Review Article

Oral Mucosal Immunology: An Overview

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Abstract

The primary function of the immune system of the mouth is to protect the teeth, jaws, gingivae and oral mucosa against infection. These host defences vary in the different oral microenvironments or domains represented by the oral mucosa, saliva and gingival crevice. This review aims to consider and contrast the main immune components in each domain and cites examples of oral diseases where the immune response is defective.

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Introduction

The primary function of the immune system of the mouth is to protect the teeth, jaws, gingivae and the rest of the oral mucosa against infection. The oral immune system is part of an extensive and specialised compartmentalised mucosa-associated lymphoid tissue (MALT).[1]

The host defences against infection vary in the different oral micro-environments or domains represented by the oral mucosa, salivary glands and saliva and the gingival crevice.

Oral Mucosa

The intact stratified squamous epithelium supported by the lamina propria presents a mechanical barrier to oral microorganisms. The continuous shedding by exfoliation of epithelial squames limits microbial colonisation of the surface. Membrane-coating granules discharged extracellularly in the granular layer, transudation of antibody through the mucosa and the barrier presented by the basement membrane contribute to mucosal defences. Intra-epithelial dendritic Langerhans cells are peripheral antigen-presenting cells which can process antigen in their MHC-Class II abundant intracellular compartments. They migrate to the regional lymph nodes to present antigenic peptides complexed to MHC-II molecules to prime naïve helper T cells. The oral epithelium also forms part of an intercommunicating network of the immune system, in which signals are regularly exchanged in dynamic interactions. Oral epithelial cells produce a range of cytokines including interleukin-1 beta (IL-Iβ), interleukin-6, tumour necrosis factor-alpha (TNF-alpha) granulocyte-macrophage colony stimulating factor (GM-CSF)[2] transforming growth factor-beta (TGF-beta) and their receptors and IL-8.[3-6] Bacteria can be a stimulus for epithelial cell production of interleukins, for example IL-6.[7] Conversely, exogenous cytokines such as IL-8 may upregulate expression of MHC-I and II antigens by epithelial cells, which may therefore function as antigen-presenting cells.[8]

Cytokines can also be secreted by macrophages, fibroblasts, dendritic cells, mast cells and intra-epithelial lymphocytes in the oral mucosa.

Saliva and Salivary Glands

The flow of saliva has a mechanical effect, flushing microorganisms from mucosal and tooth surfaces. Saliva also contains important antimicrobial agents (Table 1)[9] and patients with a significant xerostomia are prone to dental caries and candida infections.

The main specialised immunoglobulin isotype of the secretory immune system is secretory immunoglobulin A (s-IgA), the major antibody in saliva. Two IgA molecules linked by a J-chain are synthesised by plasma cells associated with salivary glands. This dimeric IgA then binds by its J-chain to a receptor for polymeric immunoglobulin (pIgR)[10,11] on the cell membrane of salivary gland epithelium. This complex is transported across the epithelial cell in an endocytic vacuole and enters the salivary duct by its

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Table 1. Antimicrobial Properties of Saliva

Antimicrobial agent	Activity
Secretory IgA (also s-IgG, s-IgM)	Inhibits adherence. Agglutinates bacteria.Virus neutralisation. IgA is the major antibody in saliva.
Lactoferrin	Iron-binding. Bacteriostatic.
Lysozyme	Effective against *S. mutans*.
Agglutinins	Glycoproteins, mucins, fibronectin, 2-microglobulin, histatins, proline-rich proteins.
Myeloperoxidase system	Bactericidal in presence of thiocyanate/halide-H_20_2.
Salivary peroxidase system	(enzyme-thioycyanate-H_20_2)
Complement (trace amounts)	C3 probably largely derived from gingival crevice fluid.
Leukocytes	>98% are neutrophils, but up to 50% may not be capable of phagocytosis.

luminal surface where cleavage of the pIgR receptor releases secretory IgA into saliva, with a portion of the pIgR receptor, the secretory piece, still attached. Of the 2 subclasses of IgA, IgA 1 and 2, IgA2 subclass predominates in secretions such as saliva.

Secretory IgA antibody blocks by "immune exclusion", inhibiting adherence of microorganisms to oral epithelium[12] or teeth. Monoclonal antibody to streptococcus mutans, a cariogenic organism, painted on the teeth in human volunteers and in animals, inhibits colonisation by the organism and could provide passive immune protection against dental caries. S-IgA can also opsonise bacteria for phagocytosis by polymorphs, activate complement by the alternative pathway, and directly neutralise some viruses.[13,14]

The plasma cells synthesising s-IgA are involved in the mucosa-associated lymphoid tissue, which forms the secretory immune system of the alimentary tract. This functions as an independent unit. The mucosa-associated lymphoid tissue (MALT) contains B & T lymphocytes whose origin, repertoire, products and probably function, are distinct.[1] S-IgA secretory precursor B cells are generated in Peyer's patches in the small bowel. They recirculate and are selectively guided by adhesion molecules expressed by mucosal postcapillary venules to home to particular secretory sites in the alimentary tract[15] including salivary glands. This independent function of the secretory immune system can be exploited diagnostically in celiac disease, in which the abnormal immune response to dietary gliadin in the small bowel is mirrored in the salivary glands and we developed an ELISA assay for salivary gliadin antibodies for the diagnosis of celiac disease.[16]

Gingival Crevice

Even in healthy gingiva, there is a continuous traffic of neutrophils from gingival capillaries into the gingival sulcus attracted by bacterial peptides from the biofilm of dental plaque and interleukin-8 from gingival epithelium.[17]

Circulating blood leukocytes accumulate in the gingival tissues in response to dental plaque. The lymphocytes first become tethered to the endothelium of high-walled post-capillary venules or other small blood vessels. This requires specific coupling of lymphocyte membrane receptor integrins such as L-selectin or LPAM-1 binding to vascular endothelial ligands such as GlyCAM-1 (glycosylation-dependent-adhesion-molecule) or MAd CAM-1 (mucosal addressin cell adhesion molecule).[18] Once tethered, the lymphocytes then roll along the endothelial surface attached by integrins such as VLA-4 (very late antigen) to fibronectin and VCAM1 (vascular cell adhesion molecule) expressed by the blood vessels. In a second phase of this transmigration of lymphocytes, LFA1-1, (lymphocyte function-associated molecule), an integrin on the non-villous surface of lymphocytes, becomes activated and adheres to endothelial cell ICAM-1 (intercellular-adhesion molecule).[19] The lymphocyte becomes flattened. Finally, LFA-1-ICAM-1 binding with PECAM-I (platelet endothelial cell adhesion molecule CD3) is also involved in diapedesis of these flattened lymphocytes between endothelial cells to exit the vessel.

Neutrophil polymorphs are induced to slow and then migrate through the blood vessel wall (diapedesis) by similar processes. In inflammation, histamine from mast cells or thrombin are released, resulting in increased expression of endothelial cell P-selectin and later E-selectin, which pair with specific ligands on the neutrophil membrane. Endothelial cell PAF-I is also upregulated and binds to a specific neutrophil receptor.

Emigration of these activated neutrophils from the blood vessels is driven by C5a fragments and leukotriene-B4. Subsequently, the inflammatory reaction is continued by macrophages elaborating a spectrum of molecules including interleukin-1 (IL-1) and tumour necrosis factor (TNF) acting on the endothelial cells which form E-selectin and P-selectin. In this later phase, neutrophil emigration is also directed by IL-8 (CXCL8) and CXCL5. MCP-1 (CCL2) is chemotactic for monocytes and is upregulated by IL-1 and TNF.

Most of the neutrophil polymorphs entering the gingival sulcus by these mechanisms are functionally active and capable of phagocytosis and the killing of micro-organisms.[20] Conversely, quantitative neutrophil deficiencies, as in neutropenias, result in uncontrolled apical extension of dental plaque and loss of periodontal attachment. Oral candida infections such as thrush are also common in neutropenia. Qualitative defects, some genetically determined, in neutrophil or monocyte

chemotaxis or phagocytosis, for example in diabetes mellitus or tobacco smoking, are also associated with aggressive forms of periodontitis.[21,22]

Activation of the Oral Immune Response

This commences with phagocytosis of antigens by macrophages and dendritic cells in lymphoid tissue or mucosal Langerhans cells. These cells process the antigens internally and present antigen peptide fragments associated with cell surface MHC-II molecules. The antigen-presenting cells first non-specifically and briefly link up with any T cells they meet by means of intercellular adhesion molecules ICAM 1 and 3 binding to LFA-1. Most T cells have surface receptors made up of a heterodimer alpha and beta chain with highly variable regions, in an immunoglobulin-like configuration, conferring antigen specificity. A minority of T cells have gamma and delta chain receptors[23] instead and this subset is relatively more numerous in sites such as the tongue. Recognition of antigen associated with the MHC-class II molecules on the antigen-presenting cell by the T cell receptor provides a first signal, but for full activation of resting T helper cells,[24] a second signal is needed from a co-stimulatory B7 (CD80 & CD86) molecule on the antigen presenting cell (APC), a ligand for CD28 on the T cell[25] and interleukin-1 from the antigen presenting cell. The helper T lymphocyte response is MHC restricted and CD4 surface molecules on the T helper cells associate with MHC-II molecules on the macrophages. T cell CD2 is also always involved in this T cell – APC interaction. The switched-on T cells now synthesise IL-2, which has an autocrine effect via specific receptors in triggering T cell proliferation. Other T cell cytokines released include IFN-gamma, granulocyte macrophage-colony stimulating factor (GM-CSF), IL-4 and TNF-beta.

The antigen-presenting cells also contribute to this phase of cytokine signalling, producing IL-1, IL-6, TNF-alpha, IL-12 and IL-15. Class I restricted T cells recognise endogenous viral or self-proteins within the target cell broken down into antigenic peptides within organelles called proteasomes.[26] These antigen peptides enter the endoplasmic reticulum and are introduced to MHC-1 molecules synthesised there.[24,27] These complexes pass through the Golgi apparatus and are carried in transport vesicles to be displayed on the cell surface.[28]

MHC-II molecules in the endoplasmic reticulum combine with polypeptide invariant chain Ii and this complex passes via the Golgi apparatus to a MIIC vesicle where the Ii is cleaved to a smaller CLIP fragment (Class II associated invariant peptide). Exogenous bacterial antigens enter the cell by endocytosis and after degradation to antigenic peptides, displace the CLIP fragment from the antigen-binding groove in the MHC-II molecules and the resulting Class II-peptide complex is then expressed on the cell surface. Helper T cells can now recognise the antigen presented by the Class II molecules.

On activation, Type I helper cells characteristically secrete TNF-alpha, IFN-gamma and IL-2 and activate macrophages and cytotoxic lymphocytes.[29] These helper cells are involved in delayed hypersensitivity reactions. They activate macrophages and promote IgG2A opsonising and complement fixing antibody formation. TH_2 cells have a different cytokine secretion profile designed for their role in providing help for the humoral immune response, particularly IgG and IgE synthesis and mucosal immunity including IgA in secretions and production of mast cells and eosinophils. Using interferon-gamma, TH_1 cells can inhibit TH_2 cell function and reciprocally TH_2 can suppress TH_1 cells with IL-10.

In HIV infection, the virus usually gains entry by infecting Langerhans cells in the vaginal or rectal mucosa and is carried to regional lymph nodes where it proliferates.[18] Via its envelope gp120 glycoprotein, the HIV virus binds to the CD4 surface molecules expressed by T helper cells and also macrophages and microglia. The depletion of CD4 T cells due to this infection correlates with the susceptibility of AIDS patients to oral opportunistic infections by candida, HSV and cytomegalovirus.

Cytotoxicity

Cytotoxic T cells have specific receptors which recognise viral antigens presented by MHC-1 on the membrane of infected cells. Other T cell ligands such as LFA-1 and CD2 help to attach the T cells to the target cell. Natural killer (NK) cells can identify viral antigens on cells lacking MHC-1 antigens, which is useful, for example, in herpes infection in which MHC-1 antigens may be suppressed. T cells and NK cells may kill target cells by discharging granules containing perforins which punch holes in the membrane of the attacked cell. Serine esterases, collectively termed granzymes, then penetrate the target cells through these pores. Some cytotoxic T cells without granules upregulate ligands which in trimerised form can in turn trimerise with Fas (CD95) TNF receptors on the target cell surface. This sends a signal through the cell membrane for the target cell to activate a family of caspase molecules, resulting in apoptosis. Antibody-dependent cytotoxicity and NK cell activity are major responses associated with mucosal lymphocytes.[30,31]

This brief overview has attempted to show how the various anatomical regions in the mouth, teeth and salivary glands have different micro-environments with specialised immune systems designed to maintain oral health. A better understanding of oral immune mechanisms from future research should lead to improved control or prevention of

viral and fungal oral infections, particularly in the immunocompromised patient, but may also suggest further measures to combat commoner oral problems such as dental caries and periodontal diseases.

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