Fundamental mechanisms of immune response to oral bacteria and the main perspectives of a vaccine against dental caries: A brief review

Mecanismos fundamentais de resposta imune contra bactérias bucais e as principais perspectivas para uma vacina anticárie: uma breve revisão

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Abstract

Dental caries may be defined as a complex multifatorial disease in that a broad group of biological, socio-economic and cultural factors interact directly or indirectly in the establishment and colonization of cariogenic microorganisms within the microbial community of the dental biofilm. Innate and adaptative immunity are two fundamental aspects of the immune system response against infections, such as dental caries. Besides, the majority of pathogenic infectious agents enter the organisms by the oral route. Consequently, the mucosal tissue, associated exocrine glands and saliva contributes to the protection of the oral cavity because contain cells responsible for antigen internalization and antibodies specific to oral bacteria. Macrophages are phagocytic cells that can internalize and kill bacteria by several mechanisms of internalization, including endocytosis, macropinocytosis and phagocytosis. *Streptococcus mutans* is the major pathogen of dental caries due to its ability to adhere and accumulate on tooth surfaces, using different virulence factors (AgI/II, Gtf, Gbps). Recent studies demonstrated protection against experimentally induced dental caries for vaccines containing intact or peptides from antigen I/II, Gtf or Gbp and vaccines containing a combination of antigens. The present review summarizes the fundamental mechanisms of host immune responses to oral bacteria and the main perspectives of a vaccine against dental caries.

Key words: Dental caries; innate immunity; adaptative immunity; immunization; mutans streptococci

Resumo

A cárie dentária pode ser definida como uma doença complexa multifatorial causada por fatores biológicos, socioeconômicos e culturais que interagem direta ou indiretamente na colonização e estabelecimento de microrganismos cariogênicos na comunidade microbiana do biofilme dentário. As imunidades inata e adaptativa são os dois aspectos fundamentais de resposta do sistema imune contra infecções, como a cárie dentária. Além disso, a maioria dos agentes infecciosos patogênicos entra no organismo por via oral. Consequentemente, o tecido mucoso, associado com as glândulas exócrinas e a saliva contribuem para a proteção da cavidade bucal por conterem células responsáveis pela internalização de antígenos ou anticorpos contra as bactérias bucais. Os macrófagos são células fagocíticas que podem internalizar e eliminar bactérias por diversos mecanismos de internalização, como a endocitose, macropinocitose e fagocitose. *Streptococcus mutans* é o principal patógeno da cárie dentária por sua habilidade em aderir e acumular nas superfícies dentárias, usando diferentes fatores de virulência (AgI/II, Gtf e Gbps). Estudos recentes têm demonstrado proteção contra cárie induzida experimentalmente utilizando vacinas contendo antígenos intactos ou peptídeos a partir de AgI/II, Gtf ou Gbps ou uma combinação de antígenos. A presente revisão sumariza os mecanismos fundamentais de resposta imune contra bactérias bucais e as principais perspectivas de uma vacina anticárie.

Palavras-chave: Cárie dentária; imunidade inata; imunidade adaptativa; imunização; estreptococos mutans

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Introduction

Dental caries is an infectious disease that occurs because of imbalance in the homeostasis between the host and microbiota. This imbalance is created by the emergence of cariogenic microorganisms in the complex community known as dental biofilm. Host and environmental factors contribute to transmission and colonization of cariogenic organisms in the dental biofilm and they may be defined as risk factors for caries development (1). The ingestion of carbohydrates mainly derived from diet, such as sucrose, associated with a poor oral hygiene, can favor bacterial accumulation in the dental biofilm. Besides this, individuals may have teeth with higher susceptibility to caries development, for example with developmental defects on enamel in primary and permanent teeth. The frequency and intensity of contact with infected subjects are initial factors in the transmission of cariogenic microorganisms. All these factors influence the composition of the dental biofilm community. The establishment of pathogenic organisms depends on the antagonisms and cooperative interactions that occur among inter-genera and inter-species during the dynamic process of microbial competition. Salivary innate and adaptative immune defenses may influence in the bacterial colonization and some disorders can affect these systems such as general immune deficiencies associated with malnutrition, inherited or medication disorders, or other factors that affect salivary flow and saliva composition (2).

Among the oral bacteria, *Streptococcus mutans* (SM) is the main pathogen of dental caries because of its capacity to accumulate, produce and tolerate extremely low pHs in the dental biofilm resulting in dental demineralization. The synthesis of the water-insoluble glucan is necessary for the accumulation of SM on the tooth surface. These glucans are synthesized from sucrose by the action of multiple glucosiltransferases (Gtfs) (3). Besides the Gtfs, SM produce glucan-binding proteins (Gbps) that contribute to the initial adherence of SM by binding to glucans synthesized in the salivary pellicle by adsorbed Gtfs. There are three non-enzymatic glucan-binding proteins: GbpA, GbpB and GbpC. One of them, GbpB has been considered an immunodominant protein in children and adults (4,5). Besides, the immunization of rats with GbpB induces the production of specific IgG and IgA antibodies that provide protection against dental caries (6).

Initial oral colonization with mutans streptococci in a child usually occurs during a "window of infectivity" between 18 and 36 months of age (7). After this period, a significant reduction of SM acquisition occurs, but little information is known about the factors that cause this phenomenon. The presence of secreted IgA specific to proteins produced by mucosal pathogens indicates that the adaptative immune system interferes in the colonization/persistence of these pathogens. Nogueira et al. (5) demonstrated that only 36% of early SM infected children (between 6 and 21 months of age) had IgA response to GbpB, while 76% of SM non-infected children of the same population matched by age, racial background, number of teeth and total salivary IgA had high levels of IgA to GbpB, during the first 24 months of age, suggesting that the antibody production against this protein may really have a protector effect against dental caries.

Macrophages, dendritic cells and B cells, known as antigens-presenting cells (APC), have an important role in the generation of antigen-specific response. Macrophages and dendritic cells located below the epithelium in the lamina propria are the first cells of the innate system to interact with microorganisms and microbial products. They are responsible by uptake, processing and presenting of peptides to T lymphocytes. The main mechanisms of internalization of antigens by macrophages are known as phagocytosis, macropinocytosis and endocytosis (8). Some species of streptococci seems to be internalized as by phagocytosis as by the macropinocytosis process (8,9).

Traditional vaccine therapy ideally induces protective immunity prior to infection with the target pathogen. As the "window of infectivity" opens in the middle of the second year of life, children might receive a caries vaccine in order to interfere in the bacterial establishment and colonization (10). Recent studies demonstrated protection against experimentally induced dental caries for vaccines containing intact or peptides from antigen I/II, Gtf or Gbp and vaccines containing a combination of antigens. The present review summarizes the fundamental mechanisms of host immune responses to oral bacteria and the main perspectives of a vaccine against dental caries.

Bacterial colonization in the oral cavity

Individuals are born with a sterile oral cavity but are colonized by maternal microbiota that can inhabit mucosal tissues and the saliva. The main components of the early microbiota are *Streptococcus salivarius* and *Streptococcus mitis*, both of which colonize an individual shortly after birth (11). These soon constitute most of the streptococci in the oral cavity, but the eruption of dentition at ~6 months of age signals a considerable change in the characteristics and distribution of the colonizing microbiota (12).

Teeth provide colonization sites for *Streptococcus sanguis* and mutans streptococci, as well as other tooth-inhabiting microorganisms. *S. sanguis* can be detected in most children by the end of the first year of life (13). Initial colonization of the mouth of a child with mutans streptococci usually occurs during a "window of infectivity" (7) between 18 and 36 months of age. However, some children can remain uninfected until the permanent dentition erupts (4).

Streptococcus mutans

Although molecular biological and cultural techniques have also incriminated other bacteria in the process and extension of dental caries in various dental habitats, *S. mutans* continues to be a public enemy, especially for early childhood dental disease. The molecular pathogenesis of mutans

streptococci involves several phases, each of which offers targets for immunological intervention. These acidogenic streptococci require the hard surfaces furnished by teeth for sustained colonization and accumulation. To colonize the oral cavity, these streptococci must first bind to pre-existing receptors within dental biofilms. Initial attachment to the tooth occurs by the interaction of bacterial proteins with host-derived components in the dental pellicle covering the tooth surface (14). These bacterial adhesines referred to as antigen I/II in *Streptococcus mutans*, bind acidic, mucin-like glycoproteins found in parotid and submandibular saliva. These components are found in salivary pellicles that coat both the tooth surface and early colonizing bacteria such as *Streptococcus sanguis* and *Actinomyces* species (14).

The ultimate pathogenicity of mutans streptococci occurs through erosion of the dental enamel by lactic acid, a metabolic end product of bacterial growth. However, significantly destructive concentrations of this acid require the substantial accumulation of these acidogenic streptococci in dental plaque. This accumulation process is initiated by the activity of extracellular glucosyltransferases (Gtf) of which several are secreted by mutans streptococci. In the presence of dietary sucrose, Gtfs synthesize several forms of high molecular weight branched extracellular glucans. Gtfs that synthesize insoluble forms of glucan (Gtf-B and Gtf-C) have been most closely associated with pathogenicity (2). These glucose polymers provide scaffolding for the aggregation of mutans and other oral streptococci through interaction with bacterial cell-associated glucan-binding proteins. Furthermore, glucans modify the porosity of the dental biofilm, thus increasing the availability of nutrients for continued bacterial metabolism. Some glucan-binding proteins have been described in mutans streptococci such as GbpA, GbpB, GbpC and GbpD (3). These glucan-binding proteins (Gbps) have the ability to bind to certain forms of glucan and some have been shown to be cell-associated (1). One of them, GbpB has been considered an immunodominant protein in children and adults (4,5,15). The interactions of glucans with cell-associated glucan-binding domains of Gtfs and Gbps combine to cause extensive accumulation of mutans streptococci in dental biofilm. Since Gtfs and Gbps are also secreted into the extracellular environment, their specific or nonspecific incorporation into the salivary pellicle would also provide binding sites for mutans streptococci (14).

Innate and adaptative immunity

The immune system is organized in terms of cells and molecules that have specialized roles for defending against infection. Innate (natural) immunity and adaptative (acquired) immunity are two fundamental aspects of the immune system response to eliminate microorganisms (16). The mammalian innate immune system serves as a powerful barrier to invading bacterial pathogens by employing direct antimicrobial mechanisms, and indirectly, by stimulating the potent and antigen-specific adaptative immune response. The innate system is likely protective against many opportunistic pathogens and may increase the infections dose of a successful pathogen required for symptomatic disease to occur (17). Innate immunity lies behind most inflammatory responses; these are triggered in the first instance by macrophages, polymorphonuclear leukocytes, and mast cells through their innate immune receptors (18). The components through the sensing structures ("pattern recognition receptors" – PRRs) initiate innate immunity responses. An important class of these molecules are the Toll-like receptors (TLRs), which enable mammalian cells to recognize bacterial lipopolysaccharides (LPS) and other characteristic microbial molecules ("pathogen-associated molecular patterns" – PAMPs) (19).

Adaptive immune responses are mediated by the generation of antigen-specific T and B cells. Antigens induce T cell in effector T cell that produce various cytokines or elicit cytolysis to eliminate target cells. On the other hand, B cells secrete immunoglobulins, which are responsible for eliminating extracellular microorganisms (16). T lymphocytes are primarily responsible for cell-mediated immunity, and B lymphocytes are responsible for humoral immunity, but they work together and with other types of cells to mediate effective adaptative immunity. Along with the natural killer cells, these specialized lymphoid cells are derived from committed progenitors in hematopoietic tissues, which then undergo rearrangements of their antigen receptors to become clonally diverse lymphocytes. Newly formed T and B lymphocytes bearing autoreactive receptors can be eliminated by self-antigen contact in the thymus and bone marrow, respectively. The surviving T and B cells then migrate via the blood-stream to peripheral lymphoid tissues, where following antigen recognition, they may undergo clonal expansion and differentiation into effector T lymphocytes or antibody-producing plasma cells or otherwise become memory cells that await re-exposure to their specific antigens (20).

Defense mechanisms of the oral tissues to resist microbial invasion

Various mucosal surfaces evolved a broad array of protective mechanisms to resist microbial invasion. The majority of pathogenic infectious agents enter the organisms by the mucosal route. Consequently, to cope with the enormous and highly variable antigenic load, resident cells are involved in uptake, processing and presentation of antigens, production of antibodies and cell-mediated defenses are strategically distributed at the front line of defense – the mucosal tissues and associated exocrine glands (19). The parotid duct transports saliva from the gland into the oral cavity. The lamina propria of this gland presents granulocytes, T lymphocytes and macrophages distributed in the lamina propria and the epithelium and were encountered in the subepithelial connective tissue close to the oral cavity (21). Moreover, the saliva contributes to the protection of the oral environment. Saliva contains

several types of antimicrobial peptides and proteins (AMPs) including peroxidases, lactoferrin, lysozyme, histatins, phospholipaseand, calprotectin that mediate the innate immune response. Recently, another group of antimicrobial peptides called defensins (α- and β-defensins) has been detected in saliva (22).

The majority of immunologically active cells of oral tissues are the same present in tissues of the gastrointestinal tract (19). Macrophages and dendritic cells located below the epithelium in the lamina propria are the first cells of the innate system to interact with microorganisms and microbial products. They play an important role in the protection of the host against foreign pathogens and regulation of the response to commensal bacteria. Macrophages are professional phagocytic cells that can internalize and kill bacteria by several mechanisms, some of which are part of the innate immunity such as phagocytosis, macropinocytosis and endocytosis (8), while others require the presence of specific antibodies against the bacterium and should be considered part of the effector arm of specific, acquired immunity. Bacteria that are new to the host may activate the complement system by the alternative pathway, resulting in their opsonization and in a most efficient form of phagocytisis. Macrophages may also serve as antigen-presenting cells – APC in the essential initial steps of the induction of acquired immunity. They process the antigen and present it to the T-helper lymphocytes of an MHC II molecule on the macrophages. Moreover, macrophages are considered a main source of the cytokines IL-1α, IL-1β and TNFα, which contribute to the initiation and regulation of the inflammatory process (23).

Mechanisms of pathogen internalization by macrophages

Endocytosis

Endocytosis is a process by which cells internalize portions of plasma membrane, including associated proteins and a portion of the extracellular space. It occurs in all eukaryotic cells, and is the route by which cells absorb nutrients, recycle membrane proteins and lipids, receive some chemical and molecular signals, and down-regulate membrane receptors in response to ligands and/or environmental changes (24). All eukaryotic cells exhibit one or more forms of endocytosis. In mammalian cells, wherein endocytosis is most thoroughly characterized, several distinct endocytic pathways for internalization exist. These pathways include the multimeric protein clathrin, which consists of three heavy chain and three light chains noncovalenty bound to form a symmetric complex termed a triskelion, these triskelia can polymerize to form rounded baskets that coat invaginated pits and vesicles (25).

Micropinocytosis × Macropinocytosis

Eukariotic cells have the capacity to internalize fluid (pinocytosis) and particles (phagocytosis) from the extracellular environment. Micropinocytosis, operates is all cells, produces small vesicles <200 nm in diameter and is important for internalization of plasma membrane localized receptors. It has been demonstrated to be regulated by clathrin and other regulatory proteins. The other major fluid internalization process, macropinocytosis, functions under more restrictive conditions in a limited number of cells, such as dendritic cells, and generates large vesicles > 1 μm in diameter (26). Macropinocytosis and clathrin-mediated endocytosis differ in several important respects. Macropinosomes are large endocytic vesicles that form primarily at sites of ruffling, usually at the margins of spread cells, and are heterogeneous in size, sometimes as large 5 μm in diameter. By contrast, coated pits appear more or less uniformly over cell surface and are restricted to a uniform size by the clathrin coat (85-110 nm in diameter). This coat, together with associated proteins, also concentrates some receptors for growth factors and nutrients into vesicles (27).

Phagocytosis

Phagocytosis involves the entry of large particles, typically 1 μm or more, including particles as diverse as inert beads, apoptotic cells, and microbes (28). In mammalian phagocytes, the study of phagocytic pathways has been focused mainly on those triggered by the Fc receptors and complement receptors, which mediate, respectively, phagocytosis of antibody-opsonized and complement-opsonized particles (29). Integration of these signaling events triggers a dynamic process of cytoskeletal rearrangement accompanied by membrane remodeling at the cell surface that leads to engulfment (30). Membrane remodeling leads to the complete wrapping of the particles and their release in the cytoplasm as novel membrane-bound organelles: the phagosomes. Newly formed phagosomes are unsuited to perform their basic tasks, the killing and degradation of their content, and acquire these properties by a complex maturation process characterized by the sequential fusion with endosomes of increasing age and ultimately with lysosomes. In addition to acidification, one of the hall-marks of phagosome maturation is the acquisition of hydrolytic enzymes, wich digest and degrade the content of the internalized particle (31).

Mechanisms of internalization of oral bacteria by macrophages

Unfortunately, there is little information about the mechanisms of internalization of oral bacteria by macrophages. Mechanisms of phagocytosis has been described in group A streptococcal, human pathogens which infection cause a variety of pyogenic infections that can be mild, such as pharyngitis, impetigo, and erysipelas, to extremely severe, such as cellulites, necrotizing fasciitis, septicemia, pneumonia, meningitis, and streptococcal toxic shock syndrome (32). Macrophages efficiently take up and kill *Streptococcus pyogenes* during *in vivo* infection (33). However, sometimes GBS can enter in macrophages and survive (9). In another study, *Streptococcus gordonii* (one of the most common streptococcal species currently recognized as etiological agents of infective endocarditis)

survive in polymorphonuclear following adhesion-mediated phagocytosis (34).

In relation to other mechanisms of internalization by macrophages, there was found some examples in non-oral bacterial species. *Mycobacterium avium* invades the host by translocatting across the mucosal membrane. These bacteria invade macrophages by macropinocytosis mechanism (35). *Sphingomonas* are environmental organisms that have recently been implicated in a variety of community-acquired and nosocomial infections. Mechanisms of cell interaction and entry into epithelial cells were investigated by electron microscopy, immunofluorescence, and biochemical inhibitors and this study showed that the microorganism *Sphingomonas* is internalized in part via a macropinocytosis. Periodontal microbial pathogens can be internalized by nonprofessional phagocytes, such as epithelial cells. Internalization of putative periodontal pathogens as *Prevotella intermedia* (36), *Porphyromonas gengivalis* (37), *Actinobacillus actinomycetemcomitans* (38), and *Fusobacterium nucleatum* (39) has been observed by epithelial cells *in vitro*. *Streptococcus mutans* organisms are occasionally from the blood of patients with infective endocarditis, though the mechanisms of invasion and survival remain to be elucidated. *S. mutans* serotype K strains are present in the oral cavity in humans and may be able to survive longer in blood owing to their low susceptibility to phagocytosis (40). In a study in our laboratory, preliminaries results (data not yet published) demonstrated that different genotypes of *Streptococcus mutans* may be internalized by the three mechanisms of internalization by murine macrophages, observed in transmission electron microscopy (TEM).

Streptococcus mutans × adaptative immune system

The saliva of newborns is devoid of secretory IgA, however the concentration of secretory IgA rapdidly increases and is close to that of adults by 1-2 years of age and certainly by 4-7 years (4). Therefore, colonization with oral bacteria occurs in a mucosal environment that is immunologically responsive to infections challenge. For example, by 3-5 weeks of age, salivary IgA responses specific for microorganisms that colonize the oral cavity at this time (such as *S. mitis* and *S. salivarius*) can be detected (4). By 12 months of age, both secretory IgA1 and secretory IgA2 specific for antigens of early colonizing streptococci are present. The IgA antibody responses to *S. mutans* antigens were characterized in 21 pairs of 5- to 13-month-old children. This study provides evidence that a robust natural response to *S. mutans* antigens can be archived by 1 year of age and that IgA antibody specificities may be critical in modulating initial *S. mutans* infection (5).

Immunization against dental caries

The principle of immunization against dental caries was first described by Bowen in 1969 (41). He showed that monkeys that were immunized intravenously with *S. mutans* developed little carious disease (41). Following this principle, another studies used rats that were immunized subcutaneously in the vicinity of the salivary glands with mutans streptococci that induced salivary secretory Ig A responses. The levels of IgA were correlated with a reduction in the number of bacteria recovered after infection, as well as a reduction in the development of subsequent disease. Parenteral immunization with a mutans streptococci protein antigen induced detectable specific serum IgG in rats and experiments with intraductal immunization of non-human primates with intact mutans streptococci induced salivary IgA and marked reduction in dental caries. Several cell-surface antigens of mutans streptococci and their recombinant fragments have been studied as possible candidates for dental caries vaccines (42). Besides, synthesis and the sequencing of virulence genes have led to the development of synthetic peptide vaccines. The main cell-surface substances are antigen I/II-Pac, glucosyltransferases (Gtfs) and glucan-binding proteins (42). Some studies demonstrated protection against experimentally induced dental caries for both vaccines containing a single antigen – antigen I/II (43), intact GTF (4) or Gbp (44) and vaccines containing a combination of antigens – a Gtf-glucan conjugate: Gtf and antigen I/II administered as a mixture or a fusion protein (45) diepitopic constructs of peptides derived form the catalytic and glucan binding regions of Gtf (46) and diepitopic construction of peptides derived from the catalytic region of Gtf and a 20-amino-acid peptide derived from the N-terminal region of GbpB (47).

This active vaccination has the advantage of inducing the endogenous production of salivary antibodies and the establishment of immune memory but requires a commitment to performing the human trials necessary to establish safely and efficacy. Because of this, passive immunization techniques, using transfer of milk antibody specific for mutans streptococci from mother to suckling infant have been investigated in animal models and shown to be useful to protect against dental caries. Positive results were observed with dietary antibody supplements, including chicken-egg-yolk antibody specific for S. mutans Gtf (48) or GbpB (44) or topic application of monoclonal antibody (49). Other studies, using transgenic plants to produce artificial antibodies, have been developed (50). Ma et al. (50) used human secretory Ig A specific for antigen I/II obtained by a transgenic tobacco plant in humans and verified that after treatment with chlorexidine and passive application of these antibodies, humans remained free of mutans streptococci for 4 months or longer. Passive immunization of preformed exogenous antibodies offers the advantage of evading risks, however, it is necessary to provide a continuous source of antibodies to maintain protection over a prolonged time. Ideally, the target population for a vaccine against dental caries should be infants aged around 12 months, because of the natural history of oral bacterial colonization and the salivary immune response of young children (14).

References

1. Mattos-Graner RO, Li Y, Caufield PW, Duncan M, Smith DJ. Genotypic diversity of mutans streptococci in Brazilian nursery children suggests horizontal transmission. J Clin Microbiol 2001;39:2313-6.
2. Mattos-Graner RO, Smith DJ. The vaccination approach to control infections leading to dental caries. Braz. J Oral Sci 2004;3: 595-608.
3. Smith DJ, Shoushtari B, Heschel RL, King WF, Taubman MA. Immunogenicity and protective immunity induced by synthetic peptides associated with a catalytic subdomain of mutans group streptococcal glucosyltransferase. Infect Immun 1997;65: 4424-30.
4. Smith DJ, King WF, Akita H, Taubman MA. Association of salivary immunoglobulin A antibody and initial mutans streptococcal infection. Oral Microbiol Immunol 1998;13:278-85.
5. Nogueira RD, Alves AC, Napimoga MH, Smith DJ, Mattos-Graner RO. Characterization of salivary IgA responses in children heavily exposed to the oral bacteria Streptococcus mutans: influence of specific antigen recognition in infection. Infect Immun 2005;73:5675-84.
6. Smith DJ, Taubman MA. Experimental immunization of rats with a Streptococcus mutans 59-kilodalton glucan-binding protein protects against dental caries. Infect Immun 1996;64:3069-73.
7. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res 1993;72:37-45.
8. von Delwig A, Bailey E, Gibbs DM, Robinson JH. The route of bacterial uptake by macrophages influences the repertoire of epitopes presented to CD4 T cells. Eur J Immunol 2002;32:3714-9.
9. Valentin-Weigand P, Benkel P, Rohde M, Chhatwal GS. Entry and intracellular survival of group B streptococci in J774 macrophages. Infect Immun 1996;64:2467-73.
10. Smith DJ. Caries vaccines for the twenty-first century. J Dent Educ 2002;67:335-49.
11. Pearce C, Bowden GH, Evans M, Fitzsimmons SP, Johnson J, Sheridan MJ et al. Identification of pioneer viridans streptococci in the oral cavity of human neonates. J Med Microbiol 1995;42:67-72.
12. Kononen E, Asikainen S, Saarela M, Karjalainen J, Jousimies-Somer H. The oral gram-negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth. Oral Microbiol Immunol 1994; 9:136-41.
13. Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin JM. Natural history of Streptococcus sanguinis in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun 2006;8:4018-23.
14. Smith DJ. Caries vaccines for the twenty-first century. J Dent Educ 2003;67:1130-9.
15. Smith DJ, Akita H, King WF, Taubman MA. Purification and antigenicity of a novel glucan-binding protein of Streptococcus mutans. Infect Immun 1994;62:2545-52.
16. Azuma M. Fundamental mechanisms of host immune responses to infection. J Periodontol Res 2006;41:361-73.
17. Radtke AL, O`Riordan. Intracellular innate resistance to bacterial pathogens. Cell Microbiol 2006;8:1720-9.
18. Janeway CAJr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197-216.
19. Tlaskalova-Hogenova H, Tuckova L, Mestecky J, Kolinska J, Rossmann P, Stepankova R et al. Scand J Immunol 2005;62:106-13.
20. Pancer Z, Cooper MD. The evolution of adaptative immunity. Annu Rev Immunol 2006;24:497-518.
21. Kutta H, May J, Jaehne M, Münscher A, Paulsen FP. Antimicrobial defense mechanisms of the human parotid duct. J Anat 2006;208:609-19.
22. Abiko Y, Nishimura M, Kaku T. Defensins in saliva and the salivary glands. Med Electron Microsc 2003;36:247-52.
23. Metzger Z. Macrophages in periapical lesions. Endod Dent Traumatol 2000;16:1-8.
24. Baggett JJ, Wendland B. Clathrin function in yeast endocytosis. Traffic 2001;2:297-302.
25. Ungewickell E, Branton D. Assembly units of clathrin coats. Nature 1981;289:420-2.
26. Cardelll J. Phagocytosis and Macropinocytosis in Dictyostelium: phosphoinositide-based processes, biochemically distinct. Traffic 2001;2:311-20.
27. Hewlett LJ, Prescott AR, Watts C. The coated pit and macropinocytic pathways serve distinct endosome populations. J Cell Biol 1994;124:689-703.
28. Jutras I, Desjardins M. Phagocytosis: At the crossroads of innate and adaptative immunity. Annu Rev Cell Dev Biol 2005;21:511-27.
29. Swason JA, Hoppe AD. The coordination of signaling during Fc receptor-medaited phagocytosis. J Leukoc Biol 2004;76:1093-1103.
30. Brumell JH, Grinstein S. Role of lipid-mediated signal transduction in bacterial internalization. Cell Microbiol 2003;5:287-97.
31. Silverstein AM. Darwinism and immunology: from Metchnikoff to Burnet. Nat Immunol 2003;4:3-6.
32. Efstratiou A. Group A streptococci in the 1990s. J Antimicrob Chemother 2000;45:3-12.
33. Goldmann O, Rohde M, Chhatwal GS, Medina E. Role of macrophages in host resistance to group A streptococci. Infect Immun 2004;72:2956-63.
34. Lee SY, Cisar JO, Bryant JL, Eckhaus MA, Sandberg AL. Resistence of Streptococcus gordonii to polymorfonuclear leukocyte killing is a potencial virulence determinant of infective endocarditis. Infect Immun 2006;3148-55.
35. Bermudez LE, Petrofsky M, Sangari F. Intracellular phenotype of Mycobacterium avium enters macrophages primarily by a macropinocytosis-like mechanism and survives in a compartment that differs from that with extracellular phenotype. Cell Biol Int 2004;28:411-9.
36. Dorn BR, Leung KL, Progulske-Fox A. Invasion of human oral epithelial cells by Prevotella intermedia. Infect Immun 1998;66:6054-7.
37. Lamont RJ, Chan A, Belton CM, Izutsu KT, Vasel D, Weinberg A. Porphyromonas gingivalis invasion of gingival epithelial cells. Infect Immun 1995;63:3878-85.
38. Meyer DH, Lippmann JE, Fives-Taylor PM. Invasion of epithelial cells by Actinobacillus actinomycetemcomitans: a dynamic, multistep process. Infect Immun 1996;64:2988-97.
39. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, Genco RJ. 2000. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun 2000;68:3140-6.
40. Nakano K, Nomura R, Nakagawa I, Hamada S, Ooshima T. Demonstration of Streptococcus mutans with a cell wall polysaccharide specific to a new serotype, k, in the human oral cavity. J Clin Microbiol 2004;42:198-202.
41. Bowen WH. A vaccine against dental caries. A pilot experiment in monkeys (Macaca irus). Br Dent J 1969;126:159-60.
42. Koga T, Oho T, Shimazaki Y, Nakano Y. Immunization against dental caries. Vaccine 2002;20:2027-44.
43. Hajishengallis G, Russell MW, Michalek SM. Comparison of an adherence domain and a structural region of Streptococcus mutans antigen I/II in protective immunity against dental caries in rats after intranasal immunization. Infect Immun 1998;66:1740-3.
44. Smith DJ, King WF, Godiska R. Passive transfer of immunoglobulin Y antibody to Streptococcus mutans glucan binding protein B can confer protection against experimental dental caries. Infect Immun 2001;69:3135-42.
45. Zhang P, Jespersgaard C, Lamberty-Mallory L, Katz J, Huang Y, Hajishengallis G et al. Enhanced immunogenicity of a genetic chimeric protein consisting of two virulence antigens of *Streptococcus mutans* and protection against infection. Infect Immun 2002;70: 6779-87.

46. Taubman MA, Holmberg CJ, Smith DJ. Diepitopic construct of functionally and epitopically complementary peptides enhances immunogenicity, reactivity with glucosyltransferase, and protection from dental caries. Infect Immun 2001;69:4210-6.

47. Smith DJ, King WF, Rivero J, Taubman MA. Immunological and protective effects of diepitopic subunit dental caries vaccines. Infect Immun 2005;73:2797-804.

48. Hamada S, Horikoshi T, Minami T, Kawabata S, Hiraoka J, Fujiwara T et al. Oral passive immunization against dental caries in rats by use of hen egg yolk antibodies specific for cell-associated glucosyltransferase of *Streptococcus mutans*. Infect Immun 1991;59:4161-7.

49. Lehner T, Caldwell J, Smith R. Local passive immunization by monoclonal antibodies against streptococcal antigen I/II in the prevention of dental caries. Infect Immun 1985;50: 796-9.

50. Ma JK, Hikmat BY, Wycoff K, Vine ND, Chargelegue D, Yu L et al. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med 1998;4:601-6.