The University Of Jordan

Faculty of Dentistry

fourth Year

Periodontics

☐ slides ☐ handout ☐ sheet

Website:

http://dentistry2018.weebly.com/

LECTURE # : 6

DOCTOR :

NAME

DONE BY :

NAME

CORRECTED BY :

NAME

DAY & DATE :

PRICE :

we have something called Koch's postulate we use it to identify pathogen as an cuastve of infection diasese

it does not apply a perio disease because as we said earlier sometimes you have the bacteria but you dont have the disease ; thats why these criteria were modified

what we have to know for us to identify a pathogen as a periodontal pathogen they have to be these conditions

1.increased numbers in diseased sites

2.decreased numbers in clinical resolution

3.induce an immune response

4.causes disease in exp. animal models

5.demonstrate virulence factors

most important you need to know that we have certain bacteria well established as periodontal pathogens and the most important its AA (A actinomycetemcomitans) and porphyromonas gingivalis.

you need to know the microbiology of specific periodontal conditions ..usually when we have periodontal health most of the bacteria gram positive facultative aerobes so even in health situation you have a microflora usually its mostly streptococcus and actinomyces genera or species and vary small proption of gram negative bacteria where as start to transmission from health to diasese which start with gingivites you start seeing equal proportion of gram positive and gram negative bacteria ....as this advances in to more destructive disease the perdontites you see predominantly gram negative anaerobic bacteriawe took last time about composition of bacteria and how we have stages fromation and how we have shift if you from gram positive towards gram negative anerobic bacteria

the chronic gingivites usually it mostly anerobic gram negative bacteria we have spirochets like treponema denticola

AA..aggregatebacter

whats the name of bacteria that help in aggregation of bacteria ?

rod shaped ..fusobacterium nucleatum And peptostreptococcus micros

as you move towards more aggressive disease you have more anaerobic and gram negative bacteria .

the localized aggressive periodontitis.... usually this bacteria (AA) classically have been identified associated with localized aggressive form to the disease.sometime you dont have AA sometime other types of bacteria like b E colonies,campylobacter .

slides :***Periodontal health: (in case of periodontitis)

1. Primarily G+ve Facultative species

2. Streptococcus and Actinomyces genera

3. Small proportions G-ve species

***Gingivitis:

1. Initially -->G+ve cocci and rods & G-ve cocci

2. Later --> G-ve rods

-Equal proportions of G+ve and -ve

*** Chronic periodontitis:

1. Anaerobic (90%), G-ve bacteria (75%)

2. Spirochetes

3. Pg(p.gingivalis) , Tf, Td, Cr, Pi, Ec, Aa, Fn, Pm

*** Localized aggressive periodontitis:

1. Capnophilic(favors co2) ,

2. Anaerobic like A actinomycetemcomitans

3. Pg, Cr, Ec, ..

know you need to understand how does plaque accumulation ,the presence of plaque actually ends up alveolar bone loss (what the molecular and cellular changes that happen with alveolar bone loss ?)most of the destruction that associated with periodontal disease(bone loss) it mostly due yo host immune response .

teeth is the only hard tissue structure that penetrate the humen line(epithelial mucosal membrane) .the teeth are non sheding surface thats the proplem ,you have a microflora in muocsa and microflora in tongue but usually one of protective mechansim is sheding of surface epithilium . the teeth are non sheding surface so they give bacteria chanse to mature to change it in to more pathogeneic bacteria

what the end outcome of periodental diasese (bone loss) ?

tooth loss , so from philosophical stand point the body even in periodontitis is actually doing his job(geeting rid of sheding surface) but from apractical stand point ,i dont want to lose my teeth . you have to understand that as if the body is attaching the bone and removing the bone im losing my tooth but what really happen in the dentogingival complete as if trying to run away from plaque which ends up in bone loss and pocket formation and of course plaque is advances in the bone surface .

now we are going to talk quickly about immune response (cell, mediators) to know how accumulation of bacteria will end up with bone loss.

we also going to take about now the microbial periodontal factors cotribute to this response and mediators, now we will took quickly about two types of immunity (innate immunity and adaptive immunity)

and how all this process will end up with bone resorption and the concept of bone susceptibility and hyper inflamatory types .

Dr-showed a slide represnt periodontal disease cases and said its an inflammatory disease that causes alveolar bone destruction and loss of attachment

mast cell:

involved in immediate inflamation and usually have relation with anapylactic shock .

they usuallyhave reseptors for antibodies , usually they release vasoactive substances that cuse vasodilation exudate.

Dendritic cells :

they basically leukocyte with cytoplasm projection

Second part of lecture includes all the slides. (Any underlined sentence means that it is not mentioned in the slides).

Immune cells:-

Mast cells:-

Immediate inflammation (involved in anaphylactic shock).

It has receptors for 1) complement 2) IgG 3) IgM .

Produce vasoactive substances: vasodilation and vascular permeability. (Increase the inflammatory exudates).

Produce histamine, heparin, ECF (Eosinophil chemotactic factor), NCF (neutrophil chemotactic factor) & others.

1) Dendritic cells:-

They are leukocytes with cytoplasmic projections.

They are professional APCs (antigen presenting cells : they represent the antigens to T-cells usually).

Expresses MHC II and other cell adhesion and costimulatory molecules.

2) Neutrophils and monocytes/macrophages:-

2/3 of leukocytes, phagocytic, APC's.

PMN: phagocytic, lysosomes, receptors for complement and IgG (so they can recognize the antibody-antigen complex and phacocyte it).

Monocyte/Macrophages: blood & tissue, chronicity, receptors for complement, Ig's, MHC II & others.

3) Lymphocytes (T-cells):

Produce cytokines (mediators and signaling molecules).

After activation they are either CD4, CD8.

Recognizes antigen associated with MHC I or II on APC's.

CD4: humoral(antibodies) response / CD8: cytotoxic.

5) Lymphocytes (B-cells):

APC's.

Plasma cells & memory cells (memory for adapted immunity).

Antibodies.

Produce cytokines.

6) Lymphocytes (natural killing cells):

They are phagocytes that are involved in cellular killing.

Recognize antigens with MHC I, MHC I or other surface GP (glycoprotein).

They are responsible for auto-regulation of the immune response.

NOW we'll move to complement system:-

They are a group of glycoproteins (30 soluble or membrane-associated GP).

They have three pathways for activation (classical, lectin and alternative pathway).

They have four main functions:
- Vasoactive function.
-Anaphylactic function.
-Chemotactic function.
-Opsonization (coating the surface of the microorganism to ease the phagocytosis of it).

Activation pathways:-

Classical : starts with the antigen-antibody complex.

Lectin.

Alternative : starts with pathogen surface (mostly LPS toxin (lipopolysaccaride)).

Eventually we end up with mediators for vasodilation and chemotaxis – then we have the cell membrane complex to destroy the pathogen – and we have C3b for Opsonization mainly.

CHECK the figure below for more details.

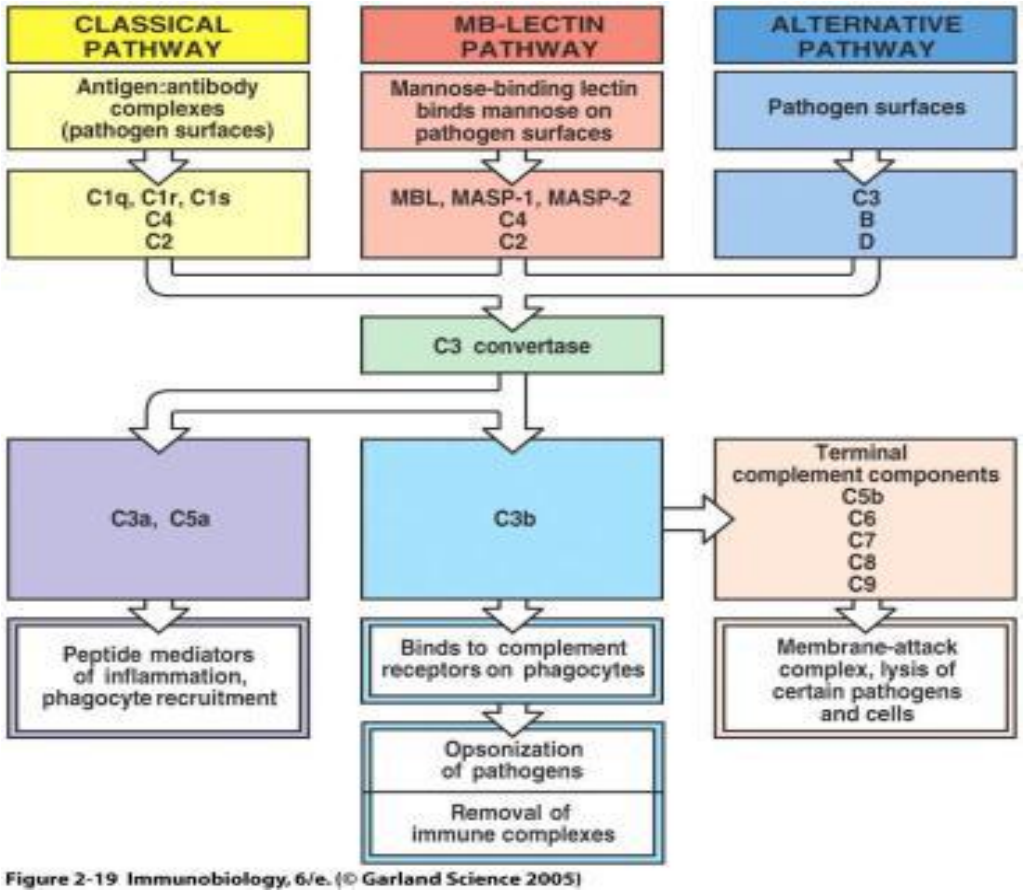


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

NOW we'll move to the leukocyte (mainly neutrophils) functions.

Chemotaxis

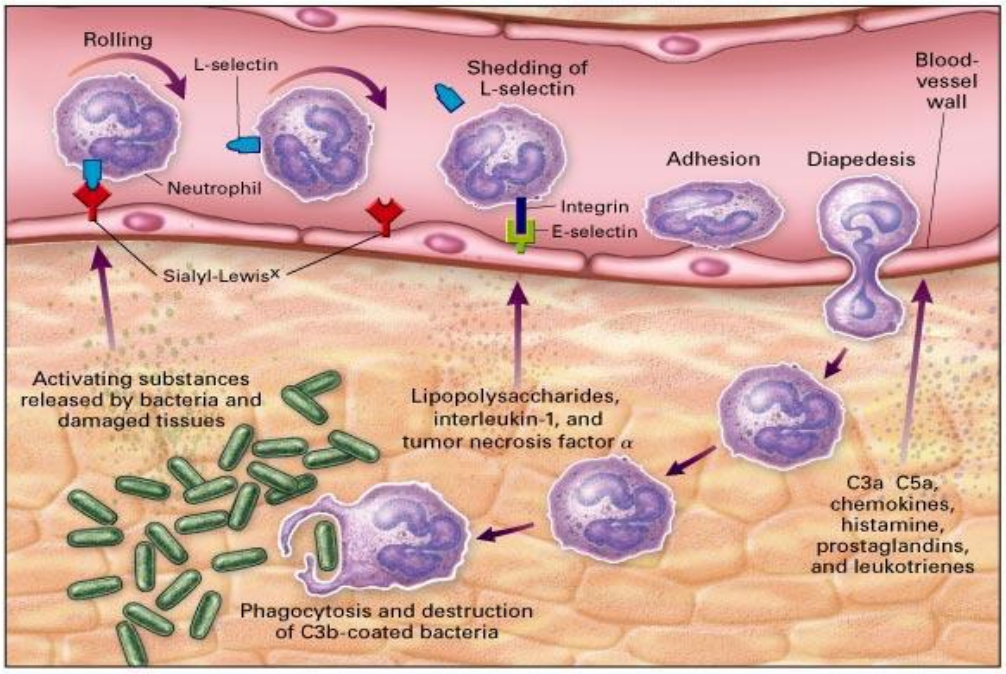
Phagocytosis

Antigen processing and presentation

Chemotaxis:-

Movement of leukocytes along a chemotactic gradient (bacterial or host derived).

Once we have release of chemotactic agents, we'l have expression of adhesive molecules on the inner side (the side inside the blood vessel), then cells from circulation will attach to them and start rolling on the endothelium until they do what is called **diapedesis** (which is getting out of the blood vessel and going towards the source of releasing chemotactic agents). And that's how Chemotaxis attract the immune cells to the site of inflammation.



Phagocytosis:-

The process by which cells ingest particles of a size visible to light microscopy.
Killing mechanisms after ingesting:

Oxidative killing (using supra-oxides that destroy the antigen)

non-oxidative killing (by lysosomes that secrete enzymes that degrade the antigen).

Antigen processing and presentation:
once they engulf the antigen, the present it on the surface associated with MHC II molecules. (humoral type of immune response).

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T-Lymphocytes, B-lymphocytes and antibodies

T lymphocytes can be of two main types (depending on its specific cell surface molecule) ; CD4 or CD8.

In periodontal disease **CD4 T lymphocytes** are the predominant type of T lymphocytes.
B-cell-dominated destructive lesion.

We don't have clinical implication on this type of information because we don't usually do sampling

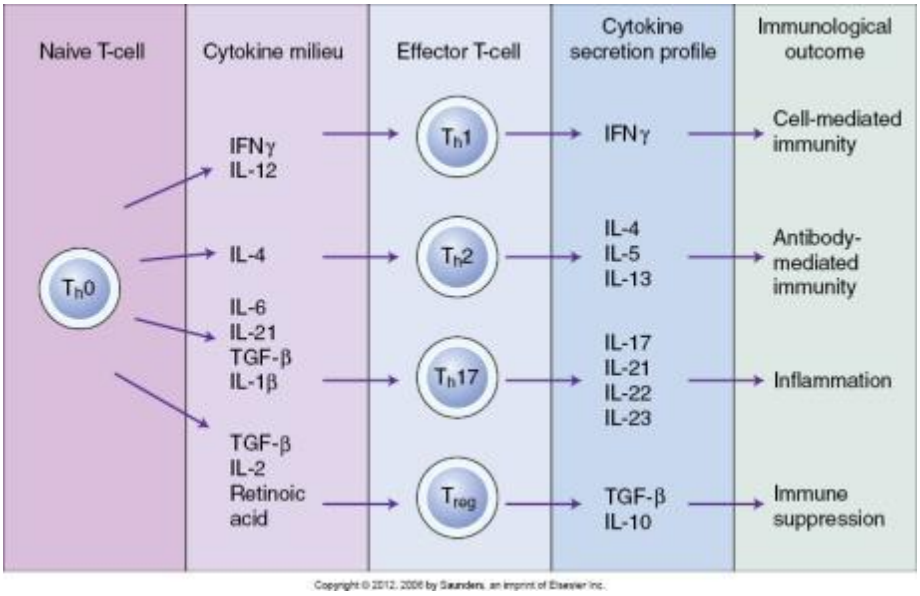
and send it to the histology lab. It's doesn't has great clinical value But from histological point of view this is the type of cell mediated immunity that we have.

Regarding T-Lymphocytes; One of the most important roles to prevent the disease; is the production of cytokines.
this cytokines will stimulate other inflammatory and immune cell.
Depending on what type of cell we get whether it was T_h1, T_h2, T_h 17, etc. and depending on what type of cytokines were released by the immune cells, the naïve T cell differentiation and maturation will be determined.

Each type of mature fully differentiated T cell has a certain group of cytokines responsible for its maturation after its activation.

The type of T helper cell produced will determine the type of immune response the body will express; T helper cells are involved in the regulation of immune response.

See this picture



B-Lymphocytes & Antibodies
Plasma cells are involved in antibody production and usually most of the antibodies seen in periodontitis are IgG, and there are some IgM and IgA.
BUT actually IgG has low biologic activity against periodontal diseases.
NOTE: it's not yet understood that the role of antidodies of B-Lymphocytes are protective or the opposite !!

Microbial Virulence factors

They are factors that induce an immune response (main role) and cause destruction of the periodontal tissue (minimal role).

So, the primary virulence is come from its ability to activate or stimulate the immune response, by:

1) LPS (Lipopolysaccharide):

-It is an endotoxin

-Usually found in the outer membrane of G-ve bacteria

- LPS activate the internal pathway of the complement system. So there will be activation of (Vasoactive function, Anaphylactic function, Chemotactic function, Opsonization) but also it will be detected by neutrophils using toll-like receptor-4 (TLR-4).

-P.gingivalis LPS is atypical in being recognized by TLR-2 and TLR-4).

- NOTE: Lipotechoic acid is present in G+ve bacteria instead of LPS (they don't have LPS). Lipotechoic acid will stimulate TLR … BUT it is not as strong as LPS.

Most of the destruction that we see in periodontal disease is due to the immune response, not to the offending agent directly (bacterial toxins and enzymes, etc.) which has a very minimal role in the destruction process.

2) Bacterial enzymes and noxious products:
that cause damage **directly** (where the host cells are damaged) in a minimal manner or **indirectly** by potentiating the immune response which is the dominating role.
These enzymes will be identified as antigens in the host.

These include:

1) **Ammonia** (NH3)

2) Hydrogen sulphide (H2S)

3) Butyric and propionic acid.

4) Proteases

5) P.gingivalis secrete certain types of proteases (which break down some matrix proteins).The most effective of which is a special set of proteases known as **Gingipains**, these are secreted by P.Gingivalis.

Microbial invasion

Where actual bacteria and microorganisms invading the cells and connective tissues.

In periodontal diseases, Microbial invasion has been shown to take place mostly with:
***P.Gingivalis** is found within epithelial tissue not inside them.
* Only **AA (Aggregatibacter actinomycetemcomitans)**, has been found **within the gingival connective tissues**.
But in fact AA is in the green complex does not indicate that it is not aggressive or dangerous (not a major bath of destruction to tissues).

***Fimbriae**: are proteinous structures that coat cells (sticky layer of glycoprotiens) that help the bacteria to attach cells.
P.Gingivalis has a special type of fibrae called **FimA** which is a very potent activator of the immune system (that identified by neutrophils and epithelial cells as well) and that is why it is considered as a virulence factor.

* **Bacterial DNA and exrta-cellular DNA:** by doing transmission of DNA that will do resistance or stimulation of immune system (from antigenic standard).

Host derived inflammatory mediators:

- **Cytokines**

- **Prostaglandins** (found in cell membranes)

- **Matrix metalloproteinases** (similar to proteases)

Cytokines
are Soluble proteins that function as messengers that transmit signals between cells, binding to receptors initiates an intra-cellular signalling cascade resulting in altering gene regulation and ultimately affecting the cell phenotype and function.

Cytokines act as signals, they are soluble proteins and when they bind to receptors they change the pathway a cell is following to another pathway.

They are:

- Produced by many cells; (epithelial cells, neutrophils,..)

- They mainly act **locally** (opposite to hormones that get produced somewhere and then acts in another place).

- They give +ve feedback; it potentiates itself; certain types of cytokines stimulate the release of other types of cytokines that inturn will also stimulate the release of other types of cytokines eventually the primary cytokines will be re-stimulated and so on.

- Significant overlap and redundancy; each individual cytokine has more than one function and its functions overlap with the functions of other cytokines.

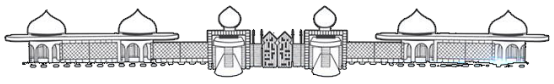
- examples of cytokines:
1) IL-1β:
*One of the most important cytokines in periodontal diseases..
*produced by almost all cells, but mainly by monocytes, macrophages and neutrophils
*elevated in sites affected by periodontal disease

2)TNF alpha (Tumor necrosis factor alpha):

- Is secreted by activated macrophages

- Induces MMP (Matrix metalloproteinase) secretion, development of osteoclasts, apoptosis of fibroblasts (which leads to lesser deposition of a matrix), leukocyte recruitment, stimulation of IL-1β & PGE2secretion.

PGE2 is very important in bone resorption and degradation of connective tissue protiens.



Prostaglandins:

- Lipid compounds derived from the degradation of Arachidonic acid found in the cell membranes of most cells.

- We have many types of prostaglandins, the most important of which is PGE2 because it activates MMPs and Osteoclasts.

- two pathways: COX-1 & COX-2

- COX-2 upregulated by IL-1β, TNFα, LPS

- PGE2 : the most important because it induces MMP's & osteoclasts.

Matrix Metalloproteinases:
- A family of Zinc-dependent proteolytic enzymes that degrade extracellular matrix molecules such as collagen, gelatin, and elastin (which are the components of connective tissue matrix)
- In the periodontium, secreted by most cells (mostly by fibroblasts and neutrophils especially MMP1,2,8,9)
-Very important for maintenance and turnover of connective tissue.
- Upregulated by IL-1β & TNFα
- Contributes to the REMODELLING of connective tissue and bone

TABLE 21-1 -- Classification of Matrix Metalloproteinases

Group	Enzyme	Name
Collagenases	MMP-1	Collagenase 1, fibroblast collagenase
	MMP-8	Collagenase 2, neutrophil collagenase
	MMP-13	Collagenase 3
Gelatinases	MMP-2	Gelatinase A
	MMP-9	Gelatinase B
Stromelysins	MMP-3	Stromelysin 1
	MMP-10	Stromelysin 2
	MMP-11	Stromelysin 3
Matrilysins	MMP-7	Matrilysin 1, pump-1
	MMP-26	Matrilysin 2
Membrane-type MMPs	MMP-14	MT1-MMP
	MMP-15	MT2-MMP
	MMP-16	MT3-MMP
	MMP-17	MT4-MMP
	MMP-24	MT5-MMP
	MMP-25	MT6-MMP
Others	MMP-12	Macrophage elastase
	MMP-19	—
	MMP-20	Enamelysin

Adapted from Hannas AR, Pereira JC, Granjeiro JM, et al: Acta Odontol Scand 65:1-13, 2007.

MMPs, Matrix metalloproteinases; *MT*, membrane type.

Innate Immunity

1.Saliva

2.Epithelial tissues : barrier, produce

TABLE 21-4 -- Constituents of Saliva that Contribute to Innate Immunity

Saliva Constituent	Host Defense Function
Antibodies (e.g., IgA)	Inhibit bacterial adherence, promote agglutination
Histatins	Neutralize LPS, inhibit destructive enzymes
Cystatins	Inhibit bacterial growth
Lactoferrin	Inhibits bacterial growth
Lysozyme	Lyses bacterial cell walls
Mucins	Inhibits bacterial adherence, promotes agglutination
Peroxidase	Neutralizes bacterial hydrogen peroxide

IgA, Immunoglobulin A; *LPS*, lipopolysaccharides.

antimicrobial agents called (*beta*-defensins)

3.GCF (Gingival crevicular fluid) : do washing, produce antibodies, complements, proteins, neutrophils)

4.Pathogen recognition: such as Toll-like receptors (TLRs) and PAMPs recognized by TLRs.
the protein surface of these pathogens is highly preserve (the protein sequence doesn't change) so TLRs and MAMPs an easily recognize them although if there is no previous exposure.

5.Neutrophil function in the sulcus of the teeth. Neutrophils are always there.

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Adaptive Immunity

T-cells

B-cells

Antibodies
========

Alveolar Bone Resorption

Osteoclast: in the past it didn't considered as part of the immune system BUT now it is called "osteomonology" and it's very important part of the immune system.

-cell responsible for bone resorption

-derived from OPC/monocytes

-resorption stimulated by wide range of mediators

-Critical factors:
1.Concentration of mediators

2.Distance from bone

- Activation of it by: RANK/RANKL/OPG pathway .. discovered in the 1990s (it is related to the post-menoposal bne resorption and bone loss in multiple myeloma)

RANK is an Acronym for : Receptor activator of NFKB
RANKL: Receptor activator of NFKB ligand
OPG: osteoprotegerin

Mechanism of action:

Pre-osteoclast has RANK receptor
RANKL binds to RANK … so the transformation of pre-osteoclast to osteoclast happen.

if OPG binds the RANKL … will prevents the RANKL to bind into RANK

So if we have more osteoprotegerin (OPG) we have less osteoclast formation .While if we have higher ratio of RANKL we have more activation of RANK Receptor.

Vit D and Estrogen increase the amount of OPG so less bone resorption . whereas glucocorticoids have osteoporosis because it decreases the availability of OPG.

Parathyroid hormone , Vit D , Glucocorticoids and inflammatory cytokines increase the amount of RANKL

Slide 65:

To put things together , we have plaque accumulation on tooth structure , bacteria (MPS) , in sulcus neutrophils , dendritic cells and antibody attached to some part of bacteria.MPS activates complement system and processed as an antigen .when the is complement System activated , it causes : 1-vasodilation (greater flow of Inflamatorycells into tissues) . 2-chemotaxis which guides the Inflamatory cells to to the insult . So greater accumulation of neutrophils.

Inflamtory cells start producing cytokines which function as : chemotaxis , vasodilators, and they also stimulate production of prostaglandins . PGs comes from cell membrane of cells. They function as chemotaxis , Vasodilatory and stimulate osteoclast. So upregulation for RANKL production by pretty much all cells but mainly by osteoblast which is very important on regulation and formation of osteoclast . Also there are enzymes produced by neotrophils and cytokines called Matrix Metalloproteinases.

These enzymes activated to degrade the matrix to have space for the accumulation of inflamtory cells in dentinogingival complex . One of the ways in measuring the intensity of the inflmation is by the size of the inflammatory cell infiltrate .As the insult persists , the inflammation gets bigger , more propagation of Inflamation response and gets closer from the bone . remember in periodontitis , you never have direct bacteria cells on the bone . there is always an intact zone of connective tissue . so initially ,resorption happen while the matrix is intact and as the disease progress destruction of this CT (matrix) will take place .So first decalsification then destruction of the matrix .

So as the inflammatory cell infiltrate gets larger in size it becomes closer from bone tissue , where immune response destroys the bone by ablation of osteoblast to RANKL and OPG factor .

Iron beta which is produced by lymphocyte, neutrophils , epithelial cells , every thing and that is famous in activating RANKL .

Slide 66

This figure talks about what we have talked about , increase pack crest , vasodilation , opsanisation , further activation of complement system , complement system activation results in further chemotaxis (+Ve feedback loop).

Typically, how we treat periodontitis ?

By removing the pathogen (bacteria)

What if understanding the Immune response we can actually modulate it ,characator it down a little bit to prevent it from being so intense so we won't have so much destruction.

There are researches not only in dentistry , but also in medicine __49:15__ are also associated __49:17__.

What if we find an inhibitor for IL-1 or an inhibitor for TNF alpha . actually there is TNF alpha inhibitor but it has not been shown to be successful in periodontitis patients, or safe enough to be used .

For example MMPs are zinc dependent so by removing the zinc they become not effective . So one of the medications given to periodontitis patient is periostat group doxycycline 20 mg which is sub antimicrobial dose (doxycycline in this dose is not effective as an antibiotic) .Doxycycline gets rid of zinc , so there is insufficient of zinc available for the enzymes to function . we are changing the type of immune response by affecting the function of these enzymes .

Why this not happen to every patient ? not every one has periodontitis even if plaque available and OH is not practicing no bone loss happen because of Host susceptibility.

Hos susceptibility has many different aspects :

1-Hyper immune trait , we have neutrophils that are exaggerate in production of cytokines .

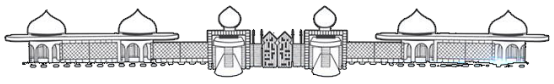
2-polymorphism : which means I have the same amount of iron 1 beta as may be you do , you have more biologic activity . so there is slight changes in protein sequence which result in more effectice in stimulating the immune response . every genetics how the (not sure of this word (environment 54:15)) affect our genes and affect translation of genes .

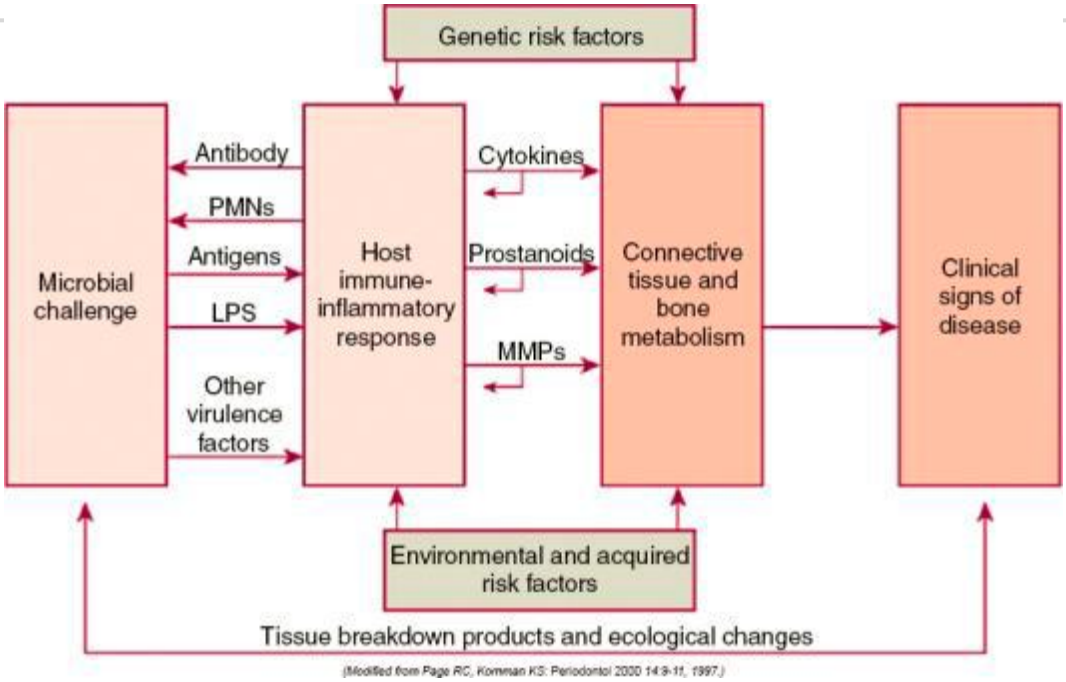
Smoking for example , when I ask you whey is it relative to periodontitis ? you always say vasoconstrictor . well it plays a very minimal role .

When smoking vasoconstrictor takes place but , once you finished reactive vasodilation happen . so it is not vasoconstriction, inhaling these toxins causes changes that affect macrophage and neutrophils which stimulate them to produce cytokines .

Slide 72 is an important slide that we should memorise and understand .

We have microbial challenge then we have host immune response . also we have all the variables genetic risk factor and envirmental like smoking risk factor and acquired risk factor




Genetic risk factors
Microbial challenge
Antibody
PMNs
Antigens
LPS
Other virulence factors
Host immune-inflammatory response
Cytokines
Prostanoids
MMPs
Connective tissue and bone metabolism
Clinical signs of disease
Environmental and acquired risk factors
Tissue breakdown products and ecological changes
(Modified from Page RC, Kornman KS: Periodontol 2000 14:9-11, 1997.)