• Mucosal & Systemic immunity of oral cavity
• Lecture (10)
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It is clear, therefore, that the tooth surface is influenced by both local salivary immune mechanisms, mediated largely through secretary IgA, and by systemic immunity involving all the varied immune components present in blood. The way in which these contributing factors interact to provide immunity within the oral cavity
• **mucosal component MALT:**

• The mucosal surfaces of the body are secretory in nature and have their own immune system, the mucosa-associated lymphoid tissue, or MALT. The main mucosal surfaces are the gastrointestinal tract (including oral mucosa and the salivary glands), the genitourinary tract, and the respiratory tract. The secretion of the mammary glands (colostrum) is also part of the MALT. This branch of the immune system is stimulated through mucosa-associated lymphoid tissue, which is organized into discrete areas in the gut known as Peyer's patches,
Figure. Follicle-associated epithelia (FAE) and M cells
Location of M cells

enterocyte

B and T lymphocytes

M cell

MØ
• just below the epithelial cells in the lamina propria. Antigen travels through specialized epithelium (M cells) to the subepithelial lymphoid tissue. The main antibody produced at these sites is secretory IgA, a dimeric immunoglobulin which acquires its secretory component during transport across the epithelium
Transport of IgA across the mucosal epithelium

IgA
poly-Ig receptor
basolateral surface
transport vesicle
luminal surface
secreted Ig
secretory component
Regulation of IgA synthesis at mucosal sites.
Mucosal inductive site

Antigen

FDC

HEV

Naive cells

MALT

Peyer's patches
Isolated lymphoid follicles (ILFs)
Appendix
Waldeyer's ring (NALT)

Mucosal effector site

Antigen

S IgA

S IgM

(IgG)

CD8

CD4

Peripheral blood

Endothelial gatekeeper function

M cell

Lymphatic vessel

Lymph node

Lymph node

DC

APC

IgG(J)

IgA+J

α/β

α/γ

HEV
Oral immunity:

Oral health is dependent on the integrity of the oral mucosa, which normally functions as an effective barrier against microorganisms. In addition, the oral mucosa is in continuity with a number of anatomical structures, if the oral defenses break down. A major area of risk is the junction between the gingival and the tooth and the various forms of periodontal disease.
CONTRIBUTORS TO ORAL HEALTH

- Integrity of oral mucosa
- Lymphoid tissue
- Saliva
- Gingival crevicular fluid
- Humoral and cellular immunity
There are several factors which may prevent penetration of intact oral mucosa by microorganisms (Fig.1).

Protective barrier:

1. Saliva
2. Keratin
3. Granular layer
4. Basement membrane
5. Leucocytes
6. Antibody
PROTECTIVE BARRIERS OF ORAL MUCOSA

I. Saliva
II. Keratin
III. Granular layer
IV. Basement membrane
V. Leucocytes
VI. Antibodies
VII. Blood vessels
Saliva is a very important component of the oral defenses, both by its mechanical washing activity and by means of the antimicrobial factors that it contains. The important antimicrobial activities and components of saliva are summarized in (Tabel.).

1. Mechanical cleaning
2. Lysozyme
3. Peroxidase
4. Lactoferrin
5. Leucocytes
6. Secratory IgA
| **Mechanical cleansing** | Muscular movements, in conjunction with saliva, maintain hygiene in accessible areas of mouth  
Swallowed microbes are inactivated in the stomach |
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<tr>
<td><strong>Lysozyme</strong></td>
<td>Bactericidal, by splitting the bond between N-acetyl glucosamine and N-acetyl muramic acid in the cell wall</td>
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<tr>
<td><strong>Peroxidase</strong></td>
<td>Heat-labile, anti-bacterial enzyme</td>
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<tr>
<td><strong>Lactoferrin</strong></td>
<td>Heat-stable protein, bacteriostatic to many micro-organisms</td>
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| **Leucocytes**           | Saliva contains many leucocytes (99% polymorphs)  
Migrate from blood via the gingival crevice                                                   |
| **Secretory IgA**        | IgA is the predominant immunoglobulin in saliva  
Produced by plasma cells within salivary glands  
Mainly in dimeric form, complexed with secretory component  
Functionally, secretory IgA prevents microbial adherence to host surfaces |
Role of Secretary IgA in oral Gavity:

Secretary IgA is by far the most important immunoglobulin in saliva. IgA is secreted by salivary gland plasma cells, two molecules of which are combined by means of a J chain (Fig.2), which is also secreted by local plasma cells. The resultant dimeric IgA is then complexes to the secretary component, synthesized by epithelial cells of the salivary acing, and the complete secretary IgA is transported into the duct lumen and thence into the mouth. Secretary IgA is more resistant to proteolysis degradation than other immunoglobulin. It probably functions by combining with micro-organisms and preventing their adherence to host surfaces.
IgA (slgA)

- VH
- VL
- CH1
- CL
- CH2
- CH3

secratory component

J chain
• **Role of Gingival crevicular fluid**

• Blood components, including leucocytes, are able to reach the oral cavity via the flow of fluid through the junctional epithelium of the gingiva. The flow of this so-called gingival crevicular fluid (GCF) increases greatly with the inflammation accompanying periodontal disease. Experiments using radiolabelled IgG, IgM, IgA and neutrophils have shown that both humoral and cellular components from blood can reach the oral cavity in GCF.
COMPONENTS OF ORAL IMMUNITY

Blood → Crevicular fluid

IgG, IgM, IgA
Proteins
Complement
Enzymes
Electrolytes
Polymorphs
B lymphocytes
T lymphocytes
Macrophages

Secretory IgA
Proteins
Enzymes
Electrolytes
Polymorphs

Whole saliva

Secretory IgA
IgG, IgA
Proteins
Enzymes
Electrolytes
Polymorphs

Salivary gland → Salivary secretion
In addition to immunoglobulin, complement components have been detected in GCF, suggesting that both the classical and alternative complement pathways may be activated in the gingival crevice. Other components include enzymes such as lysozyme, proteases and collagenases released by cells of both the host and bacteria. Specific proteases which inactivate IgA have been described.

The cellular component of GCF comprises mainly neutrophils, with small numbers of macrophages and B- and T-lymphocytes. These cells migrate continuously from the blood through the junctional epithelium into the gingival crevice. Over 80% of neutrophils in the gingival crevice are functional and can phagocytose micro-organisms.
It is clear, therefore, that the tooth surface is influenced by both local salivary immune mechanisms, mediated largely through secretary IgA, and by systemic immunity involving all the varied immune components present in blood. The way in which these contributing factors interact to provide immunity within the oral cavity is illustrated in Fig.
Immunological techniques

- the study of biological reactions relevant to the pathogenesis of disease, epidemiology, and diagnosis. Serology, the measurement of specific antibodies in serum to diagnose indirectly a specific infection, still has a major rook in clinical microbiology. Some of the most commonly used techniques involve the use of tagged, or labeled antibodies, where the label can be a fluorescent dye,
• An enzyme, or a radioactive isotope. Two of the most commonly used techniques are enzyme-linked immunosorbent assay (ELISA), and immunoblotting (Western blotting). Both techniques involve the ultimate detection of antibody-antigen complexes, where either the antigen or the antibody is being assayed. The third technique, latex agglutination (or a variation called coagglutination) is used as an extremely rapid technique for the detection of antigen or toxin in clinical specimens.
• **ELISA Principle:**

• It can be used to detect antibodies in serum or other body fluids, by binding them to an antigen coated to a solid phase (a well in a polystyrene, multi-well micro titre plate). The bound antibody can then be detected by adding a tagged second antibody, which recognizes the first as an antigen. The second antibody covalently labeled with the enzymes.
The complex of antigen, first antibody, and second antibody-enzyme conjugate is detected by adding a chromogenk substrate for the enzyme, i.e. the product of the enzyme is colored, or a different color from the substrate. The intensity of the colored produced, which is proportional to the amount of antibody bound by the antigen, can be read quantitatively in a purpose-designed micro plate spectrophotometer.
ELISA Procedure

• *The Antigen adsorbed on plastic well
• * then washed
• *Dilution of the test antibody solution are added to Antigen adsorbed onto plastic wells,
• * the complex is washed,
• *and the an enzyme-conjugated ,anti-iso- type antibody is added.
• *After that washing,
• * the enzyme substrate is added

The resulting color is measured using spectrophotometer.
The simplest form of this technique is summarized in Figar.
Steps: 1 Coat wells with antigen; 2 wash; 3 add test antibody (e.g. dilution of serum); 4 wash; 5 add enzyme conjugate (antibody to first antibody conjugated with enzyme); 6 wash; 7 add chromogenic enzyme substrate and read colour change.

- Antigen
- Test antibody
- Second antibody (anti test species antibody) conjugated with enzyme
- Colourless enzyme substrate
- Coloured product
**Enzyme Linked Immunosorbent Assay**

**ELISA**

**Identification:**
*is a method to determine the concentrations of a material in a solution.*

It was developed in 1970 and became rapidly accepted.

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**ELISA Principle:**

As its name suggests, uses an enzyme system to show the specific combination of an antigen with its antibody.

**The enzyme system consists of:**

* An enzyme which is labeled, or linked, to a specific antibody or antigen.

* A substrate which is added after the antigen antibody reaction. This substrate is acted on by the enzyme attached to the antigen antibody complexes, to give a color change.
The intensity of the color gives an indication of the amount of bound antigen or antibody.

**Figure showing principle of ELISA**

**Selecting enzyme label**
- Horseradish peroxidase
- Gall intestine alkaline phosphatase
- E. coli - D - galactosidase

**Selecting suitable substrate**
- O-phenylenediamine dihydrochloride
- P- nitrophenyl phosphate
**ELISA Types**

1) Noncompetitive binding assay or Sandwich method:
   - **A**: Antigen measuring system [Titrewell coated with antibodies; Enzyme labelled antibodies]
   - **B**: Antibody measuring system [Titrewell coated with antigens; Enzyme labelled antiantibodies]

2) Competitive binding assay [Titrewell coated with antibodies; Enzyme labelled antigens]
• **Latex agglutination**

• This is performed with microscopic latex (really polystyrene) beads which have been coated with a specific antibody. When these beads are mixed with a fluid containing the specific antigen they bind to the antigen and agglutinate, forming a visible precipitate.
Antigen-binding sites

Antibody A

Antigen

Antibody B

Antibody C

Epitopes (antigenic determinants)
Antibody-coated particles (latex or *Staphylococcus aureus*) + Antigen → Visible precipitate

Antibody-coated particles are mixed with fluid that might contain the specific antigen. If a reaction occurs a visible precipitate is produced.