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REVIEW

Antigen recognition and presentation in periapical tissues: a role for TLR expressing cells?

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Abstract

Desai SV, Love RM, Rich AM, Seymour GJ. Antigen recognition and presentation in periapical tissues: a role for TLR expressing cells?. *International Endodontic Journal*, **44**, 87–99, 2011.

Bacteria are the prime cause of periapical diseases and root canal microbiology is a well-researched area of endodontics. Antigen-presenting cells (APCs) are present in periapical lesions of endodontic origin and play a substantial role in recognizing, processing and presenting pathogenic antigens to the adaptive immune system such as an effective and long-lasting immune response is generated against the specific pathogens. Toll-like receptors (TLRs) are germ-line encoded pathogen recognition receptors (PRR) expressed by various APCs which induce their maturation, lead to gene transcription in the nucleus and the production of several pro- and anti-inflammatory cytokines. Thirteen TLRs have been discovered, 10 of which have been identified in humans so far. Preliminary studies of dental pulp tissue have demonstrated various cell types expressing different TLRs in response to commonly encountered microorganisms. However, there is little information available regarding the expression and function of the various TLRs in human periapical lesions. This review discusses the interactions of various APCs in periapical lesions and the possible roles of different TLRs and APCs in pulp/periapical pathogen recognition and presentation to the adaptive immune system in the initiation and sustaining of periapical diseases.

Keywords: adaptive immunity, antigen-presenting cells, dendritic cell, innate immunity, periapical pathology, toll-like-receptors.

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Introduction

Bacteria are the prime cause of periapical diseases (Kakehashi *et al.* 1965). Invasion of bacteria or bacterial toxins into the periapical region from an infected root canal system leads to initial non-specific inflammatory reactions followed by specific inflammatory reactions that include various host derived cells, antibodies, complement, cytokines and an array of inflammatory mediators targeted towards limiting the spread of infection and protecting the periapical tissues. Some of these mediators cause local tissue destruction in the form of bone and/or tooth resorption (Stashenko *et al.* 1998).

Periapical lesions have a diverse inflammatory and non-inflammatory cellular profile that is involved in regulation of highly complex disease processes. Numerous innate immune cells [e.g. polymorphonuclear neutrophils (PMNs), macrophages, dendritic cells (DCs)] as well as adaptive immune cells (e.g. T and B lymphocytes, plasma cells) are present in different proportions within periapical lesions. Antigen-specific adaptive immune cells have a requirement for antigen presentation and different antigen-presenting cells (APCs) are involved in the inflammatory response. The notion of separate non-specific innate and specific adaptive immunities has changed considerably with

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the recognition of receptors on various APCs that discriminate between host and pathogen. These are known as pathogen recognition receptors (PRRs) (Janeway 1989). The limited specificity of these receptors in identifying 'bad' from 'good' acts as a bridge between innate and adaptive immunities (Akira *et al.* 2001). This review examines current understanding of the various APCs and antigen presentation processes and interactions in periapical lesions. In addition the role of PRRs, mainly Toll-like receptors (TLRs), in the identification of pathogens and possible interactions in periapical diseases are discussed.

Antigen-presenting cells

In humans, the development of immunity against pathogens occurs in two distinct but overlapping phases. The basic innate or non-specific immune system is the first line of defence against invading pathogens until a more specific and long-lasting adaptive immunity develops. Development of an effective adaptive immune response relies greatly upon appropriate recognition of antigen by cells of the innate immune system and presenting it to adaptive immune cells. APCs have important pathogen recognition skills and operate at the interface of innate and adaptive immunity. Only appropriately labelled and encoded APCs can activate naive T cells. In addition, certain APCs are important for the induction of immunological tolerance and regulation of the type of T cell-mediated immune response. After experimental pulp exposure in rat molars, APCs (e.g. HLA-DR$^+$ and ED1$^+$ /OX6$^+$ cells) appeared in the periapical region as early as 1–3 days post-exposure, demonstrating their importance in the modulation of adaptive immunity in periapical disease; the expression of APCs subsequently increased in proportion to the development and size of the lesion (Okiji *et al.* 1994, Suzuki *et al.* 1999).

Dendritic cells represent a large family of APCs. They are the most potent and effective cells for presentation of antigen to naive T cells. Various dendritic cells circulate through the blood stream and have a role in immunosurveillance, particularly in the barrier zones of pathogen entry such as epidermis (Banchereau & Steinman 1998), periodontal tissues (Gemmell *et al.* 2002) and the paraodontoblastic region of the pulp (Jontell *et al.* 1998). They are traditionally considered to arise from myeloid-committed precursor cells derived from bone marrow. However, dendritic cells of lymphoid origin – plasmacytoid dendritic cells – have been characterized in both animal and human studies (Ardavin *et al.* 1993, Galy *et al.* 1995) as well as being observed in periapical granulomas (Lukic *et al.* 2006). Further subsets of dendritic cells (Table 1) have been recognized according to anatomical localization, function and expression of cell surface markers (Banchereau *et al.* 2000, Cutler & Jotwani 2004). They have been demonstrated in healthy periodontal ligament space in an animal model (Kaneko *et al.* 2008a), whilst in recent investigations Langerhans cells, interstitial cells and plasmacytoid dendritic cells have been observed in periapical granulomas and cysts (Suzuki *et al.* 2001, Colic *et al.* 2009).

Immature dendritic cells are capable of capturing and processing microbial antigens within infected tissue. Upon migrating and subsequent maturation they initiate an adaptive immune response by priming naive T cells in peripheral lymphoid organs to undergo clonal expansion and differentiation into effector and memory T cells. Such T cells migrate to the site of inflammation where upon reactivation with local APCs they perform different effector functions. In addition, during chronic inflammation, a number of immature dendritic cells are retained at the local site and undergo local maturation (identified as HLA-DR$^+$ CD83$^+$ cells) under the influence of various pro-inflammatory cytokines such as, tumor necrosis factor (TNF)-α, prostaglandin E2 and interleukin (IL)-1β (Banchereau *et al.* 2000). Approximately 10–30% of dendritic cells isolated from periapical lesions gain maturity locally

Table 1 Location and type of human dendritic cells (DC) and phenotypic expression

Location	Cell type	Expression
Blood	Myeloid DC	CD11c+, CD1c, CD14-, CD1c/BDCA1, DC-SIGN (CD209)
	Plasmacytoid/lymphoid DC	CD11c-, CD123, BDCA-2, BDCA-4, DC-SIGN (CD209)
Peripheral tissue	Langerhans cells (LC)	CD1a, Langerin-Lag, CCR6, E-cadherin, CD62L, DEC-205, intra MHCII (HLA-DR), CLA
	Interstitial DC	DC-SIGN (CD209), factor XIIIa, MMR, CD11b, intra-MHCII
Lymph stream	Veiled cells	CD80, CD83, CD86, CCR7, CD11a, CLA, surface MHCII
Secondary lymphoid organ	Interdigitating DC	DC-Lamo, DC-205, CD80, CD83, CD86, CCR7, DCIR, surface MHCII
	Geminal center DC	CD2, CD4, CD11c, CD35, CD45RO, CD64

Table 2 Antigen presentation immunological markers for macrophage subpopulations that have been utilized in various human and animal studies on periapical lesions

Marker	Study type	Reference
HLA-DR$^+$	Human	Kopp & Schwarting (1989)
ED1$^+$ /OX6$^+$	Rat	Suzuki *et al.* (1999)
OX6+ and ultrastructural analysis	Rat	Kaneko *et al.* (2001)
HLA-DR$^+$ and CD14$^+$	Human	Lukic *et al.* (2006)
CD11c$^+$, OX6$^+$ and ED1	Rat	Zhao *et al.* (2006)
HLA-DR$^+$, CD68$^+$ and TEM	Human	Kaneko *et al.* (2008b)
CD11c$^+$ and OX62$^-$	Rat	Kaneko *et al.* (2008c)

and are characterized by the expression of the CD83 protein (Lukic *et al.* 2006). In addition, a close association between dendritic cells and lymphocytes in periapical granuloma implies a local antigen presentation role for dendritic cells in these lesions (Kaneko *et al.* 2001, 2008b).

Numerous immunological cell surface markers (Table 2) have been utilized in periapical lesions studies to demonstrate the antigen-presenting role of macrophages. In an *in vitro* study, monocytes (CD14$^+$, CD1a$^-$) stimulated with granulocyte–monocyte colony stimulating factor (GM-CSF), IL4 and TNFα differentiated into mature dendritic cells (CD1a$^+$ CD83$^+$) (Zhou & Tedder 1996). This functionally different subpopulation of macrophages that express surface markers for antigen presentation (Suzuki *et al.* 1999, Kaneko *et al.* 2001) are also present in the normal periodontal ligament space, distributed mainly around the blood vessels (Zhao *et al.* 2006). The ability to activate naïve T cells during primary immune responses is limited to dendritic cells. However, both macrophages and dendritic cells share the capacity to interact with locally recruited memory T cells during a secondary immune response.

Antigen recognition

Several inflammatory cells can function as APCs; amongst the cells present in a periapical lesion macrophages, dendritic cells, B cells and certain activated T cells have all demonstrated APC markers (Lukic *et al.* 2006, Kaneko *et al.* 2008b). However, the exact mechanisms of antigen recognition and the development of immunity in periapical lesions are unclear. APCs possess special receptors on their surface that recognize specific pathogen associated molecular pattern (PAMP) and trigger appropriate intra-cellular events to continue capture of antigen and further induce co-stimulatory molecules for T cells.

The PRRs of innate immune cells are located on the cell membrane, in the intracellular compartment or secreted in the blood stream and tissue fluids. Examples of receptors on the cell membrane or endosomal membranes are TLRs and in cytoplasm are nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Secreted PRRs are mannan-binding lectin and lipopolysaccharide-binding proteins.

C-type mannan-binding lectin receptors are highly conserved carbohydrate recognition domains that are either produced as transmembrane proteins (DC-SIGN, BDCA-2, MMR) or secreted soluble proteins (collectins). Membrane bound lectin receptors recognize pathogens that leads to antigen capture, endocytosis and intracellular processing. Soluble lectins bind to microbial carbohydrates and function as opsonins (reviewed in Figdor *et al.* 2002). NLRs are evolutionary conserved proteins located in the cellular cytoplasm and involved in bacterial peptidogycan recognition (reviewed in Kaparakis *et al.* 2007). On the contrary, TLRs are germ-line encoded PRRs that recognize antigen and act to alert dendritic cells and induce their maturation.

Toll-like receptors

In humans, TLRs identify PAMPs and activate multiple steps in the inflammatory reaction. Once activated, TLRs upregulate the genes encoding inflammatory cytokines such as IL8, TNFα, IL6, IL12 and IL1β in immunocompetent cells.

There are 13 distinct TLRs identified so far, 10 of which are characterized in humans. TLRs 1, 2, 4, 5, 6 and 11 are expressed on the cell surface whereas 3, 7, 8 and 9 are present intracellularly on endosomal membranes. However, as an exception, TLR2 had been characterized in the intracellular compartment of macrophages (Underhill *et al.* 1999) whereas TLR9 expression has also been shown on the cell surface of peripheral blood mononuclear cells (Eaton-Bassiri *et al.* 2004).

TLR structure

TLRs are type I integral membrane glycoproteins with a characteristic domain architecture that comprises an extracellular N-terminal lucine-rich repeat (LRR) domain connected to a C-terminal intracellular toll/interleukin-1 receptor domain by a transmembrane

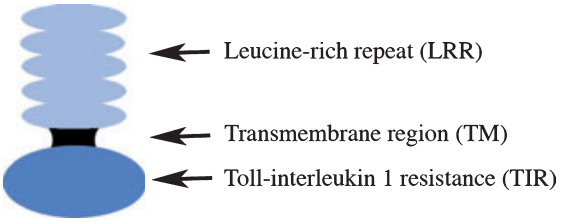

Figure 1 Diagrammatic representation of a toll-like receptor (TLR) showing the three domains.

region (Fig. 1) (Iwasaki & Medzhitov 2004). The extracellular domain is composed of between 19 and 25 repeats of a 24-residue LRR sequence. The domain is protected and stabilized at both ends by disulphide bonded capping motifs.

The extracellular domain may need support from accessory molecules to effectively bind its ligand. For example, TLR4 cannot bind to lipopolysaccharide (LPS) molecules without help from a serum protein, LPS-binding protein (LBP) and cell surface CD14 molecules (Haziot *et al.* 1996). Another component of the cell surface LPS recognition receptor complex is the MD-2 co-receptor. It is a small protein that lacks a transmembrane region and is expressed on the cell surface in association with the ectodomain of TLR4. The exact function of MD-2 is not known but it is required for LPS recognition by TLR4 (Schromm *et al.* 2001).

The intracellular domain shares a high degree of homology with that of the type 1 IL-1 receptor activation, additional protein molecules known as 'adaptor proteins' are recruited to the cytoplasmic domains of the receptors via TIR–TIR interactions (Fig. 2). The five adaptor proteins are, myeloid differentiation factor 88 (MyD88), MyD88-adaptor like (Mal), TIR domain containing adaptor protein inducing INFβ (TRIF), TRIF-related adaptor molecule (TRAM) and sterile α- and armadillo-motif containing protein (SARM).

Different adaptor proteins are utilized for different situations and selection of an adaptor protein plays a major role in determining the specificity of the immune responses mediated by different TLRs. All TLRs employ MyD88 adaptor protein. In addition, TLR2 recruits MyD88 to its cytoplasmic domain via the bridging adaptor Mal whilst TLR4 utilizes MyD88 and TRIF to employ Mal and TRAM, respectively, for downward signal propagation. A signalling cascade leads to the activation of transcription factors like NFκB, interferon regulator factor (IRF) 3 and mitogen-activated protein (MAP) kinase. Together these factors induce the pro-inflammatory response that constitutes a primary means to eradicate the threat.

TLR ligands

The extracellular domain of TLRs identify a large variety of ligands as would be anticipated considering the number and complexity of molecules that arise from infection by pathogenic bacterial, protozoan, fungal and viral organisms. TLR ligands are conserved microbial components that are essential for microbial

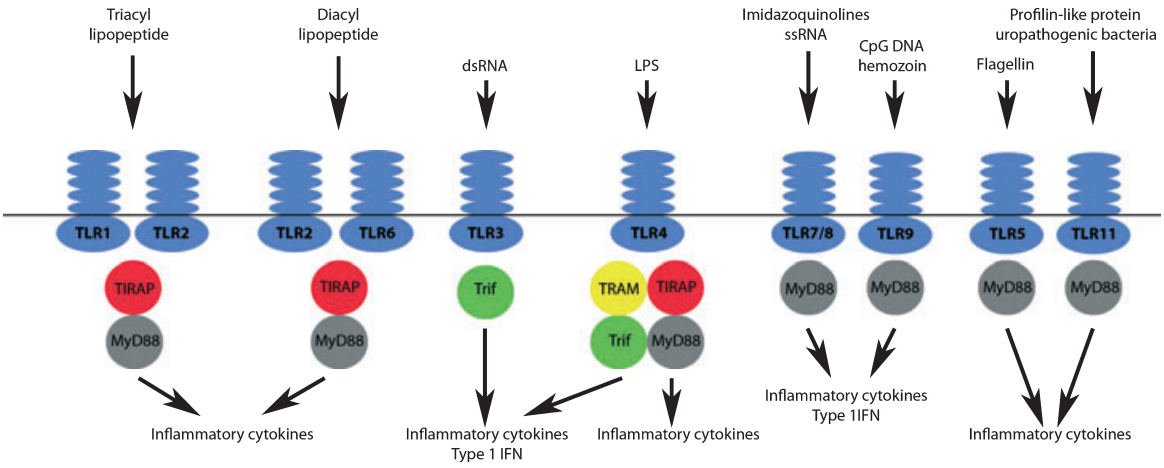

Figure 2 Schematic representation of TLR activation of inflammatory processes. Antigen recognition activates TLR leading to recruitment of intracellular adaptor proteins, for example, MyD88. Different adaptor proteins are utilized for different situations and selection of an adaptor protein plays a major role in determining the specificity of the resulting immune response.

survival, as such any structural mutation is highly unlikely without a fatal effect. All individual TLRs can identify numerous structurally unrelated ligands and some TLRs may require accessory proteins to recognize their ligands. The co-operation of different TLRs adds greater specificity and a broader range of ligand recognition capacity to the TLR proteins as well as enhancing their signal transduction capacity (Kurt-Jones *et al.* 2002, Mahanonda & Pichyangkul 2007). Any one group of pathogens is not exclusively recognized by one TLR whilst one TLR can respond to many structurally unrelated ligands derived from different groups of pathogens.

TLR2, TLR1 and TLR6

TLR2 and its associations with TLR1 and TLR6 constitute a diverse pathogen recognition complex. TLR2 is involved in the recognition of a variety of microbial products like peptidoglycan (Schwandner *et al.* 1999, Takeuchi *et al.* 1999) and lipoteichoic acid (LTA) (Schwandner *et al.* 1999) from Gram-positive bacteria, lipoproteins (Takeuchi *et al.* 2001), mycobacterial cell wall lipoarabinomannan (Means *et al.* 2000), atypical LPS of *Porphyromonas gingivalis* (Darveau *et al.* 2004) and components of spirochetes (Lien *et al.* 1999). Other experiments with TLR2 have reported its involvement in recognition of dsRNA (Nilsen *et al.* 2004) and dsDNA (cytomegalovirus) (Compton *et al.* 2003, Boehme *et al.* 2006).

The broad spectrum of components recognized by TLR2 suggests that these receptors participate in a complex pattern recognition system. TLR2 often makes functional complexes with TLR1 (Takeuchi *et al.* 2002) and TLR6 (Ozinsky *et al.* 2000, Takeuchi *et al.* 2002) for recognition of PAMPs and such TLR2 heterodimerization explains the specificity for a wide range of ligand recognition.

Lipoproteins are produced by a variety of pathogens including Gram-negative bacteria, mycobacteria and mycoplasma. The N-terminal acylated lipoprotein region is responsible for the immunostimulatory activity of bacterial and mycoplasmal lipoproteins. However, bacterial and mycoplasmal lipoproteins differ in the degree of acylation of N-terminal cysteine. Lipoproteins from bacteria are tri-acylated whereas those from mycoplasma are di-acylated (Chambaud *et al.* 1999). Experiments on the role of the TLR2/TLR6 complex in identification of lipoproteins suggested that TLR6 could detect fine differences in the structure of lipoproteins by selective discrimination in identification of the di-acylated N-terminal lipoproteins of mycoplasmal origin and tri-acylated lipoproteins of bacterial origin (Takeuchi *et al.* 2001).

TLR4

TLR4 was the first characterized mammalian TLR (Medzhitov & Janeway 1997). The presence of TLR4 has been noted on various host immune cells including dendritic cells and macrophages. The main ligand for TLR4 is LPS, an integral component of the outer membrane of Gram-negative bacteria. Structurally LPS is a complex glycol-lipid composed of a hydrophilic polysaccharide portion that is responsible for its antigenic properties and a hydrophobic domain known as 'Lipid A', which is responsible for the toxicity of Gram-negative bacteria. Although the polysaccharide portion varies between different Gram-negative bacteria, the lipid-A portion remains unchanged and acts as a PAMP that is recognized by cells of the innate immune system expressing TLR4. TLR2 was previously believed to be involved in LPS-mediated signalling but further studies using purified commercially available LPS preparations reported that it did not play any role in LPS signalling except for atypical LPS produced by *P. gingivalis* (Hirschfeld *et al.* 2001).

Another ligand for TLR4 is viral glycoprotein G (gpG). This viral envelope glycoprotein activates the TLR4-CD14 complex that results in IRF3 and translocation in the nucleus for gene expression of INFβ using an alternative-signalling pathway (Georgel *et al.* 2007). Also, TLR4 can recognize ligands like LTA (Takeuchi *et al.* 1999) and heat shock protein 60 (Ohashi *et al.* 2000).

Other TLRs

TLR5, located on the cell surface, is involved in recognition of bacterial flagella a rod-like appendage extending from the outer membrane of Gram-negative bacteria (Hayashi *et al.* 2001). Whilst TLR3, 7, 8 and 9 recognize viral nucleic acid antigens and are located in the intracellular compartment. TLR3 ligand is a double stranded (DS) RNA produced during viral replication (Alexopoulou *et al.* 2001) and TLR7 and TLR8 are involved in recognition of single stranded viral RNA as well as synthetic imidazoquinolines (Heil *et al.* 2004). TLR9 ligands are unmethylated CG dinucleotides of bacterial DNA (CpG-DNA) (Hemmi *et al.* 2000). Ligands for TLR10 and TLR11 are yet to be characterized.

TLR associated intracellular signalling pathways

Appropriate ligand recognition by TLRs stimulates intracellular signal transduction pathways and induction of different genes that function in host defence, including those for inflammatory cytokines, chemokines, major histocompatibility complex (MHC) and co-stimulatory molecules. In addition TLR activation induces multiple effector molecules, such as inducible nitric oxide synthase and antimicrobial peptides, which can destroy microbial pathogens (Thoma-Uszynski *et al.* 2001). Both TLRs and IL1R share pathways that rely upon the TIR domain to activate NFκB, MAP kinase or IRF3 and translocation in the nucleus for gene expression of different pro-inflammatory cytokines.

Experiments using adaptor protein knockout mice, which lose the ability to produce various pro-inflammatory cytokines, show that one or more adaptor proteins are mandatory to initiate signal down-streaming (Alexopoulou *et al.* 2001, Kawai *et al.* 2001, Takeuchi *et al.* 2001). Whilst most TLR intracellular signalling pathways involve MyD88 adaptor protein, some TLRs, as an addition to the standard signalling pathway or as an alternative, use other adaptor proteins to initiate various biochemical reactions in the intracellular compartment (Fig. 2).

Negative regulation of TLRs

Toll-like receptor activation can be a double-edged sword. Sustained inflammatory signalling can be harmful and the TLR family have been implicated in the pathogenesis of autoimmune, chronic inflammatory and infectious diseases. Hence, the intensity and duration of TLR responses are closely monitored with negative regulators working at different levels in TLR signalling pathways. Extra- and intracellular mechanisms that prevent overexpression of TLRs have been extensively investigated.

Intracellular negative regulation pathways generally impede adaptor proteins that are essential for downward propagation of the signal leading to inflammatory cytokine gene expression. A detailed description of complex regulatory biochemical pathways is beyond the scope of this review. Recently, Lang & Mansell (2007) comprehensively reviewed various TLR negative regulatory pathways involving interference with adaptor proteins MyD88, SARM, TRAF4, toll-interacting proteins (Tollip), IRAK-1, IRAK-M and other molecules.

Antigen presentation

Uptake and processing of antigen

Identification of pathogens by PRRs and the presence of appropriate cytokines in the vicinity initiate a series of events that leads to the uptake of antigen or opsonized particles (in case of soluble PRR) by immature dendritic cells. Intra-cellular degradation and processing follows internalization of bacterial antigen where the antigen is degraded and fragmented into smaller peptide antigen molecules. Exogenous proteins (e.g. bacterial antigens) are processed within endosome/lysosome compartments whereas endogenous proteins (e.g. tumour antigens) are processed within the cytosol/endoplasmic reticulum. The intracellular processing of antigen in the presence of cytokines TNF, IL1, IL6 and TGFβ induces maturation of dendritic cells (Rescigno *et al.* 1999).

Dendritic cell maturation is associated with several co-ordinated actions involving down-regulation of endocytic/phagocytic potential, expression of surface co-stimulatory molecules (CD40, CD80 and CD86), and appearance of higher quantities of class I or II MHC molecules on the cell surface (Banchereau *et al.* 2000). Antigen loaded dendritic cells migrate towards secondary lymphoid organs to sensitize naïve T cells. Upregulation of certain surface receptors (selectins, integrins, chemokine receptors CCR7) together with the presence of inflammatory cytokines in the microenvironment (GM-CSF, IL1β and TNFα) aids dendritic cells in their migration to a local lymph node via blood vessels or lymph channels (Sozzani *et al.* 2000).

APC interactions with effector cells

Efficient activation of effector T cells requires two signals from dendritic cells. The first signal is related to MHC-restricted presentation of antigen. T cells cannot recognize antigen in the absence of class I or class II MHC molecules on the dendritic cell surface. Exogenous antigen, processed in endosomes/lysosomes, is loaded on MHC class II molecules and transported to the surface for presentation to $CD4^+$ T cells, whereas endogenous antigen, processed in cytosol/ER is loaded on MHC class I molecules to be exported to the surface for presentation to $CD8^+$ T cells (Germain & Margulies 1993).

The second signal is related to expression of surface co-stimulatory molecules. The interaction between co-stimulatory molecules and their ligands present on

T cells is crucial to instigate T cell activation and tolerance. Mature dendritic cells express multiple accessory molecules on the cell surface, CD80 and CD86 are two such important co-stimulatory molecules (Sharpe & Freeman 2002). In addition to dendritic cells, B cells, T cells and macrophages have demonstrated CD80 and CD86 on the cell surface (Hathcock *et al.* 1994). Most immature APCs demonstrate low expression of surface CD86 and with antigen uptake CD86 levels are rapidly upregulated; however, expression of CD80 on the cell surface is much delayed until after the maturation of the APC (Hathcock *et al.* 1994). In addition, certain PAMPs (e.g. *P. gingivalis* LPS) can trigger higher expression of CD86 over CD80 molecules in DC and preferentially elicit a T cell response that is favoured towards Th2 cytokine production (IL4) (Freeman *et al.* 1995, Jotwani *et al.* 2001). Co-stimulatory molecule CD86 has been shown to be involved in induction of IL2, a growth factor essential for T cell proliferation (Hathcock *et al.* 1994, Freeman *et al.* 1995).

Other essential co-stimulatory molecules expressed on mature dendritic cells are ICAM-1-2 and -3 that pair with CD11/CD18 on T cells (Dubey *et al.* 1995). This receptor–ligand binding may favour Th1-based cytokine responses and block IL4 and IL10. CD40, a molecule on the dendritic cell surface that is related to the TNF receptor family, interacts with CD40L (CD154) on T cells to provide a maturation signal for dendritic cells, induce production of IL1β, TNFα, IL6, IL8, and IL12 and prevent dendritic cell apoptosis. Similar to the CD86-CD28 pair, CD40-CD40L binding also results in induction of Th2, but not of Th1 (van Kooten & Banchereau 2000).

It is likely that different dendritic cell subsets may provide T cells with the different cytokine profiles that determine the class of immune response. Besides activating naive T cells, dendritic cells can directly differentiate naïve $CD40^+$ B cells towards IgG secreting cells in the presence of IL6 (Dubois *et al.* 1997). Different subsets of dendritic cells may also regulate natural killer (NK) cells activation through the release of INFα, IL12, IL15 and IL18, leading to enhanced antiviral efficiency (Geldhof *et al.* 1998).

Antigen-presenting cell interactions in periapical diseases

Numerous studies have investigated APC interactions in human or animal periapical lesions using immunochemistry or flow cytometry. However, the conclusions of these investigations are difficult to compare as different studies have employed dissimilar techniques to characterize the various APCs. The heterogeneity of APCs makes such investigations complex.

A small number of APCs are present in healthy periapical tissues and in the event of bacterial stimulation the numbers increase under the influence of the altered chemical microenvironment. A source of these newly recruited dendritic cells is believed to be differentiation of circulating monocytes as well as resident macrophages being activated into antigen-presenting dendritic cells (Zhao *et al.* 2006). In addition, both phenotypically immature ($CD83^-$ cells) and mature ($CD83^+$ cells) dendritic cells have been detected in human periapical granulomas (Colic *et al.* 2009).

In an immunohistochemical study of human periapical lesions, about one-third of the total cellular composition stained positively for HLA-DR, an APC marker. Interestingly, more B-lymphocytes (18% – $HLA\text{-}DR^+/CD19^+$ cells) expressed HLA-DR, than macrophages (9% – $HLA\text{-}DR^+/CD14^+$ cells), dendritic cells (4% – $HLA\text{-}DR^+/CD3^-/CD14^-/CD19^-$ cells) or activated T-lymphocytes (0.4% – $HLA\text{-}DR^+/CD3^+$ cells) (Lukic *et al.* 2006). No difference was found in the quantitative expression of APCs in symptomatic and asymptomatic lesions, nor amongst the lesions that had a predominance of either T cells or B cells. However, amongst the APC subsets, dendritic cell expression was significantly greater in T cell over B cell predominant lesions (Lukic *et al.* 2006). Similarly, others have noted that dendritic cells are the most prominent cell type amongst the MHCII expressing APC located in the outer portion of the lesion (Kaneko *et al.* 2001).

Suzuki *et al.* (1999) demonstrated the presence of an antigen-presenting macrophage cell subpopulation along with an abundance of T cells in the periphery of periapical granulomas, whereas the macrophage subpopulation, characterizing microbial phagocytosis and killing functions, were present more towards the centre of the lesion. It has been hypothesized that the Th1 immune response may be stronger in periapical lesions when increased numbers of activated macrophages function as APC (Lukic *et al.* 2006). A comprehensive investigation of human periapical granulomas using RT-PCR, transmission and scanning electron microscopy experiments reported that mature dendritic cells ($HLA\text{-}DR^+$, $CD83^+$ cells) were more effective at presenting antigen to T cells as compared with macrophages ($HLA\text{-}DR^+$, $CD68^+$ cells) (Kaneko *et al.* 2008c). These findings suggest that dendritic cells and not macrophages primarily contribute to the local

interaction with T cells within the lesion. Others have demonstrated a similar association between $CD83^+$ dendritic cells and T cells and speculated that dendritic cells are potent stimulators of the local immune response in the periapical lesion (Colic *et al.* 2009). Further, dendritic cells in periapical lesions can stimulate both Th1 and Th17 cells with production of high levels of IL23, IL12 and low levels of IL10 and TNFα (Colic *et al.* 2009).

Langerhans cells ($CD1a^+$) are commonly seen between epidermal/epithelial cells (Banchereau *et al.* 2000), but are sparse in normal periodontal ligament (Kaneko *et al.* 2008a, c) and absent from the cell rests of Malassez in healthy periapical tissue (Suzuki *et al.* 2001). In contrast, high numbers of these cells have been observed in periapical granulomas, epithelialized granulomas and epithelium and subepithelial layers of radicular cysts (Suzuki *et al.* 2001, Santos *et al.* 2007). Additionally higher numbers of APCs were observed in periapical cysts as compared with periapical granulomas (Gao *et al.* 1988, Kopp & Schwarting 1989, Santos *et al.* 2007) with the cells being predominantly located in highly inflamed regions and adjacent to T lymphocytes (Contos *et al.* 1987, Matthews & Browne 1987, Suzuki *et al.* 2001, Lukic *et al.* 2006). These findings led to speculation regarding the roles of Langerhans cells not only for antigen recognition and triggering of an adaptive immune responses, but also possible involvement in exaggerating the proliferative potential of epithelial cells present in periapical lesions.

Recent investigations have characterized the expression of plasmacytoid dendritic cells ($CD123^+$, $BDCsA2^+$, $BDCsA4^+$) in very small numbers in periapical lesions (Lukic *et al.* 2006, Colic *et al.* 2009). The exact role of this subset in periapical inflammation is unclear.

These observations suggest that both non-specific and specific immunological responses may be working in parallel in periapical diseases. APCs are clearly important in immunoregulation of periapical lesions and cell surface receptors, such as TLRs, which are responsible for recognition of pathogens may indirectly have a complex controlling role in inflammatory periapical diseases.

Toll-like receptor interactions in periapical diseases

Mutoh *et al.* (2007) demonstrated expression of TLRs (2 and 4) by various inflammatory cells and odontoblasts in inflamed pulp tissue in response to stimulation by pathogens. Further experiments showed that upregulation of TLR (2 and 4) by innate immune cells in infected pulp tissue was not altered in immunodeficient (absent B and T cells) mice (Mutoh *et al.* 2009). However, as yet no studies have physically demonstrated expression of any TLRs by cells present in animal or human periapical lesions; however, the presence of TLR4 in periapical lesions has been indirectly shown in LPS-hyporesponsive murine models (Hou *et al.* 2000, Fouad & Acosta 2001).

Inflammatory periapical lesions are initiated by polymicrobial infections of Gram-positive and -negative bacteria whilst the continuous challenge from bacteria and/or their antigenic by-products from the infected root canal system maintains and exacerbates disease activity in the periapical area. Various innate and adaptive immune cells, including APCs have been characterized in periapical lesions. Few of these are resident cells of healthy periapical tissues with most migrating to the site from the peripheral blood in response to antigens. TLRs have been characterized in circulating leucocytes in numerous animal and human studies. Accordingly it is reasonable to assume that TLRs would be expressed by many cell types present in periapical lesions and that they would play a significant role in the recognition of endodontic pathogens and triggering of adaptive immune responses against endodontic pathogens.

TLR2 in periapical diseases

TLR2 is generally involved in detection of Gram-positive bacterial components (Takeuchi *et al.* 1999) which dominate the microflora of failed root canal treatment (Sundqvist *et al.* 1998). *E. faecalis*, a Gram-positive facultative anaerobic bacteria, led to activation of the TLR2/TLR1 complex in human odontoblasts due to its antigenic LTA and lipopeptide components. Further, gene transcription for inflammatory cytokines IL8 and TNFα was also greatly enhanced (Horst *et al.* 2009). On the contrary, chlorhexidine attenuated the ability of LTA antigen (of dead *E. faecalis*) to be recognized by TLR2, resulting in minimal production of TNFα in a Chinese hamster ovary (CHO) cell line (Lee *et al.* 2009). Refractory periapical diseases have high numbers of intraradicular Gram-positive bacteria and the findings of the above studies support the possibility of TLR2 expression by various cells in Gram-positive bacterial recognition.

Dental pulp cells have been examined for the expression of TLR2 in response to bacterial challenge. Odontoblasts and pulpal fibroblasts demonstrated

expression of TLR2 upon exposure to its ligand, LTA (Staquet *et al.* 2008). A further investigation using RT-PCR confirmed the gene transcriptions for inflammatory cytokines, TNFα but not IL1β in odontoblasts and fibroblasts. Further, when the sample cells were screened for cytokine protein production, TNFα and IL1β, none was produced at detectable levels by either cell type and the finding was attributed to a negative post-transcriptional regulatory process that is yet to be identified. In contrast, immature dental pulp dendritic cells showed significant levels of expression of TLR2 as well as inflammatory cytokine production (Keller *et al.* 2009). Dendritic cells are present in periapical granuloma and cystic lesions (Lukic *et al.* 2006, Colic *et al.* 2009) and are present in higher numbers in active and highly inflamed regions (Contos *et al.* 1987, Suzuki *et al.* 2001). It is reasonable to expect therefore, that dendritic cells will express TLR2 in these tissues.

The Gram-negative bacterial component LPS (lipid-A portion) is a ligand for TLR4 (Takeuchi *et al.* 1999) and TLR2 does not play an active role in its recognition (Hirschfeld *et al.* 2001). However, studies have reported LPS obtained from *P. gingivalis* has several different structures for the lipid-A subunit (Ogawa 1993, Darveau *et al.* 2004) and that heterogeneous LPS can activate host immune cells through either TLR2 or TLR4-dependent pathways (Darveau *et al.* 2004). *P. gingivalis* is commonly retrieved from the infected root canal system of teeth and different clonal types of this species can colonize the root canal in the same individual (Siqueira & RÔças 2010). TLR2 expression in various periapical lesions could possibly play a role in recognition of atypical LPS of *P. gingivalis* in both, symptomatic as well as asymptomatic cases.

T lymphocytes dominate the chronic periapical granuloma (Torabinejad & Kettering 1985, Lukic *et al.* 1990, Liapatas *et al.* 2003) and *in vitro* experiments have demonstrated expression of TLR2 in $CD4^+$ $CD3^+$ $CD14^-$ T cells (Komai-Koma *et al.* 2004). In addition to an indirect role through the activation of APCs, TLR2 may play an important role in adaptive immunity by directly enhancing antigen-specific Th1 responses. Sustained expression of TLR2 on memory T cells may represent an important host device by allowing an immediate strong response on encountering a previously recognized pathogen. However, TLR2 ligands alone cannot activate naive or memory T cells as stimulation by both TCR and IL2 is necessary for such activation.

The primary function of Treg cells is to regulate and dampen Th cell-mediated immune reactions. A lack of Treg activity may result in various autoimmune diseases whereas extensive immune suppression may result in inadequate development of an effective immune response during infection. Expression of TLR2 has been observed on Treg cells in direct contact with bacterial ligands. This pathogen-derived TLR2 expression promotes proliferation of Treg cells and production of IL2 which corresponds with a temporary loss of suppressive function. Thus Treg cells do not disturb the much-needed immune response aimed towards pathogen elimination. However, once the pathogen is cleared, proliferating Treg cells regain their suppressive capabilities and contribute to the balance between tolerance and immunity (Liu *et al.* 2006, Sutmuller *et al.* 2006). Similarly, in a periapical lesion, Treg cells are believed to be responsible for inhibition of Th1 mediated cytokines by producing TGFβ (Fukada *et al.* 2009). Interestingly, exposure to TGFβ abolished TLR2 mediated responses of odontoblast cells (Horst *et al.* 2009).

TLR4 in periapical diseases

TLR4 is known to be an important recognition receptor for LPS. In addition, it also modulates and co-ordinates PMN function, aids in dendritic cell maturation and various endothelial cell functions.

The TLR4 ligand LPS is a Gram-negative bacterial cell wall component and is observed frequently in the infected root canal system. A study using TLR4-gene mutated mice showed reduced bone destruction in periapical tissues 3 weeks following inoculation of root canals with anaerobic bacteria compared with normal control mice. This indicates a role of TLR4 in the production of inflammatory bone-resorptive cytokines at the site of inflammation. Interestingly, TNFα production remained unaffected in these mice. However, these TLR4 deficient mice did not develop any systemic symptoms from the periapical infection suggesting that TLR4 function is unrelated to infection dissemination (Hou *et al.* 2000). A similar but milder difference in periapical bone loss was noticed in an experiment with LPS-hyporesponsive mice at 4 and 8 weeks (Fouad & Acosta 2001).

Expression of TLR4 by odontoblasts (Jiang *et al.* 2006) and fibroblasts (Staquet *et al.* 2008) has been shown in response to antigen challenge. In an experimentally inflamed pulp model TLR2 and TLR4 were expressed on pulp macrophage and dendritic-like cells in a time-dependent manner, with TLR4 expression being lower and slower (Mutoh *et al.* 2007). Similarly,

when $TLR2^+$ and $TLR4^+$ murine macrophages were stimulated by root canal pathogens in an *in vitro* experiment, higher production of nitric oxide and reactive oxygen species was observed (Marcato *et al.* 2008). Further, murine cementoblasts have shown functional expression of TLR4 in response to LPS that was associated with alteration of gene expression related to cementum formation and upregulation of osteoclastogenesis-associated molecules, such as receptor activator of NFκB ligand (RANKL) (Nemoto *et al.* 2008). Expression of TLR4 by various cell types present in human inflammatory periapical lesions awaits further research.

Other TLRs in periapical diseases

The expression of other TLRs in pulp or periapical disease may not be evident because of their expression pathways. The ligand for TLR5 is flagellin a bacterial component of flagellated microorganisms. These organisms are generally not involved in the root canal microbiota so it is unlikely that TLR5 expression is involved in periapical disease. Similarly TLR3, 7, 8 and 9 are expressed in response to various viral antigens. Although it has been speculated that viruses may be associated with exacerbation of a periapical lesion (Sabeti *et al.* 2003), and TLR3 or TLR9 can be experimentally induced on odontoblasts (Durand *et al.* 2006) and pulp fibroblasts (Staquet *et al.* 2008) it is unlikely that expression of these pathways play a role in inflammatory periapical disease.

Conclusion

Research into the immunological interactions between bacterial antigen and inflammatory cells in pulp tissue has revealed interesting findings regarding the role of specific APCs in recognizing and presenting antigens to appropriate cells for initiating and sustaining the inflammatory process (Staquet *et al.* 2008). However, immunological aspects involving various cellular molecular interactions in recognising microbial agents in the development of periapical lesions await thorough research. Specifically very little information is available regarding the role of various TLRs in the induction and perpetuation of periapical diseases.

Antigen-presenting cells are present in periapical lesions of endodontic origin and play very significant roles of recognising, processing and presenting pathogen so that effective adaptive immune responses can be triggered. Although expression of various TLRs has not been fully characterized in the cells of human periapical lesions, the general evidence for TLR expression and cytokine production by inflammatory cells in response to bacterial antigens supports the probability that functional TLRs are involved with many cell types present in periapical lesions. The characterization of TLR expression in inflammatory cells of periapical lesions would expand our understanding about the complex pathogenesis of periapical diseases as well as aid in the diagnosis and development of novel treatment modalities for effective management of endodontic infection.

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