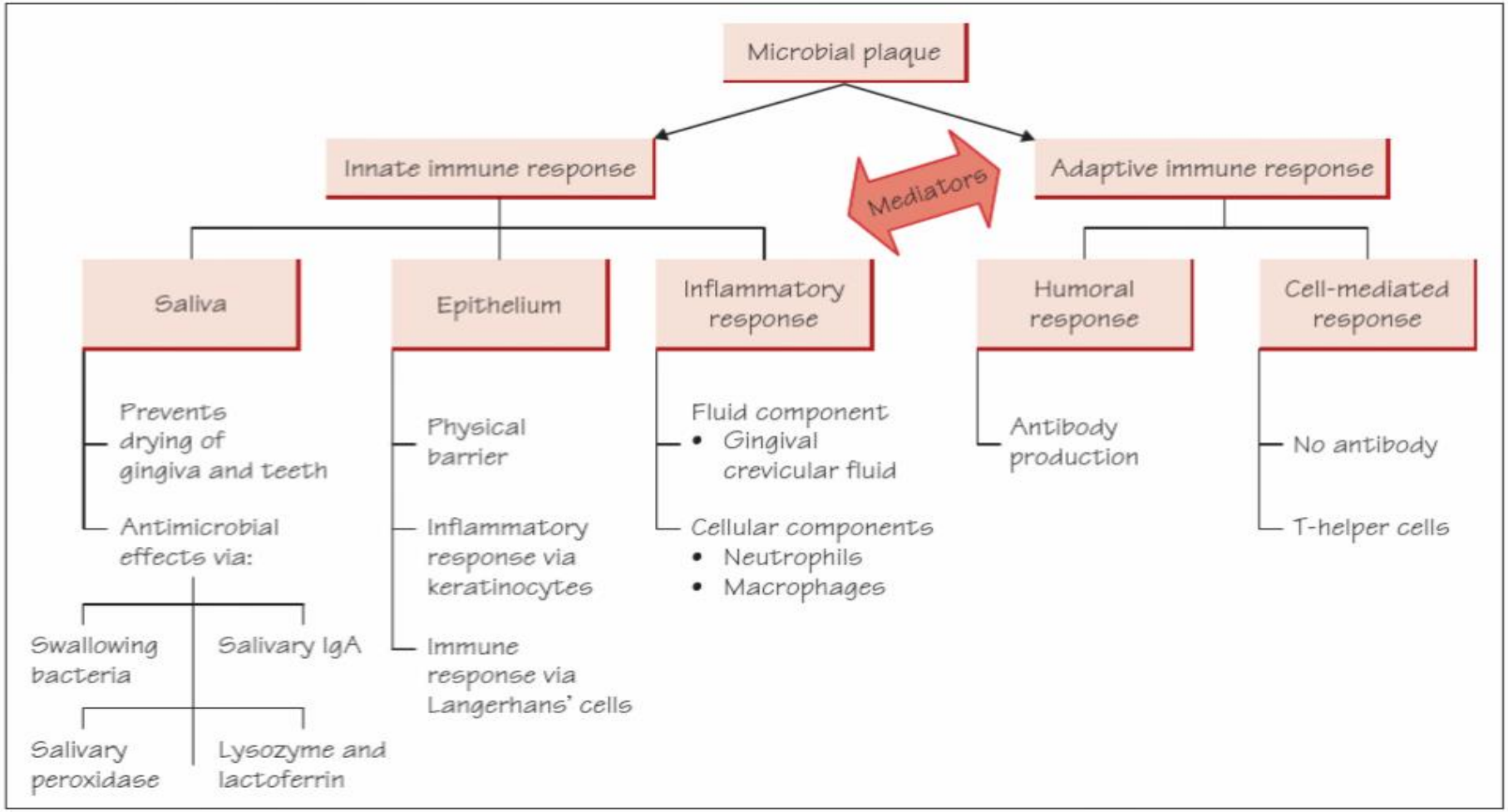


Figure 7.2 Host defences against microbial plaque.

Clerehugh et al 2013

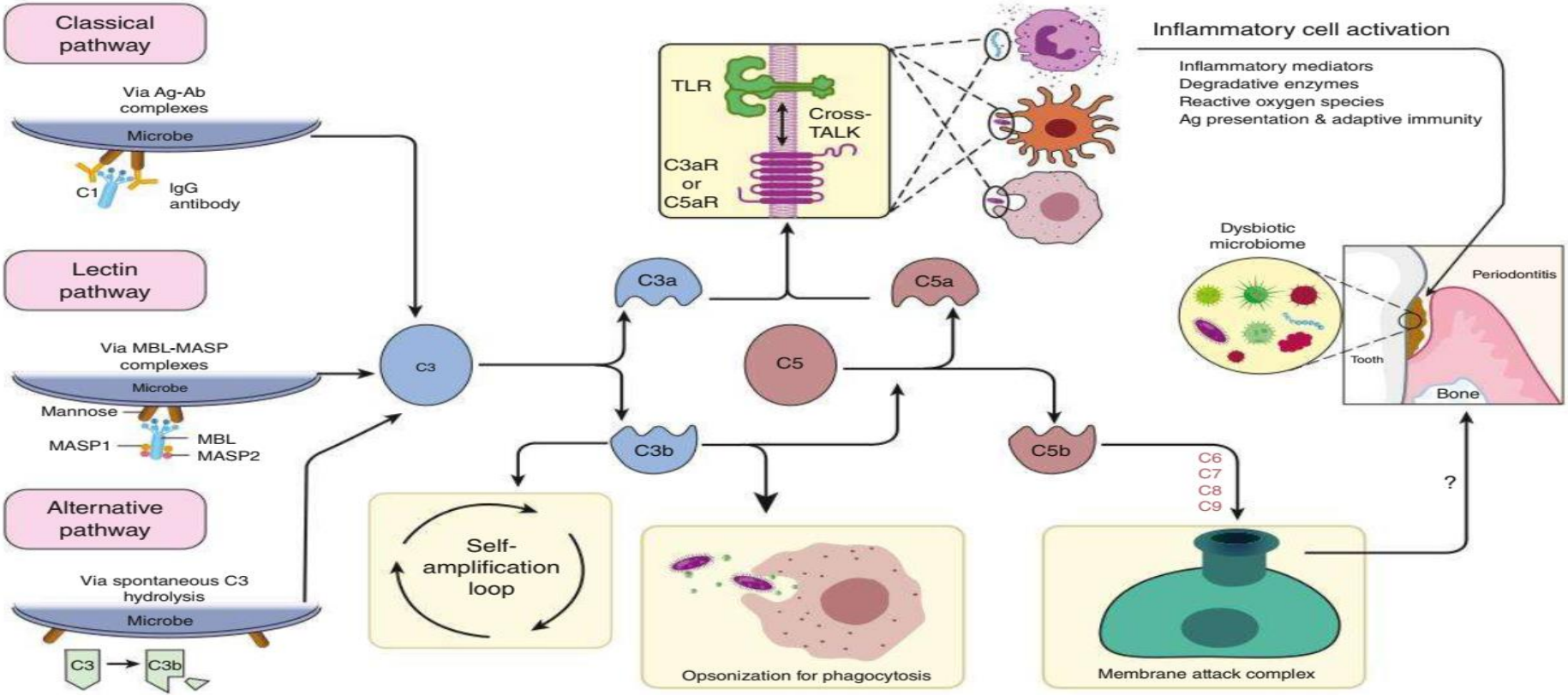
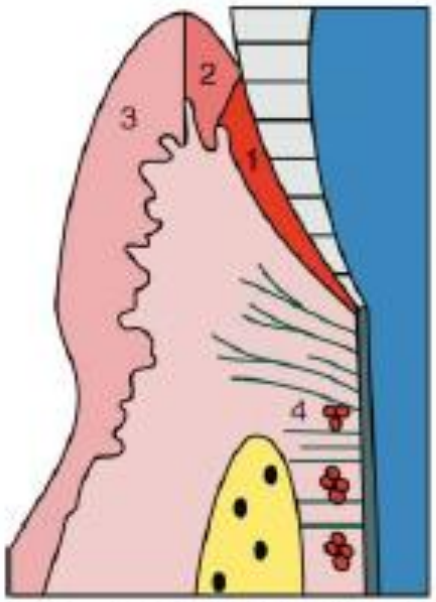


Fig. 11.3 Complement activation and periodontal disease. The complement system can be activated by distinct initiation mechanisms: The classical pathway is triggered by antigen-antibody complex-mediated activation of the C1 complex. The lectin pathway is initiated when complexes of mannose-binding lectin *(MBL)* and MBL-associated serine proteases *(MASPs)* recognize and adhere to microbial surfaces. The alternative pathway is triggered by a "tick-over" mechanism that involves spontaneous C3 hydrolysis, which occurs in the absence of complement regulatory molecules (as is typically the case with foreign surfaces such as microbial cells). In the so-called alternative pathway-amplification loop, additional C3 is cleaved into even more C3b which further fuels the loop, thereby amplifying complement activation irrespective of the initiating mechanism. All three mechanisms of complement initiation and amplification converge at C3. The downstream effects of C3 activation include the generation of effectors that promote inflammation *(C3a and C5a)*, opsonization for phagocytosis (C3b) and the generation C5b-C9 membrane attack complex (MAC). MAC can lyse susceptible targeted bacteria but has also been implicated in destructive inflammation. Whereas the role of MAC in periodontitis is uncertain, C3a and C5a activate specific G-protein-coupled receptors *(C3aR and C5aR1)*, which cross-talk with Toll-like receptors *(TLRs)*. This cross-talk interaction between complement and TLRs activates synergistically inflammatory leukocytes, which directly or indirectly mediate destructive inflammation that leads to periodontal tissue breakdown and alveolar bone loss in periodontitis.

Epithelium



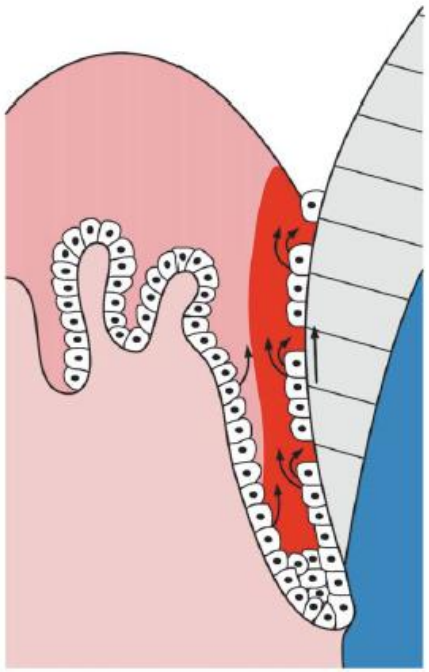
Dento-gingival junction

1. Junctional epithelium (JE)
2. Sulcular epithelium
3. Oral epithelium
4. Epithelial rests of Malassez

JE functions

- attachment to tooth
- barrier
- rapid turnover
- antimicrobial defence
- GCF flow

Fig. 1. Schematic illustration of the different epithelia at the dentogingival junction. The junctional epithelium (JE) exhibits a distinct phenotype that allows the tissue to attach to the tooth surface and participate in the host defense in a number of ways.



Physical barrier: turnover + peeling

Biological barrier: defensins, IL-8, PMN

Gingival crevicular fluid (GCF)

- Inflammatory exudate released through the crevice, resulting from the increase in permeability of vessels next to the JE and SE.
- Composed of: plasma derived substances (**antibodies, cytokines, enzymes**), epithelium and immune cells, bacteria.
- GCF volume and flow increase with increasing inflammation.

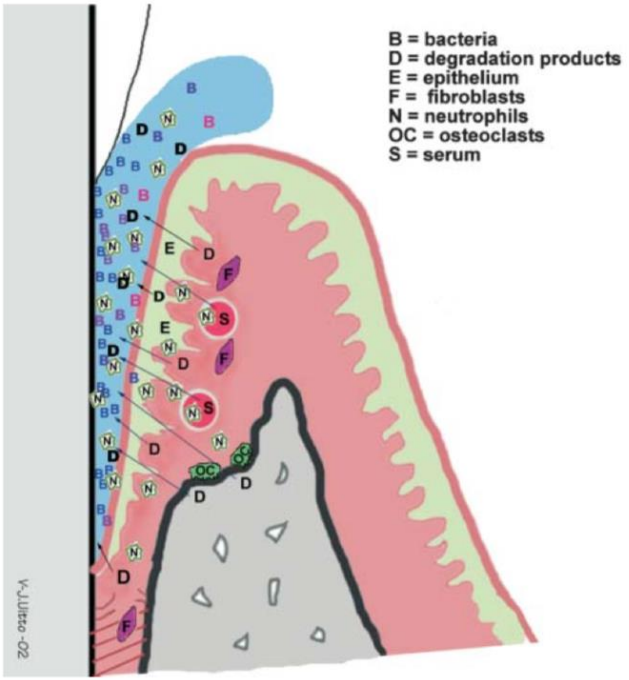
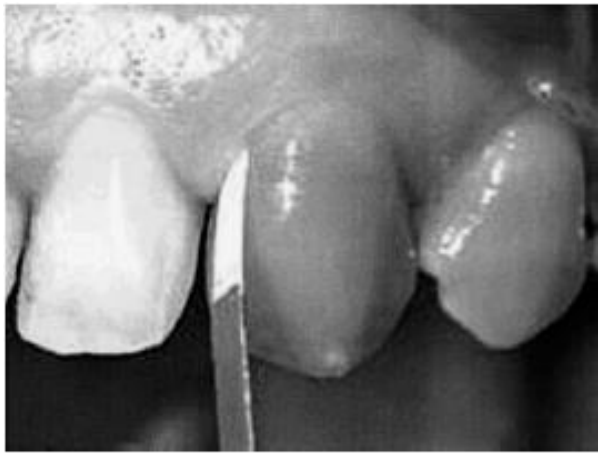
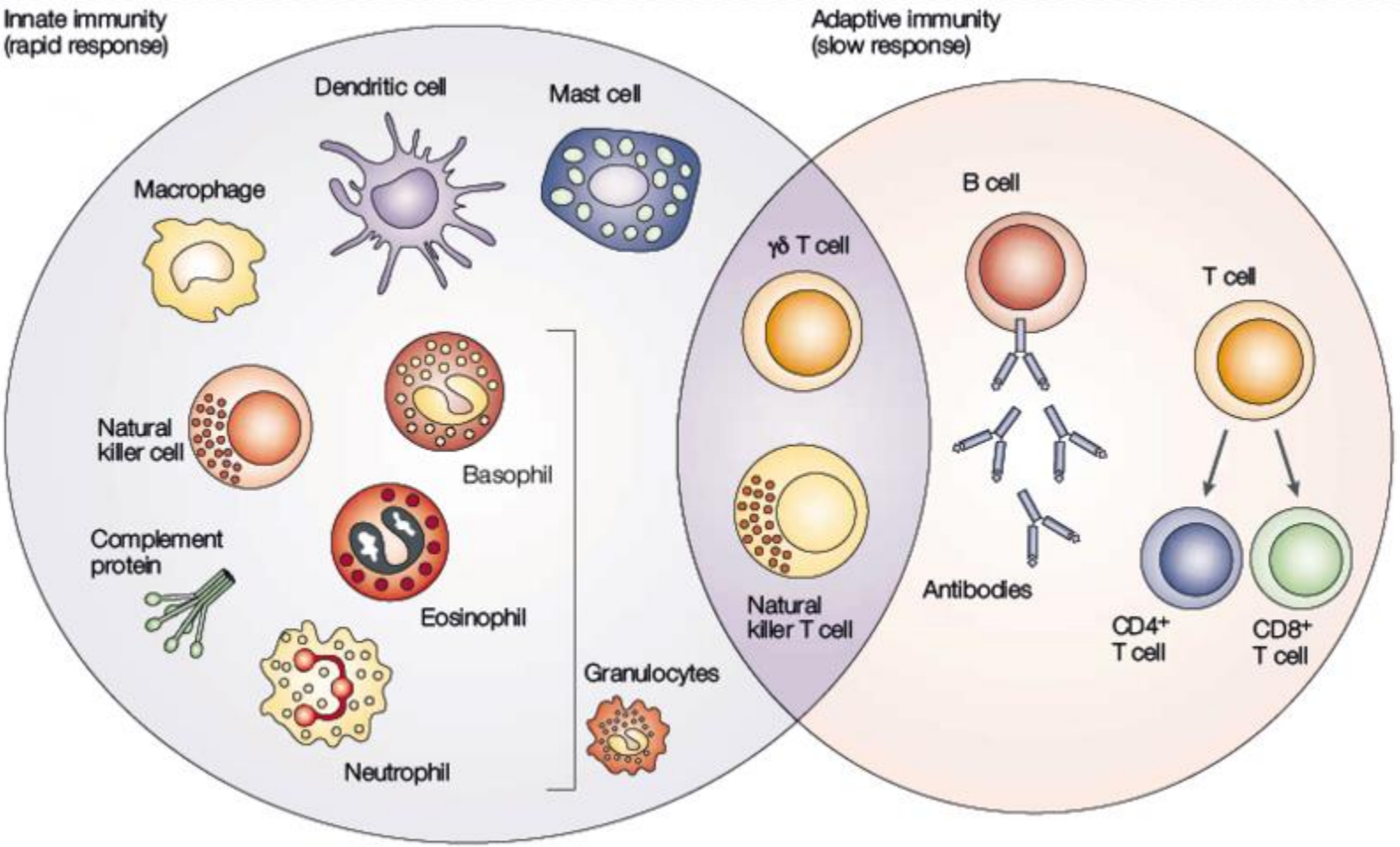


Fig. 1. Gingival crevice fluid – a window to periodontal disease. Gingival crevice fluid is composed of substances derived from serum, leukocytes, bacteria, activated epithelial cells, connective tissue cells, and bone cells. Tissue destruction during periodontal inflammation results in production of tissue fragments and growth factors released from tissue stores. All these substances reflect the periodontal disease process and can be potentially used as indicators of periodontal condition.



Uitto 2013


Innate immunity
(rapid response)
Adaptive immunity
(slow response)
Dendritic cell
Mast cell
Macrophage
γδ T cell
B cell
T cell
Natural
killer cell
Basophil
Complement
protein
Eosinophil
Antibodies
Natural
killer T cell
CD4+
T cell
CD8+
T cell
Granulocytes
Neutrophil

First defense cells (innate immunity)

Neutrophil

If bacteria are in plaque matrix:
- Neutrophils attach to plaque matrix
- Neutrophils secrete externally:
  - Enzymes
  - Hydrogen peroxide and hypochlorous acid
- Neutrophils kill bacteria without phagocytosis
- Plaque matrix dissolved
- Washed away by gingival crevicular fluid
- Bystander damage caused

If bacteria are unattached:
- Neutrophils recognise and bind to bacteria which are phagocytosed into phagocytic vacuole
- Neutrophils produce and release:
  - Antibacterial granules
  - Hydrogen peroxide and hypochlorous acid
- Enzymes digest microorganisms
- Remnants expelled
- Bystander damage caused

Figure 7.3 The role of neutrophils in phagocytosis or the killing of microorganisms.

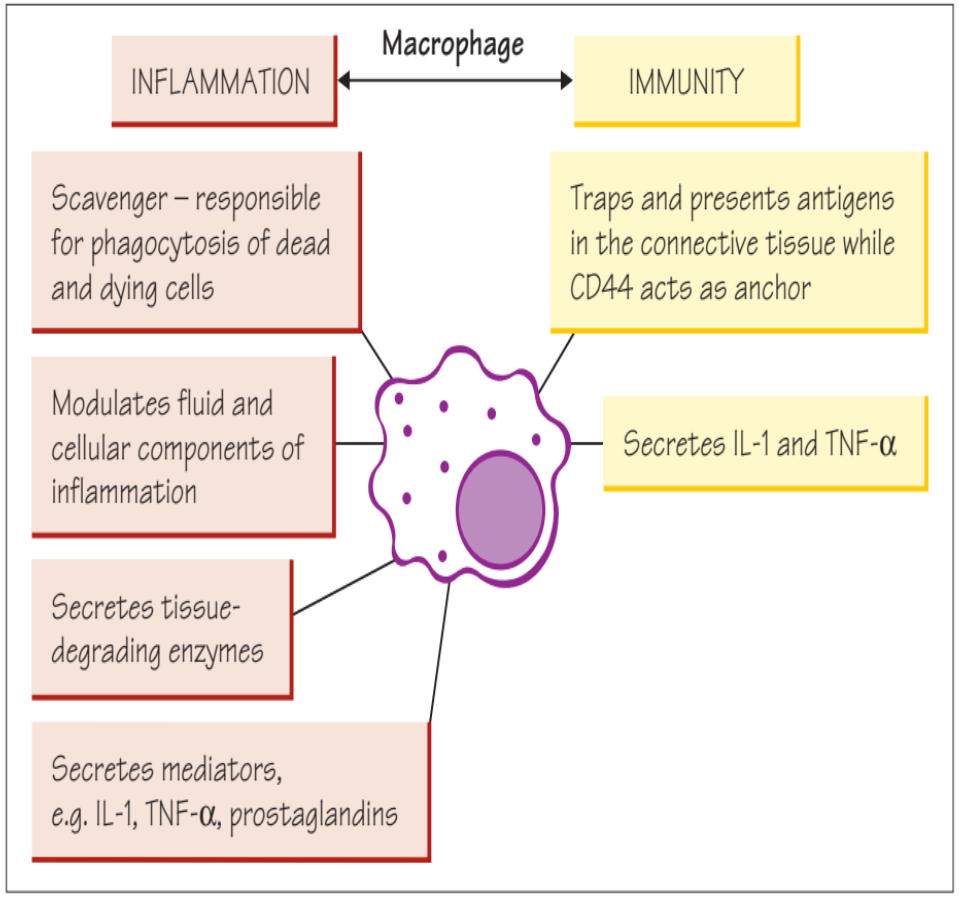
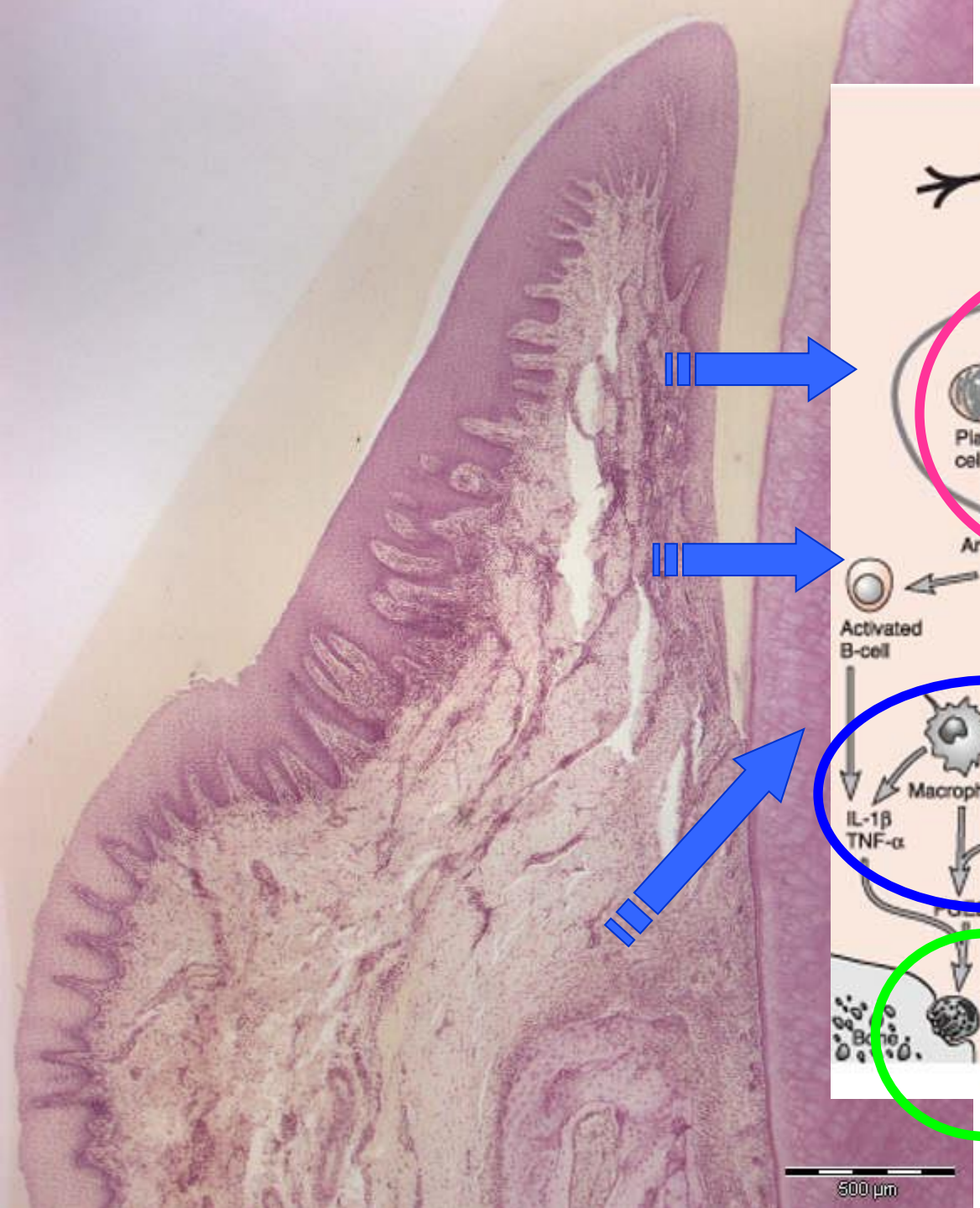
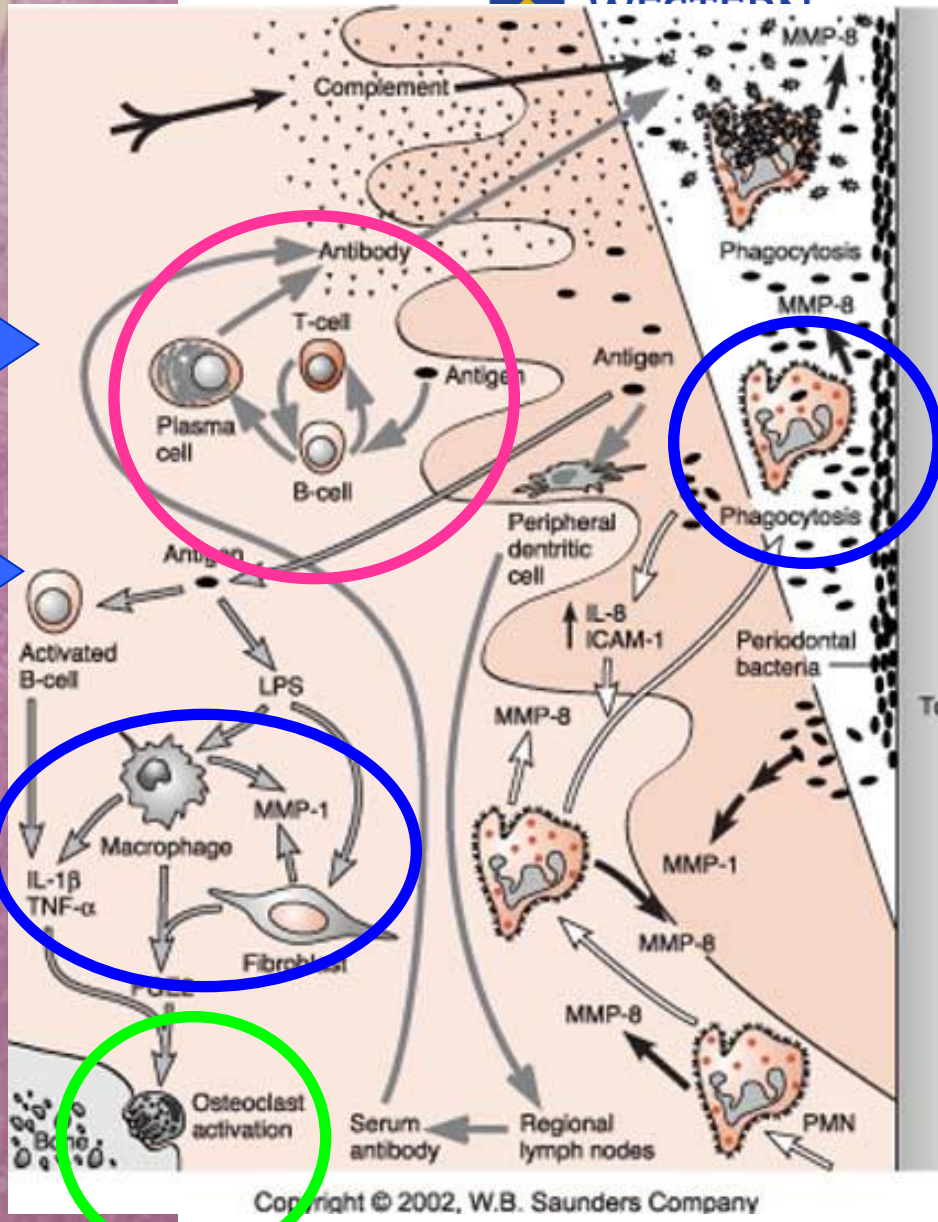


Figure 7.4 The role of macrophages in inflammation and immunity.

Clerehugh et al 2013


500 µm


Complement
MMP-8
Phagocytosis
Antibody
T-cell
Antigen
Plasma cell
B-cell
Peripheral dentritic cell
IL-8
ICAM-1
Periodontal bacteria
Activated B-cell
LPS
MMP-1
Macrophage
IL-1β
TNF-α
Osteoclast activation
Serum antibody
Regional lymph nodes
PMN
Copyright © 2002, W.B. Saunders Company

Adaptive immunity

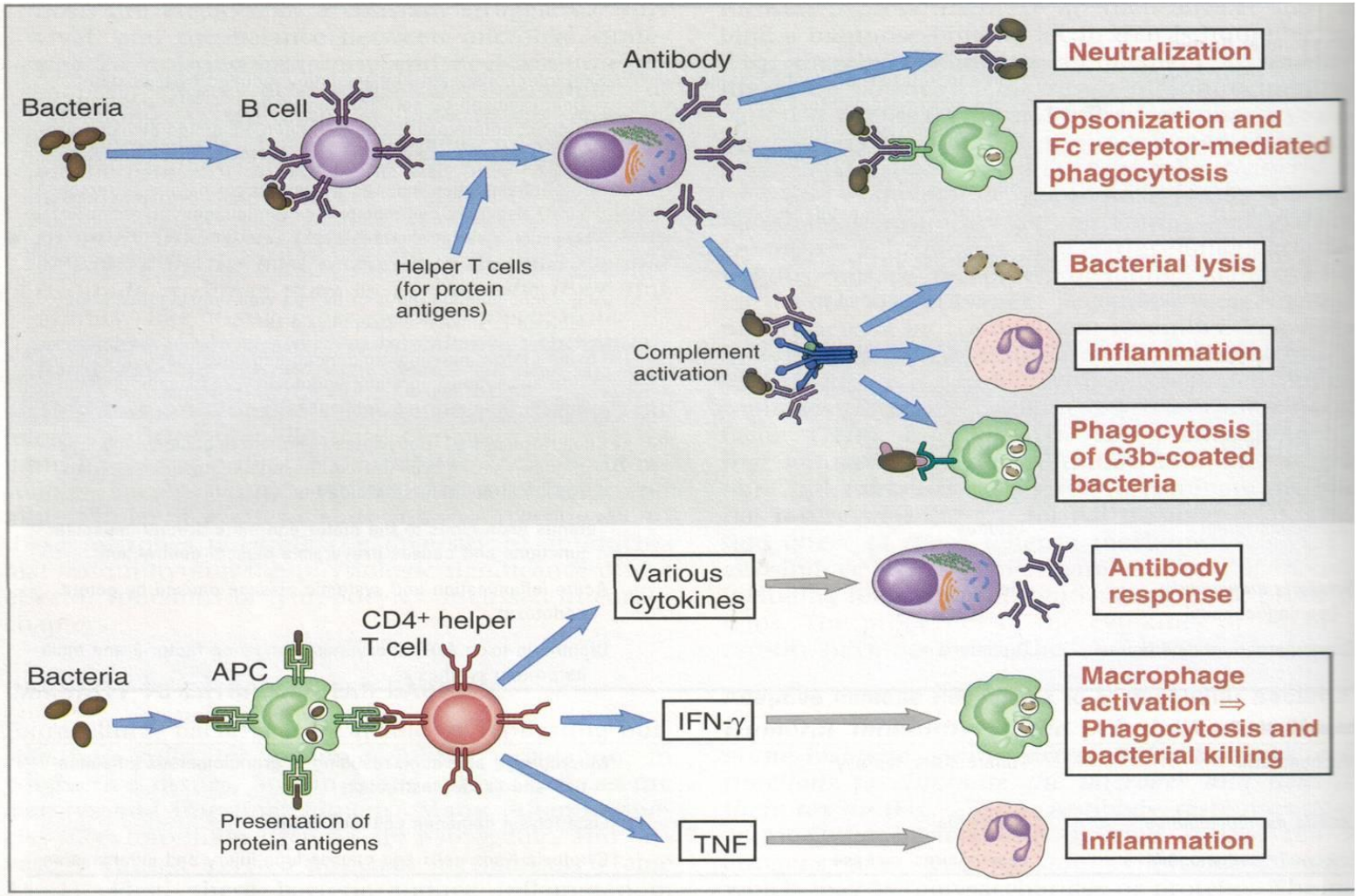


Figure 15–1 Adaptive immune responses to extracellular microbes.
Adaptive immune responses to extracellular microbes, such as bacteria, and their toxins consist of antibody production and the activation of $CD4^+$ helper T cells. Antibodies neutralize and eliminate microbes and toxins by several mechanisms. Helper T cells produce cytokines that stimulate B cell responses, macrophage activation, and inflammation. APC, antigen-presenting cell; INF-γ, interferon-γ; TNF, tumor necrosis factor.


INNATE & ADAPTIVE
IMMUNITY

HUMORAL RESPONSE

THE UNIVERSITY OF WESTERN AUSTRALIA

1. Plaque antigens diffuse through the junctional epithelium

2. Langerhans cells within the epithelium capture and process the antigens

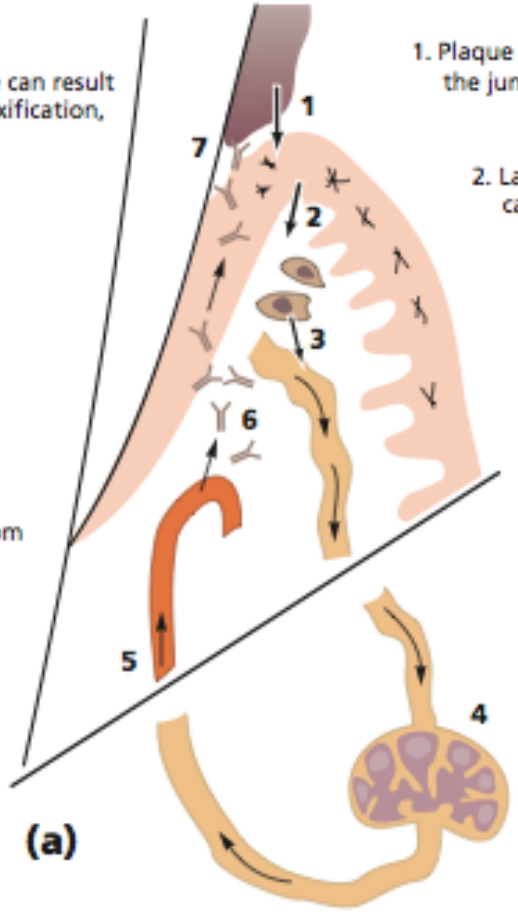
3. Antigen-presenting cells (Macrophages and Langerhans cells) leave the gingiva in the lymphatics

4. Antigen-presenting cells reach the lymph node and begin to stimulate lymphocytes to produce a specific immune response

5. Periodontal microbe specific antibodies are produced by plasma cells within the lymph nodes and travel back to the gingiva via blood vessels

6. Antibodies leave the circulation and are carried to the crevice in the transudate from the inflamed and dilated blood vessels

7. Antibody action on microbes in the crevice can result in killing, aggregation, precipitation, detoxification, opsonization and phagocytosis of bacteria



Adaptive - Cell-mediated response

Antigen penetration of JE => APC + T cell naïve =>

Different subsets of **T-helper cells** (**Th1, Th2, Th17, T reg**) proliferate and release different cytokines profiles

Th1: IL-2, IFN-g,

Th2: IL-4, IL-5

Th17: IL-17

T reg: IL-10, TGF-b

Cytokines act on other cells (PMN, macrophages, B or T cells) to stimulate, inhibit or kill

T-helper cells on re-exposure proliferate and produce cytokines (memory)

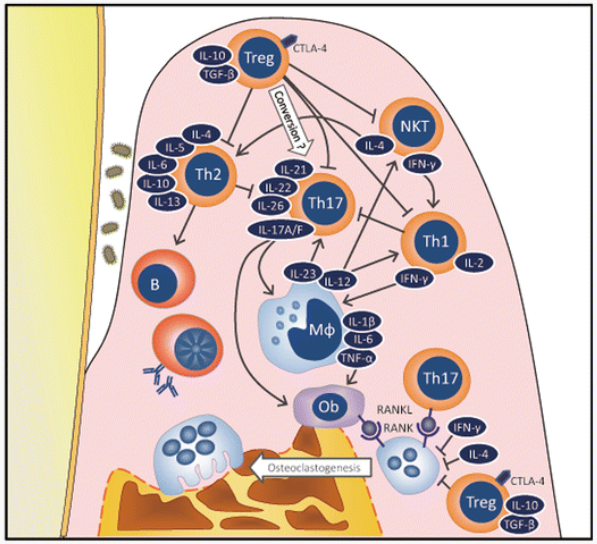
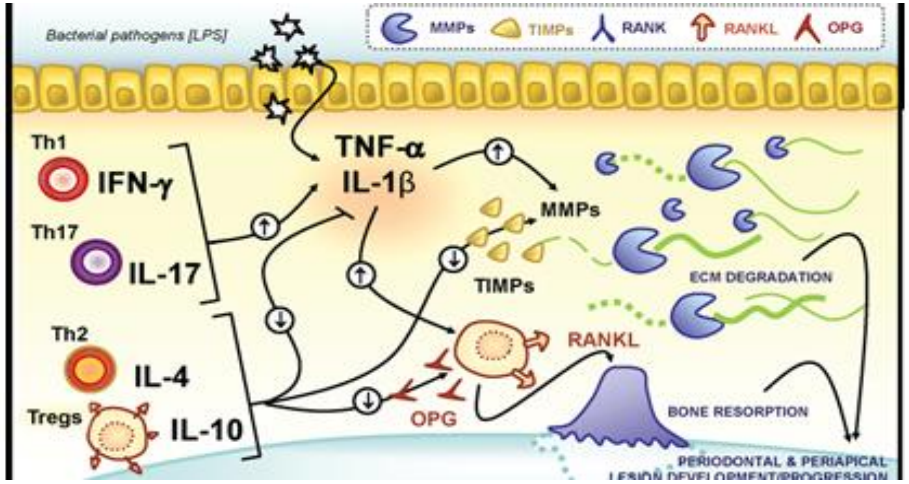
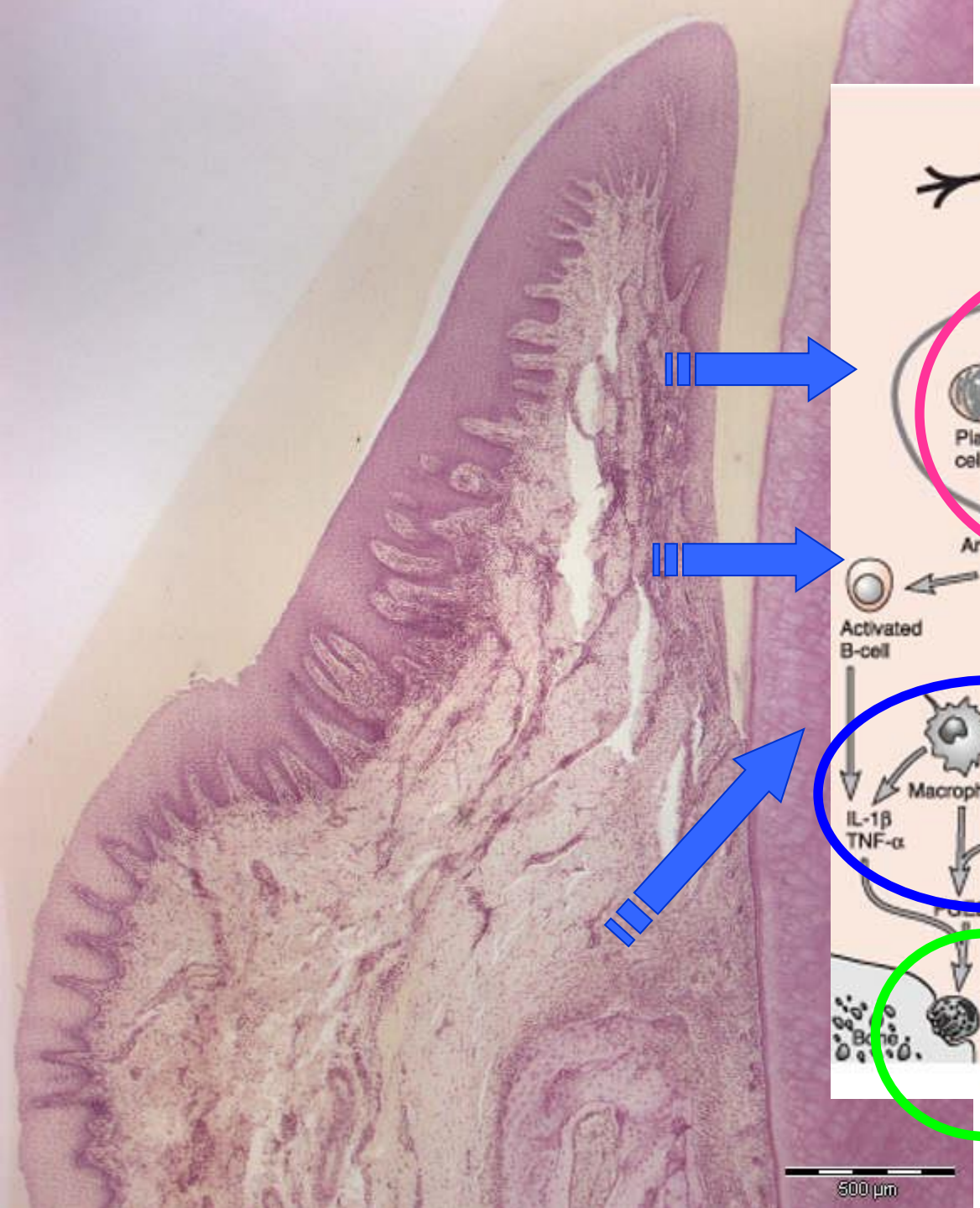


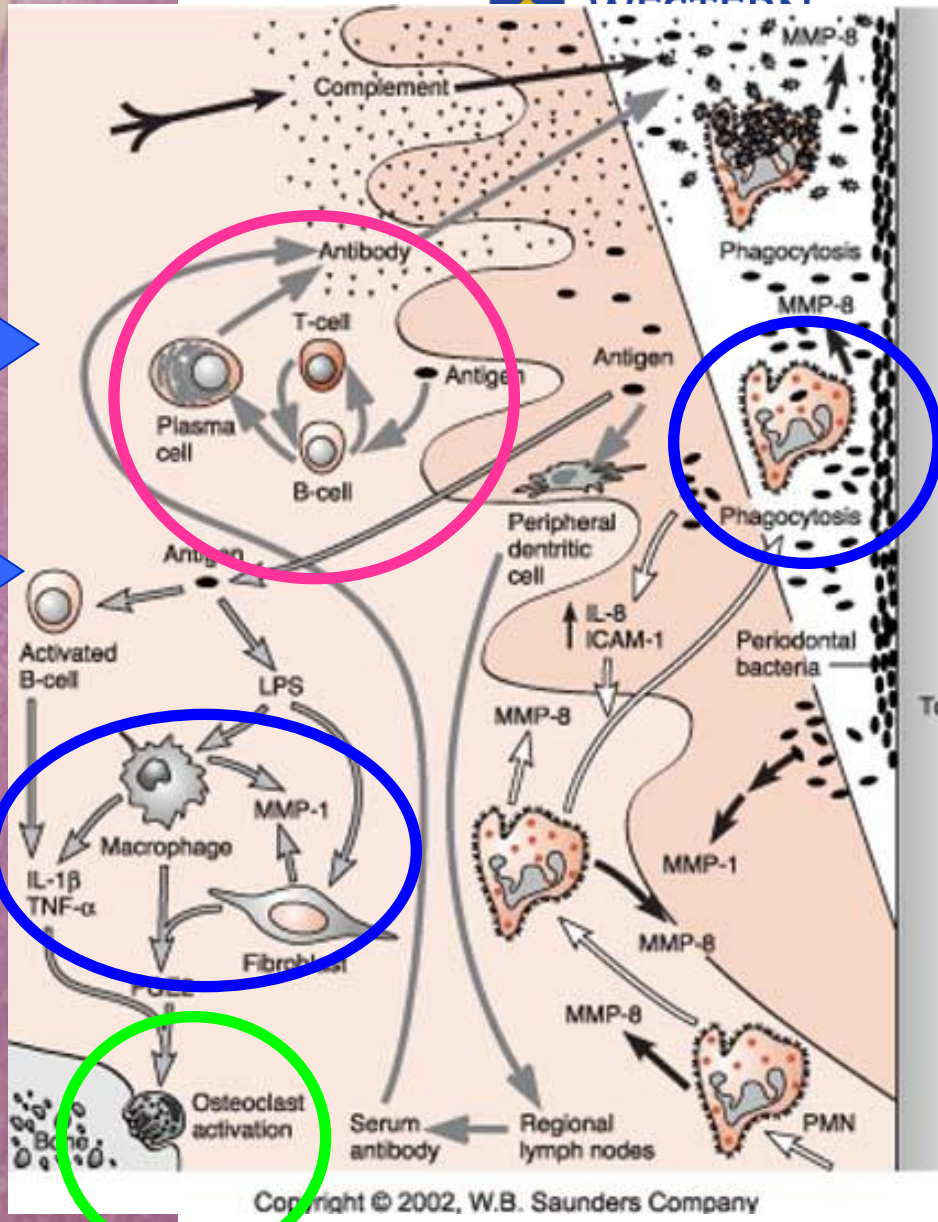
Fig. 1
The potential role of distinct T cell subsets in inflammatory responses and alveolar bone resorption in periodontitis lesions. As a consequence of sustained infection by periodontopathic bacteria, an adaptive immune response is established. NKT cells are involved in shaping the course of the immune response. Th1, Th2, and Th17 cells contribute to infection control in different ways because of their distinct cytokine profiles. However, their action also enhances inflammatory responses that lead to periodontal tissue destruction. Particularly, Th17 cells have a high potential to facilitate osteoclastogenesis thorough the production of IL-17 to induce RANKL expression on osteoblasts, enhancement of local inflammation, and RANKL expression on themselves. However, Tregs attenuate the inflammatory responses by suppressing other immune cells and inhibit osteoclastogenesis; therefore, they could protect against tissue destruction. Enhanced inflammation may convert a fraction of Tregs to IL-17-producing cells. Mφ macrophage, Ob osteoblast

Immune response = cells+mediators

- ✓ Main aim is to control infection in different ways
- ✓ Connective tissue destruction in periodontitis occurs as a “side-effect” of the immune-inflammatory response
- ✓ Perio ligament and bone destruction in periodontitis:
  - ❖ Biofilm essential, but not sufficient
  - ❖ High levels of pro-inflammatory cytokines (IL1b, TNFa), prostaglandins, MMP’s, RANKL
  - ❖ Low levels of anti-inflammatory mediators (IL-10, TGF-b, TIMPs and OPG




500 µm


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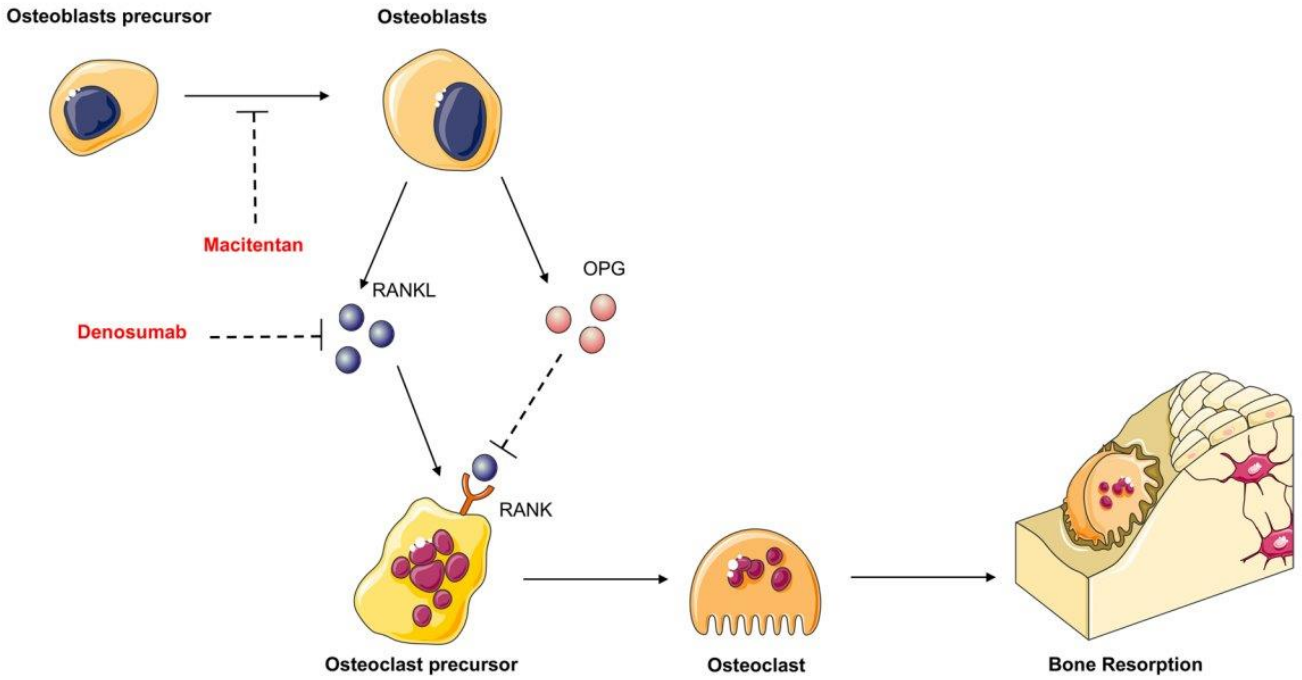

Osteoblasts precursor
Osteoblasts
Macitentan
RANKL
OPG
Denosumab
RANK
Osteoclast precursor
Osteoclast
Bone Resorption

Adaptive immunity and bone destruction

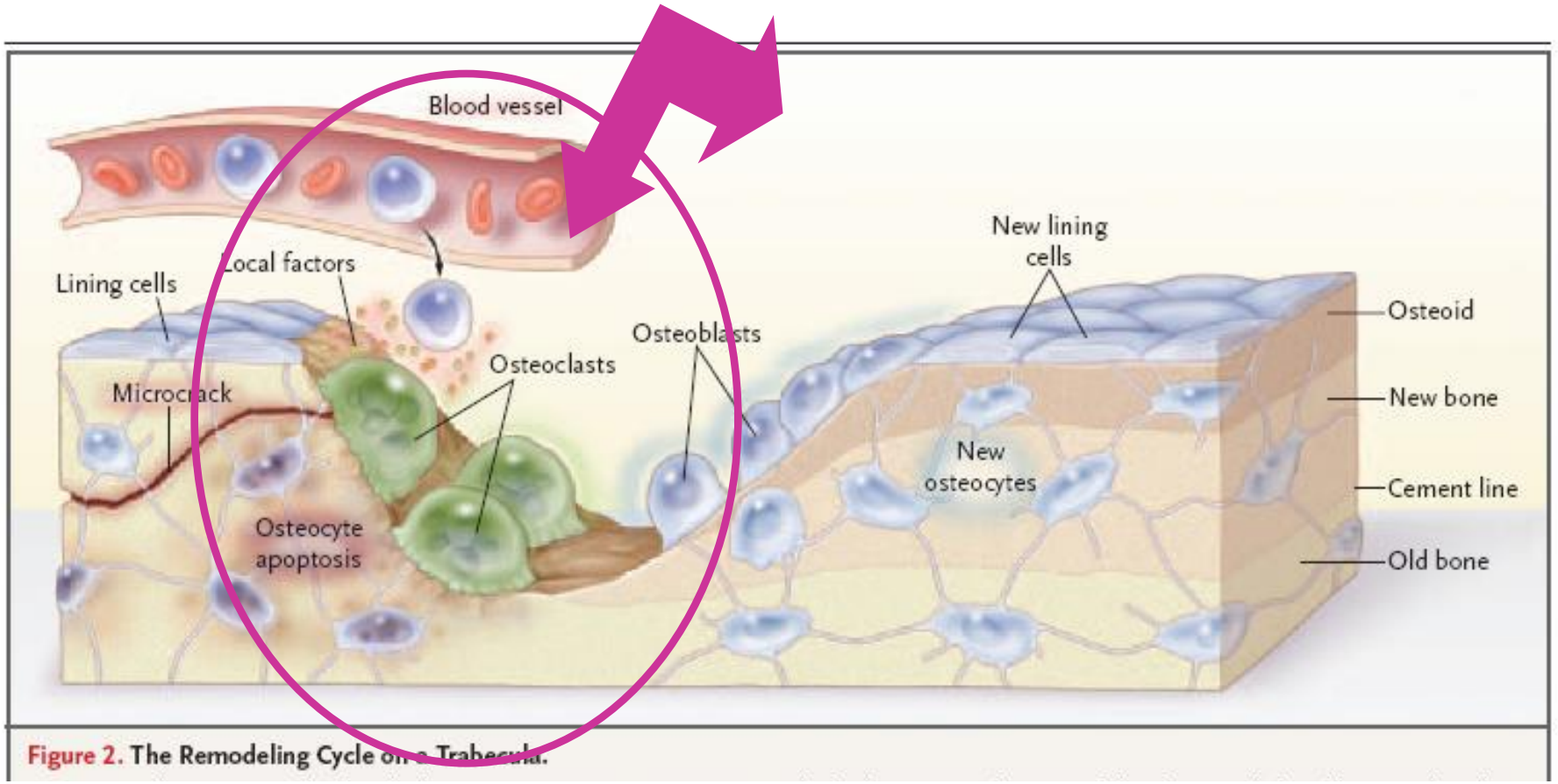


Figure 2. The Remodeling Cycle on a Trabecula.

Seeman et al 2006
Yamasaki et al 2004

OSTEOCLASTS ACTIVATION

RANKL & OSTEOPROTEGERIN OPG STIMULATE OSTEOCLASTO-GENESIS AND BONE RESORPTION.

RANKL /OPG HIGH RATIO PRORESORPTIVE.

RANKL /OPG LOW RATIO ANTIRESORPTIVE.

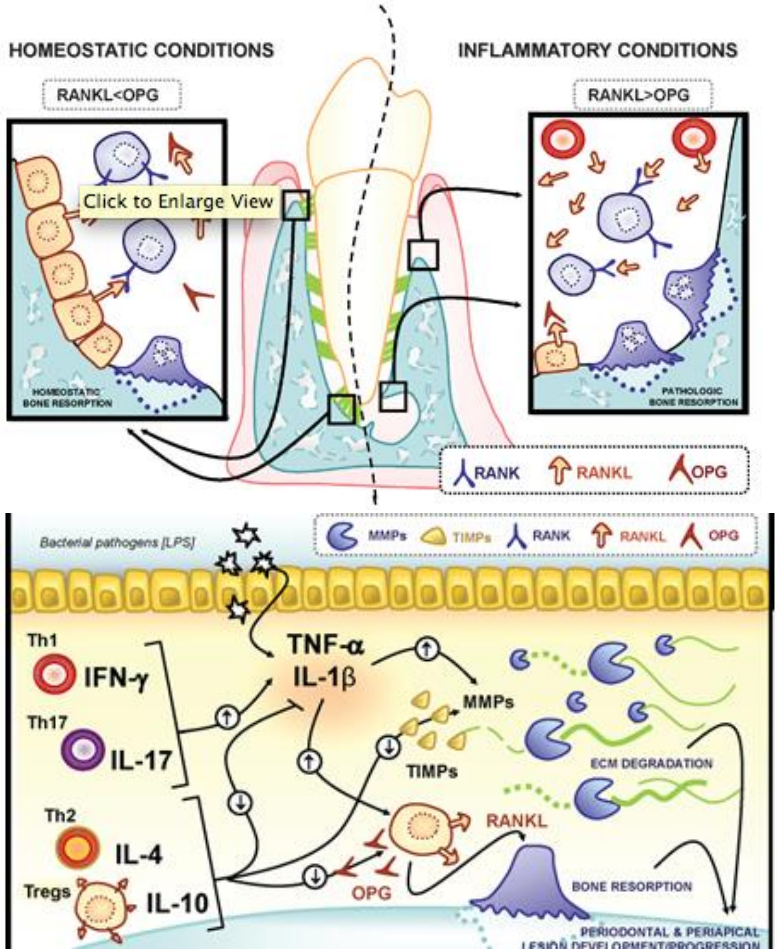
ACTIVATED T AND B CELLS CELLULAR SOURCE FOR RANKL

IFN-γ AND IL-17 INCREASE RANKL

IL-4 7 IL-10 REDUCE RANKL/OPG RATIO

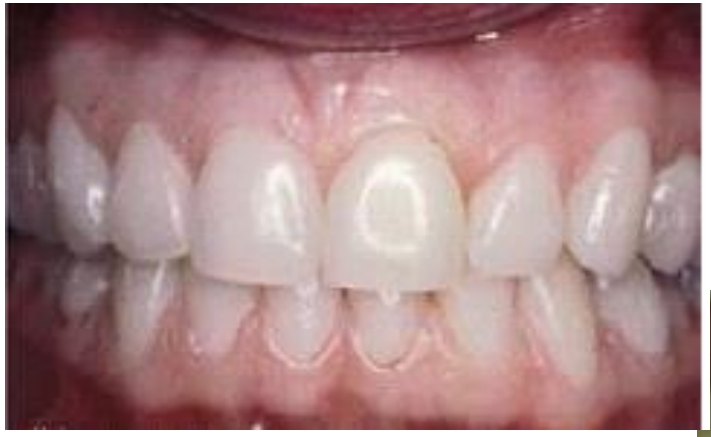
LYNPHOCYTES SUBSETS TH1 & TH17 PRO RESORPTIVE

TH2 & Tregs (Regulatory T) antiresorptive

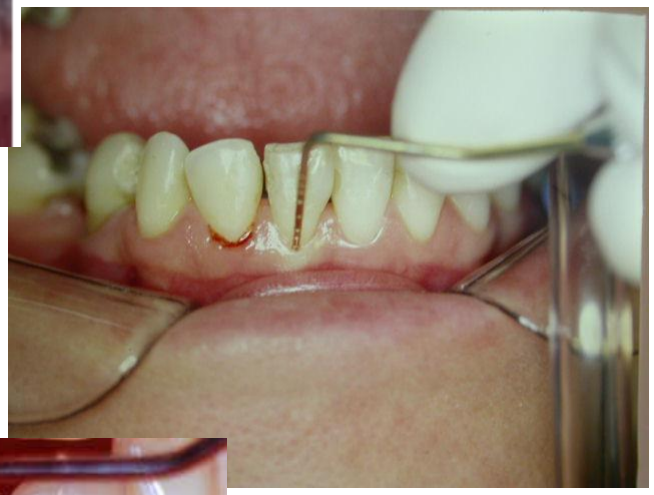


Stages of perio health/disease process

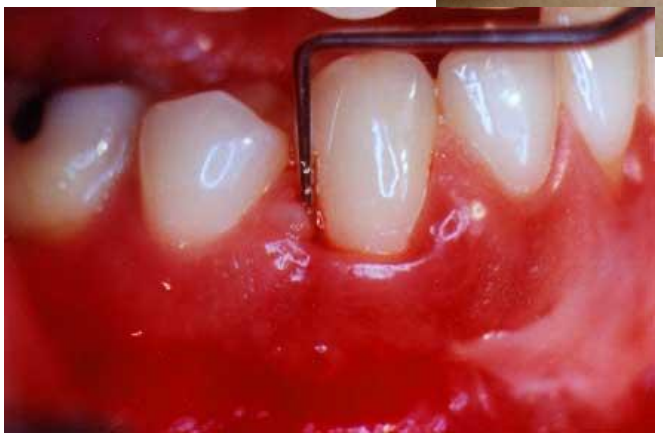
- Health



- **Gingivitis**



- **Periodontitis**



Histopathology of periodontal diseases

PRISTINE GINGIVA x Histologic Perfection

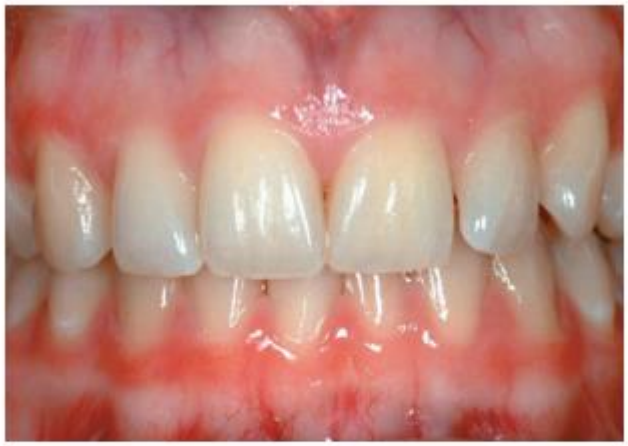


Figure 8.3 Clinically healthy gingiva in a 19-year-old female.

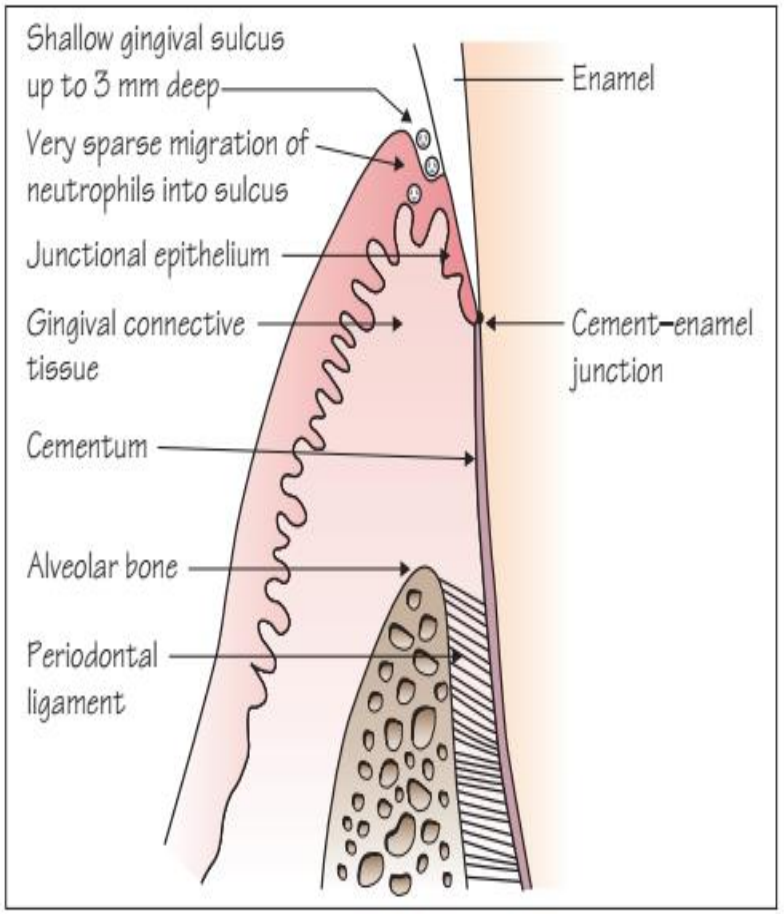


Figure 8.1 Pristine gingiva. This state of fastidious oral hygiene is rarely achieved clinically. There is very sparse neutrophil migration into the sulcus and no inflammatory response.

INITIAL x Clinically Healthy Gingiva

- Clinically healthy gingiva with a limited coronal infiltrate (5-10% of the connective tissue area)
- Neutrophils and monocytes in JE
- Lymphocytes in the connective tissue
- Increase in vascular structures near JE
- Exudative fluid from vessels to tissues= GCF

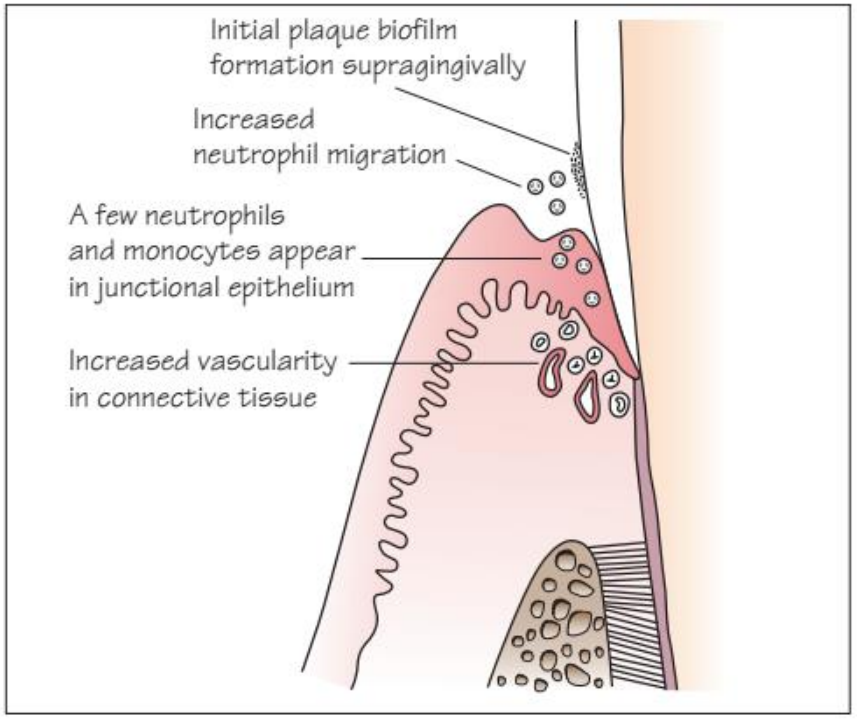
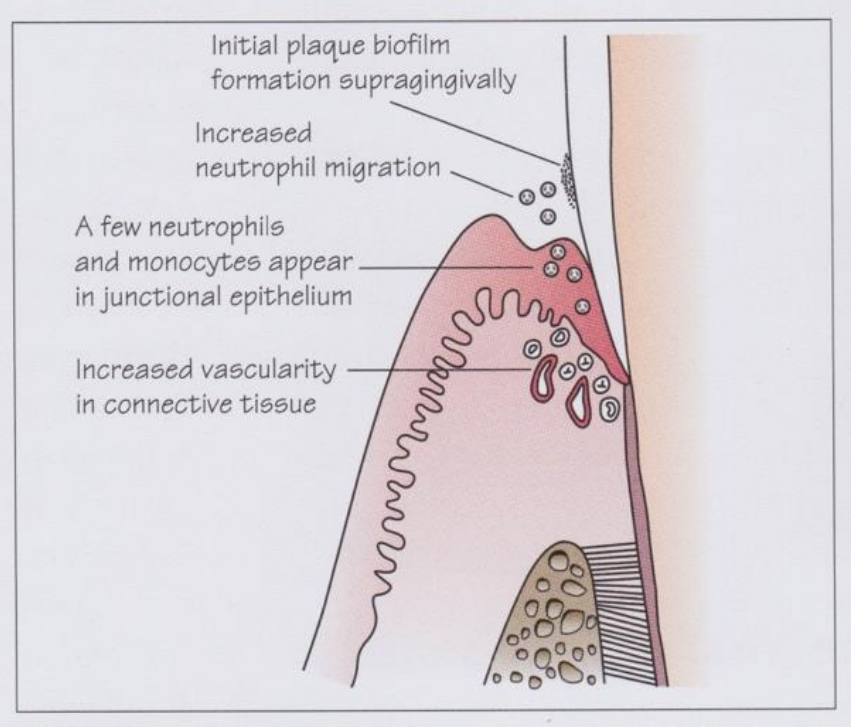


Figure 8.2 An initial lesion.

INITIAL LESION histologically = gingival health clinically

- 24 HOURS PLAQUE ACCUMULATION
- CHANGE IN MICROVASCULAR PLEXUS JE
- ARTERIOLAS, CAPILLARIES AND VENULES DILATION
- HYDROSTATIC PRESSURE INCREASE
- INCREASED PERMEABILITY
- EXUDATE OF FLUIDS & PROTEINS
- INCREASED GCF
- ENHANCED PMNS MIGRATION
- PMNS ACCUMULATE IN JE AND SULCUS



EARLY LESION x Early Gingivitis

- One week after plaque accumulation
- JE blood vessels remain dilated
- Increased in number and size of vasculature
- Infiltrate predominant PMNs and

  lymphocytes = now 15-20% volume
- Very few plasma cells
- Initial fibroblast degeneration and collagen destruction
- Proliferation of basal and JE cells
- Coronal rete pegs
- Inflammation clinically detected – bleeding – due to pocket epithelium ulceration

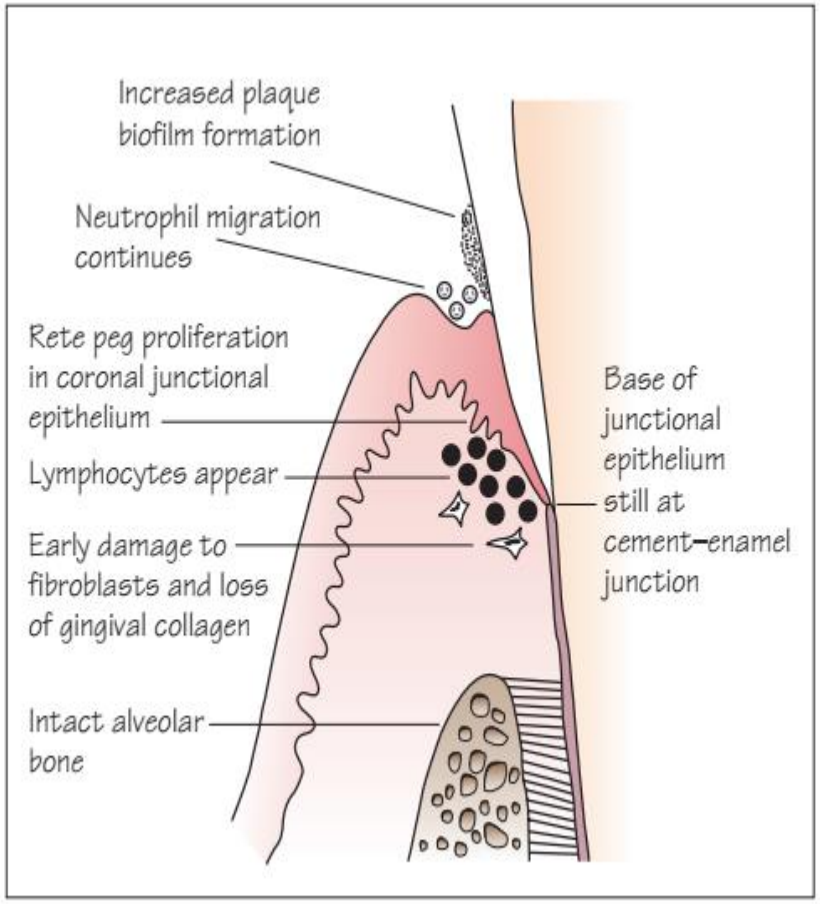


Figure 8.4 An early lesion.

ESTABLISHED LESION x Chronic or established Gingivitis

- Increased fluid and leukocyte migration
- **More edematous swelling clinically**
- Plasma cells 10-30 % on coronal connective tissue
- Collagen loss in apical and lateral directions
- Inflammatory cell infiltrate expands
- Extension of rete pegs into connective tissue
- **JE deattached to tooth surface**
- Pocket epithelium with heavy cell infiltrate PMNs
- **Permeable and ulcerated pocket epithelium**

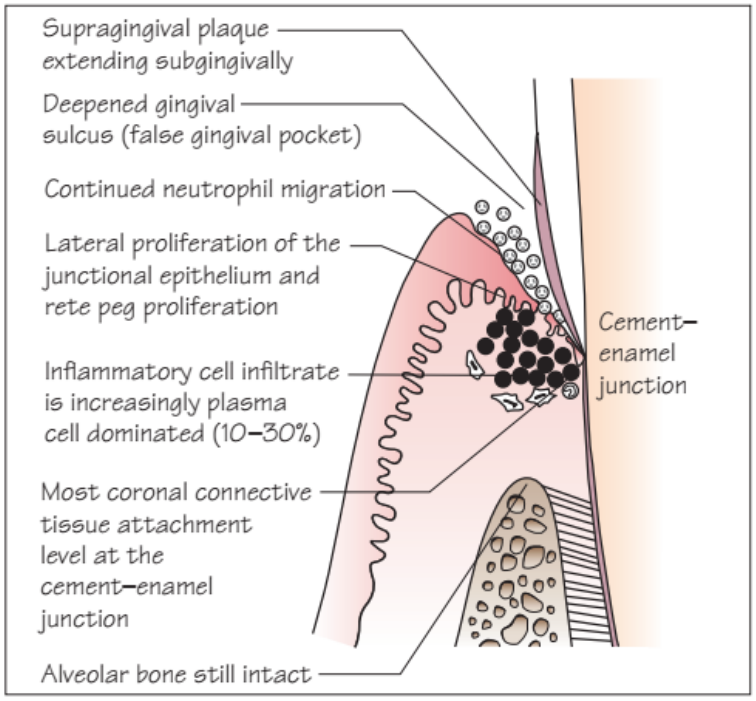


Figure 8.5 An established lesion.

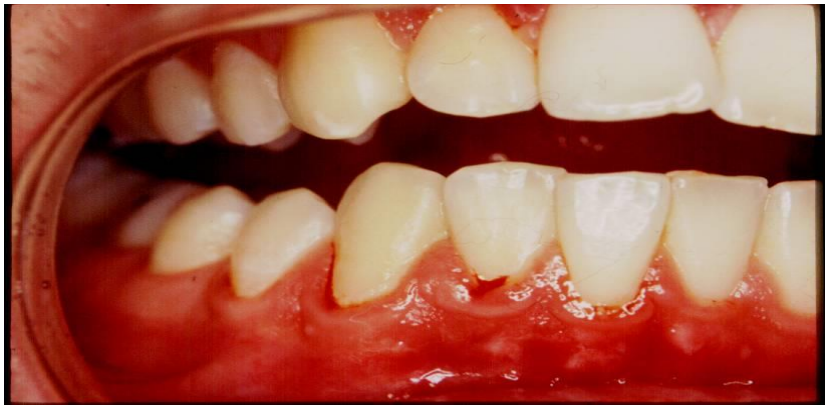


TABLE 15.2 Hallmarks of Gingivitis

Feature	Healthy Gingiva	Gingivitis
Color	Coral pink	Red
Contour	Knife-edged and scalloped	Rolled with bulbous papillae
Consistency and texture	Firm and resilient with stippling of the attached gingiva	Edematous and with loss of stippling

TABLE 15.1 Stages of Gingivitis

Stage	Time (Days)	Blood Vessels	Junctional and Sulcular Epithelia	Predominant Immune Cells	Collagen	Clinical Findings
I. Initial lesion	2–4	Vascular dilation Vasculitis	Infiltration by PMNs	PMNs	Perivascular loss	Gingival fluid flow
II. Early lesion	4–7	Vascular proliferation	Same as stage I Rete pegs Atrophic areas	Lymphocytes	Increased loss around infiltrate	Erythema Bleeding on probing
III. Established lesion	14–21	Same as stage II, plus blood stasis	Same as stage II but more advanced	Plasma cells	Continued loss	Changes in color, size, texture, and so on

PMNs, Polymorphonuclear leukocytes (neutrophils).

ADVANCED LESION x Periodontitis

Advanced lesion

- Apical plaque growth
- Apical migration of JE from CEJ
- Lateral & apical extension of infiltrate
- Alveolar bone loss starts
- Extensive collagen fiber damage
- **Plasma cells predominantly (>50%)**

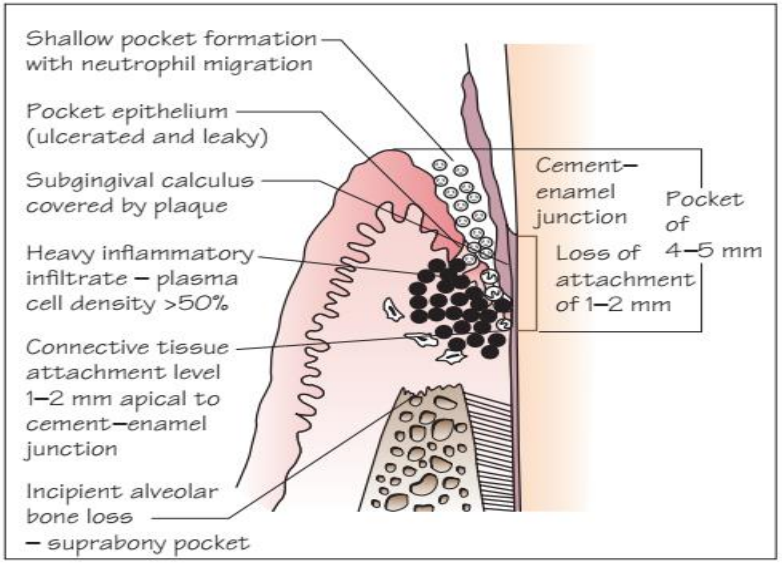


Figure 8.7 An 'advanced' lesion (note 'advanced' here is a descriptor of the stage of histological lesion not a descriptor of clinical severity) showing incipient periodontitis: true shallow periodontal pockets (4–5 mm); CAL of 1–2 mm; and incipient alveolar bone loss (horizontal), with the formation of a suprabony pocket.

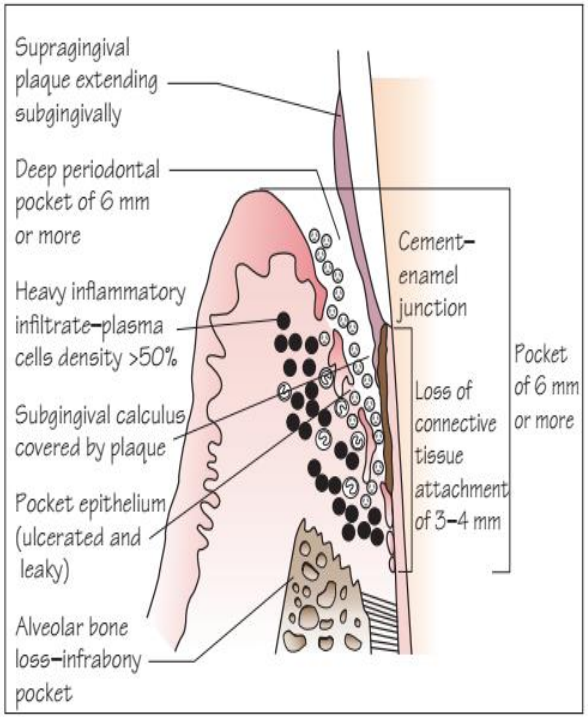


Figure 8.8 An 'advanced' lesion (note 'advanced' here is a descriptor of the stage of histological lesion not a descriptor of clinical severity) showing moderate periodontitis: typically, true deep periodontal pockets (6 mm or more) and CAL of 3–4 mm are present; alveolar bone loss may be vertical, rather than horizontal, with the formation of an infrabony pocket. Note that 5 mm or more of CAL is considered to be severe.

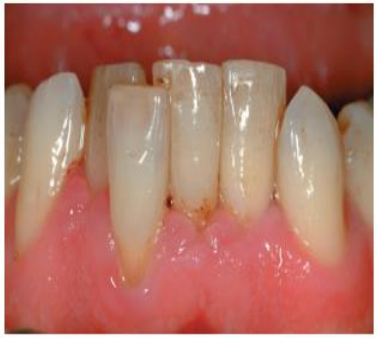


Figure 8.9 Patient with moderate chronic periodontitis showing swollen, inflamed gingivae and recession/clinical attachment loss. The gingival margins are blunted and there is loss of contour.

Thank you!!!

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