Microbiological Aspects and Immunity Response of Bacteria Causing Pulpitis

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Abstract

Oral cavity is a very wide distributed ecosystem in which several hundred microbial species normally cohabit harmoniously. Under special conditions some micro-organism with a potential is promoted, leading to inflammation & infection by induced demineralization of dental enamel that normally constitutes an impermeable barrier that protects the underlying dentin and the connective tissue situated in the centre of the tooth & dental pulp such as pulpitis, dental caries, periodontal infection. In inflammation process in the dental pulpitis the immunity response against oral infection follow leading infection & it is resulting in high level of morbidity and economic burden to society.

Introduction

The human tooth is the target of a substantial number of oral bacteria agents that are responsible for the development of oral inflammation. These agents induce demineralization of enamel that normally constitutes an impermeable barrier that protects the underlying dentin and the connective tissue situated in the centre of the tooth, and dental pulp (Love and Jenkinson 2002). When the enamel barrier is disrupted the dentin exposed to the oral environment. The pulpitis tissue is mainly composed of cells, loose connective tissue and ground substances. The main components are fibroblast, odontoblast, undifferentiated cells and defence cells. Dental pulp is also consists of nerves and blood supply

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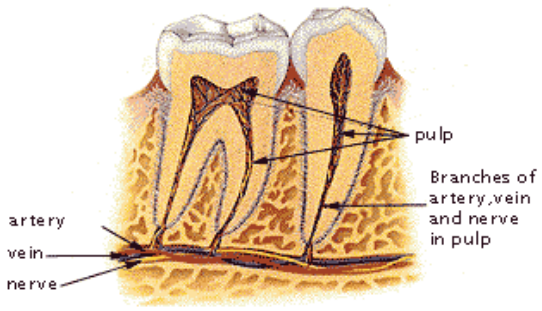

Figure-1, Structural overview of Pulp

Invasion of the pulp and the periapical areas can promote the development of pulpitis and dento-alveolar abscess and spread of the infection to other anatomical areas. Pulpitis can occur when caries progresses deeply into the dentin, when a tooth requires multiple invasive procedures, or when trauma disrupts the lymphatic and blood supply to the pulp. Untreated pulpitis may lead to pulp necrosis,

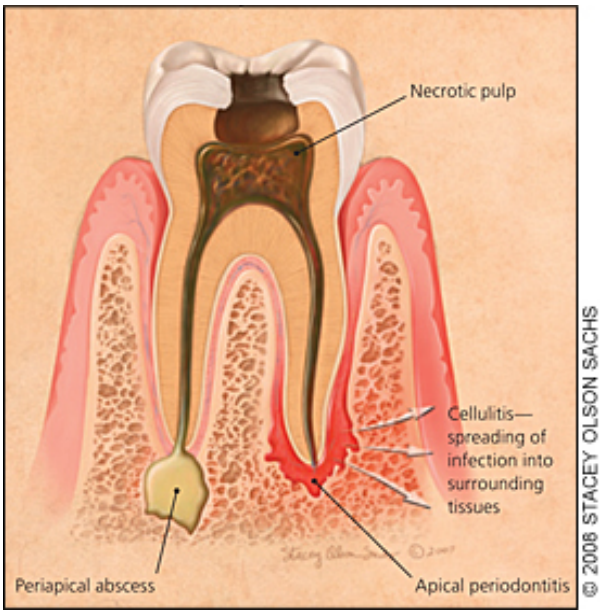

Figure-2, Mechanism of inflammation in pulp

associated with the root canal infection finally lead to apical periodontitis, destruction of the bone surrounding the root apex of affected tooth. Pulpitis begins as a reversible condition in which the tooth can be saved by a simple filling. It becomes irreversible as swelling inside the rigid encasement of the dentin compromises circulation Several oral acid producing aerobic and anaerobic bacteria.

There is a chamber in the centre portion of each tooth, and that chamber contains a mix of nerves, blood vessels and soft, spongy tissue called pulp. Healthy pulp causes no sensations, but when it is attacked by bacteria, pressure builds up in the chamber and you experience pain.

Pulpitis is typically caused by untreated caries (decay) that has inched its way down into the pulp chamber. Your "simple toothache" could have also been the result of a loose filling, periodontal disease, an injury to the tooth or an extensive, invasive dental procedure that came close to the pulp chamber.

Predominant isolated bacteria from necrotic infected pulp

Obligate anaerobic bacteria

	Genus	Common species
Gram negative rods	Porphyromonas	P. gigivalis , P.endodolis
	prevotella	P.oralis, P.oris, P.intermidius
	Fusobacterium	E.alactolyticum, E.lentum,
	Propionibacterium	P.propionicus
	Lactobicillus	L.catenaforme etc.
	Actinomyces	A.naeslundii etc.
Gram positive cocci	Peptostreptococcus	P. anaerobius
Gram negative cocci	Veillonella	V.parvula

Facultative anaerobic bacteria

Gram positive cocci	streptococcus	S.mitis, S.oralis etc.
	Enterococcus	E.faecalis
Gram negative cocci	Neisseria	
Gram positive rod	cornibacterium	
Gram negative rod	Eikenella	

The interaction between micro flora and eukaryotic cell is highly complex and involves active processes allowing both type of partner to co-exist. The microbes encounter with the host and bacterial surface protein termed "adhesions" mediate this step. Bacteria also secret mediators that bind or invade the host, he microbial attachment as well as the encounter with the secreted protein serve as signals that are deciphered through multi mediator cascades ultimately affecting gene expression in the host.

Many receptors recognizing the pathogens and mediating the host response, as well as the variety

of microbial molecular triggering the host response have been demonstrated.

Toll like receptor and bacteria present in pulpitis –

Host defence against invading microbial pathogens is elicited by the immune system, which consists of two components: innate immunity and acquired immunity. Both components of immunity recognize invading microorganisms as non-self, which triggers immune responses to eliminate them

As above described in caries causing pulpitis both gram positive and gram negative bcteria are present and TLRs function as signal transducers that mediate innate immunity and inflammatory response to pathogen through pattern recognition of virulence molecules. Mammalian innate immune system recognizes bacteria and their cell wall components through and pattern recognition receptors CD14 and TLR 2and 4, Although the exact mechanism of immune activation by Gram-positive bacteria remains unknown, recent studies of immune activation by bacterial LPS provide a clue. TLR2 recognizes a variety of microbial components. These include lipoproteins/lipopeptides from various pathogens, peptidoglycan and lipoteichoic acid from Gram-positive bacteria. Whereas TLR4 seems to serve as the primer LPS receptors. Some researchers have shown that TLR 2 may not be exclusively pattern recognition receptors for LPS from Gram negative bacteria (Schwandner *et al* 1999).

To study the expression of TLRs by odontoblasts, we used a pure population of cells generated in vitro that are similar in many aspects to in vivo odontoblasts (Couble et al 2000,Lucchini et al 2002).We observed the constitutive expression of *TLR1–6* and *9* genes but not *TLR7*, *8*, and *10* genes. This large range of TLRs expressed by odontoblasts appears comparable to what has been reported for cultured epithelial cells, including keratinocytes intestinal epithelial cells , bronchial epithelial cells (sha *et al* 2004)and gingival epithelial cells . Interestingly, the pattern of TLRs expressed by odontoblasts was similar to the one reported for gingival fibroblasts in primary cultures . Whether a common TLR expression profile exists for all oral mesenchymal cell types remains to be determined.

Thus, odontoblasts might be involved in the recognition of bacterial products such as triacetylated lipoproteins (TLR1+TLR2), LTA (TLR2), diacetylated lipoproteins, peptidoglycans (TLR2+TLR6), LPS (TLR4), flagellin (TLR5), and unmethylated CpG motif-containing DNA (TLR9), and also of viral dsRNA through TLR3 (Takeda et al 2002, Iwasaki 2004).

Indeed, we found in the present work that odontoblasts responded in vitro to the TLR2 ligand LTA but also to TLR3 and TLR4 ligands (our preliminary data). It remains to be determined whether these cells can actually detect and react to TLR5 and 9 ligands.

Although the dental pulp is equipped with cells of the immune system (Jontell 1986), the immune response in the pulp to caries pathogens is poorly understood.

The basis for the earliest step in innate immune response to Gram positive bacterial infection is poorly understood. We hypothesized that Gram positive bacteria might also be recognized by TLRs.

Although the exact mechanism of immune activation of Gram positive bacteria remains unknown, recent studies of immune activation by bacterial LPS provide a clue. Like Gram negative bacteria, major component of the gram positive bacterial cell wall employ CD14 for immune recognition. Both peptidoglycan and lipoteichoic acid have been demonstrated to activate macrophages in a CD14-dependent manner (Gupta 1996)

Gram positive bacteria entering the dentinal tissue during the caries process are suspected to influence the immune response in human dental pulp. Odontoblasts situated at the pulp are the first cells encountered by these bacteria and therefore could play a crucial role in this response.

The predominant role of Gram-positive bacteria in dental caries, we analyzed further the odontoblast response to LTA. LTA was found to strongly up-regulate the expression of its own receptor, TLR2, and to a lesser extent, of TLR3, 5, and 9. The up-regulation of TLR2 by its ligand may increase the sensitivity of odontoblasts, as previously reported in hemopoietic cells (Ray *et al* 2003). The absence of TLR2 detection in resting odontoblasts is probably because the protein is present at a level below the sensitivity threshold of flow cytometry, as reported for iDCs (Visintin *et al* 2001).

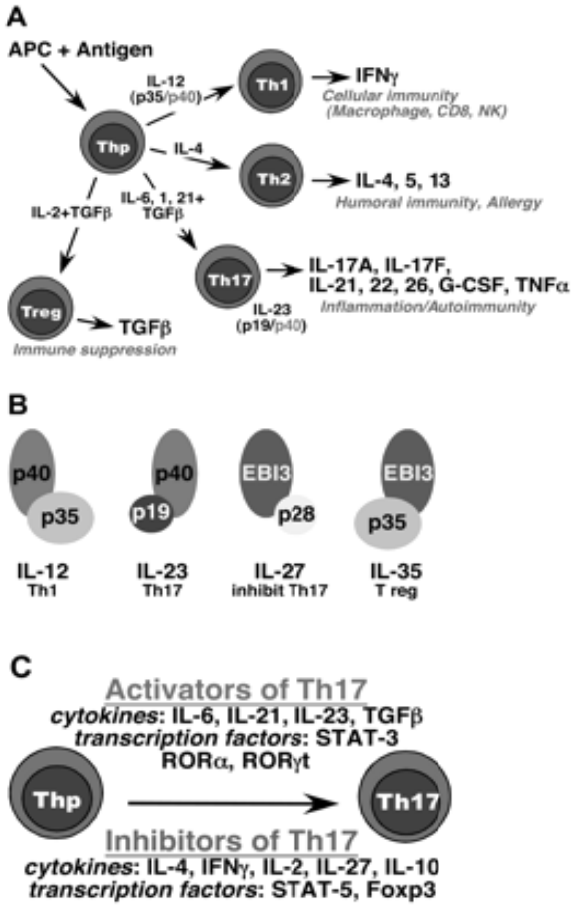

Figure-3 Role of TLR's in oral Inflammation

Immunity against pulpits causing bacteria –

Pulpitis is characterized as the immune response that is mainly triggered by the invasion of caries-related micro-organisms into dentinal tubules and pulp.

The innate immune response associated with infections is linked to the adaptive immune response by cytokine production.

Cytokinesproduction in pulp by bacteria -The lesions in dental pulp under shallow caries are T-cell dominated, with $CD8^+$ T cells predominating. As caries progresses and the deep lesion emerges, the $CD8^+$ T cell continues to dominate, but $CD4^+$ T cells, B cells, and plasma cells appear in substantial numbers (Hahn *et al* 1989;Izumi *et al* 1995).

A feature of pulpal immune responses is the predominance of type 1 cytokine mRNA under shallow caries and a mixed (type 1/type 2) profile under deep caries. These results prompted an examination of the cytokine profiles induced by bacteria in shallow caries (*Streptococcus mutans* and *Actinomyces viscosus*) and deep caries (*Lactobacillus casei, Pseudoramibacter alactolyticus*, and *Prevotella intermedia*). All isolates induced interferon-γ and interleukin-10, *S. mutans* induced substantially more interferon-γ than interleukin-10, suggesting strong type 1 polarization. *P. alactolyticus* induced significantly more interleukin-10 than interferon-γ, suggesting polarization toward type 2. The high titers of IL-12 induction by *S.mutans* agree with previous studies using different *S.mutans* strains (Jiang,Magli&Russo 1999, Plitnick *et al* 1998).Hahn,Best & tew,2000 first time demonstrated the presence of multiple inflammatory cytokine mRNAs (IFN-γ, IL-4, and IL-10) in the human dental pulp in carious lesions.

It is known that T-cells are most likely the predominant lymphocyte population in inflamed dental pulp tissue (Hahn *et al* 1989;Izumi *et al*

1995). Chemokines are responsible for the recruitment and subsequent activation of particular leukocytes, such as activated T-cells, into inflamed tissues *via* specific chemokine receptors expressed on the cells (Sallusto *et al* 2000); however, little is known about the role of chemokines in the Increased expressions of chemokines such as interleukin-8, C-C chemokine ligand (CCL) 20, and CCL2 are found in inflamed dental pulp (Huang *et al* 1999,Nakanshi *et al* 2005,Durand *et al* 2006); results indicate that CCL20 expression is induced by stimulation with caries-related bacteria that have invaded deeply into the dentinal tubules as well as by proinflammatory cytokines in the inflamed pulpal lesions. It may be involved in the progression of pulpitis via accumulation of inflammatory cells. however, the mechanism of activated lymphocyte infiltration in dental pulp tissues was unclear. In periapical granulomas, the presence of other chemokines (such as CCL3, CCL4, and CCL5) related to lymphocyte recruitment has been demonstrated (Marton *et al* 2000,Kabashimo *et al* 2001). These chemokines might contribute to the formation of chemokine networks in activated T-cell infiltration in inflamed dental pulp lesions, although they have not yet been elucidated. In the present study, we first demonstrated that CXCL10 expression in inflamed dental pulp tissues was significantly increased compared with that in healthy dental pulp. In addition, many CXCR3-expressing T-cells were observed in inflamed pulp. These findings suggest that CXCL10 may act as a key chemokine in the accumulation of activated lymphocytes in pulpitis. accumulation of lymphocytes into the dental pulp lesion.

Bacteria can initiate cell-mediated immunity by stimulating macrophages to produce interleukin-12 (IL-12), which can then promote gamma interferon (IFN-γ) production by natural killer cells. IFN-γ-activated macrophages then secrete more IL-12 as a result of positive feedback, leading to cell-mediated Th1 responses. IFN-γ also activates macrophages to produce tumor necrosis factor alpha (TNF-α), which in turn maintains the activated state of the macrophage (samaranayake 1996). TNF-α is a potent proinflammatory cytokine that can stimulate chemokine production by endothelial cells and fibroblasts in vitro (Sylvester et al 1990). In addition to its protective role in the host defence against infectious agents, TNF-α contributes to bone resorptive activity and has been implicated in pathologic bone resorption (Pfizenmaiener et al 1993, Stashenko et al 1987, Thompson, Mundy, Chambers 1987). An effective host defense against bacterial invasion is characterized by the vigorous recruitment and activation of inflammatory cells, which are modulated by the coordinated expression of both pro- and anti-inflammatory cytokines. IL-10, which is produced by monocytes and Th2 lymphocyte subsets, inhibits IFN-γ synthesis by Th1 cells and inhibits the production of proinflammatory cytokines such as IL-12 and TNF-α. The inhibiting effects of IL-10 on cytokine production correlate with their antiinflammatory effects in vivo (Li, Elliott & Monsmann1994). Therefore, IL-10, IFN-γ, IL-12, and TNF-α have important and cross-regulatory roles in infection (Trinchieri 1997).

It is known that immune cells in inflamed dental pulp produce a variety of cytokines, which can modify the pathogenesis of pulpitis.Streptococcus mutans elicite multiple inflammatory cytokines IFN –y , IL- 4, IL-10 are detected in human dental pulp (Hahn et al 2000). Lipoteichoic acid and peptidoglycan are know to induce IL – 12 by dendritic cells or monocytes and monocytes and IL-12 promotes inductions of Th 1 responses and IFN-Y production (Rissoan *et al* 1999)

P. gingivalis possesses bioactive materials such as cytoplasmic membranes, peptidoglycans, outer membrane proteins, lipopolysaccharide (LPS), capsules, and fimbriae on their cell

surface(Offenbacher,1996). These materials induce excessive production of cytokines and may modulate the cytokine network in periodontal tissues (Genco and Slots, 1984). Several cytokines are involved in inflammatory as well as immunological responses, and are designated as inflammatory cytokines.

P. gingivalis LPS enhances the production of inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α).Marked infiltration of inflammatory cells, such as activated T-cells, is observed in the progression of pulpitis; Both *S. mutans* and *L. plantarum* are Gram-positive bacteria, which have bacterial cell wall components such as LTA and PGN. It is known that LTA and PGN are recognized by TLR2 (Michelesen et al 2001). Adachi et al 2007examined,HDPF (human dental pulp fibroblasts) stimulated with PGN, but not LTA, were able to produce CXCL10.

The macrophage appears to be a key cell involved in host response to LPS. After release from bacteria, LPS is initially bound to a plasma protein called LPS-binding protein (LBP) and is then delivered to CD14, a cell receptor for LPS on the surface of macrophages. Subsequent activation of the macrophage is a result of signal triggered by a signal-transuding receptor called Toll-like receptor (TLR). The Toll family of receptors encompasses trans membrane molecules linking the extracellular compartment, where contact and recognition of pathogens occurs, and the intracellular compartment, where signalling cascades leading to cellular responses are initiated. TLRs are responsible for cell signalling to a variety of bacterial components. TLR-4 is involved in cellular activation by LPS from most bacteria. However, TLR-2 may be involved in cell signalling to some types of LPS, such as that from *Porphyromonas gingivalis*. Engagement of the receptor activates transcription factors, which induce activation of genes encoding several cytokines.

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