

# Role of Cytokines, Endotoxins (LPS), and Lipoteichoic Acid (LTA) in Endodontic Infection

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Received: September 04, 2014; Accepted: November 219, 2014; Published: December 03, 2014

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## Abstract

The most common etiological factor for pulpal and periapical disease is microorganisms. Apical periodontitis is considered as an inflammatory reaction in the peri-apical tissues to the presence of micro-flora and their components within the root canal system. The composition of micro-flora of root canal has been studied extensively over the years and the presence of gram-positive, gram-negative bacteria and their components are considered to be the main reason for endodontic treatment failure. Bacterial components such as Lipopolysaccharide (LPS) and Lipoteichoic Acid (LTA) can enter the peri-apical tissue and initiate the inflammatory process by increasing the level of cytokines. Therefore, this review was done to understand the role of Lipopolysaccharide (LPS), Lipoteichoic acid (LTA) and cytokines in endodontic infection and study their relationship between each other. Data from various resources were gathered and analyzed. The activation pathway and biological effects of cytokines were also studied and it was observed that cytokines especially TNF- $\alpha$  (Tumour Necrosis Factor), IL-1 $\beta$  (Interleukin), IL-6 and IL-8 are up-regulated as a consequence of the immune response generated against the LPS and LTA. Chemokines CXCL2 (Chemokine (C-X-C motif) ligand) and CXCL10 were also shown to have increased level due to the presence of LTA. Cytokines are required to combat the foreign material during an infection, but when the level of cytokines is too high, it can cause injuries to the host itself through peri-apical bone destruction. More studies should be carried out in order to better understand the significance of each cytokine during an endodontic infection and to improve the treatment given for the patients depending on their infection status.

**Keywords:** Cytokines; Endotoxins; Lipoteichoic Acid; Endodontic Infection

## Introduction

Bacteria and their byproducts are considered to be the major cause of pulpal and peri-apical diseases [1,2]. Therefore, their elimination is one of the most important steps in root-canal treatment. In most cases failure of root-canal treatment occurs when treatment procedures have not met a satisfactory standard for control and elimination of infection [3]. The association of bacterial species with specific clinical conditions has been

investigated in a number of studies and it has been reported that bacterial species recovered from asymptomatic teeth are different from that isolated from clinically symptomatic teeth [4-7] and from primary infection to that of failed endodontic treatment [8-11]. Primary infected root canals are untreated canals, where microorganisms are able to access and colonize the pulpal tissue and progresses to cause apical periodontitis. The root canal microbiota responsible for primary infection is mainly mixed, comprising gram-negative and gram-positive and mostly anaerobic microorganisms. On the other hand, persistent intraradicular or secondary infections are the major causes of the failure of root-canal treatment and facultative anaerobic, gram-positive bacteria predominate in the canals with failed endodontic treatment [11-13]. It is generally believed that persisting gram-positive bacteria in root canals have ability to survive under harsh, nutrient-limited conditions of the root-filled teeth [13,14]. These gram-positive and gram-negative bacteria contain various virulence factors including Lipoteichoic acid (LTA), Lipopolysaccharide (LPS), peptidoglycan, aggregation substance, surface adhesions, sex pheromones, lytic enzymes such as gelatinase, hyaluronidase and cytolysin, responsible for survival of these microorganisms in a very harsh environment of the root canal system [15]. Each of these factors may be associated with various stages of endodontic infection as well as peri-apical infection. Among the virulence factors, LTA, a glycerol phosphate surface polymer, seems to have high pathogenic potential similar to Lipopolysaccharide (LPS) (i.e. endotoxin) released from gram-negative bacteria [16]. LTA from gram-positive bacteria acts as an adhesion molecule binds to host cells via its lipid moiety and promote bacterial colonization and invasion. It acts as a stimulator of respiratory burst and exocytosis of lysosomal enzymes, as an inducer of nitric oxide and cytokines, as a mitogen for T cells, and as a transcription factor [17]. LTA can also synergise with peptidoglycan and in mixed bacterial infections, also with lipopolysaccharide to induce generation of nitric oxide and cytotoxic cytokines [18]. LPS and LTA activate the innate immune system by similar mechanisms. Both LPS and LTA bind to CD14, activate signaling by Toll-Like Receptors

(TLRs), and induce proinflammatory cytokines such as Tumor Necrosis Factor alpha (TNF- $\alpha$ ), Interleukin-1 (IL-1), Interleukin-8 (IL-8), Interleukin-12 (IL-12), and anti-inflammatory cytokine interleukin-10 (IL-10) [19,20]. Studies have demonstrated that LTA induces bone resorption [21], TNF- $\alpha$  production by TLR 2 (Toll-like receptor 2) [22], apoptosis of cultured pulp cells (mainly fibroblasts) that contributes to the initiation of and/or progression of pulpitis [23], exhibits high potency in the induction and rapid rise of macrophage Vascular Endothelial Growth Factor (VEGF) expression [24,25] that may lead to pulpal necrosis or even to the maintenance of the inflammatory reaction after root canal therapy. Today, the term 'endotoxin' is used synonymously with the term lipo-polysaccharide, which is a major constituent of the outer cell wall of gram-negative bacteria. LPS is considered the major etiological component responsible for pathophysiology of inflammation and post infectious sequelae. Correlations have been found between the high concentration of LPS content of infected root canals and clinical endodontic symptoms such as spontaneous pain, tenderness to percussion, exudation and periapical radiolucent areas [26,27] but not much importance is given to LTA content present in the infected canals, which can also lead to severe inflammation and tissue damage. There are findings that do not clearly support the role of LTA as a major virulence factor because quantities of the LTA (microgram to milligram) necessary to induce generation of proinflammatory agonists are many times greater than those of lipopolysaccharide (nanogram) [17]. Although lipopolysaccharide is almost the exclusive virulence factor in gram-negative organisms, those that are gram-positive can induce tissue damage by elaborating many cytotoxic factors, of which LTA would be only one. The literature has indicated that the success of re-treatment of teeth with apical periodontitis is lower with an overall success rate of 66% [28]. This poorer prognosis in root-canal re-treatments is associated with difficulties in the elimination of the particular microorganism in cases of root-canal treatment failure because different strains from the same bacteria species can present variations in their virulence and resistance influencing the treatment outcome. Therefore, a thorough knowledge of virulent microorganism present in failed endodontic cases is necessary to guide new strategies to combat infection, leading to a better prognosis for root-canal re-treatments. Therefore, this review was carried out to understand the role of (LPS), (LTA) and cytokines in endodontic infection and study their relationship between each of them.

## Discussion

The approach of review was taken to compile current best evidences for this review. After formulation of a research question on the role of LPS, LTA and cytokine in an endodontic infection, a comparison was made between data collected from various databases using the Medical subject headings (Mesh) or DeCS vocabulary. The search engines that were utilized for electronic data from the internet were MEDLINE, PUBMED, GOOGLE, and the Cochrane Collaboration Database for original research articles in the English literature published from 1<sup>st</sup> January 2000 to 31<sup>st</sup> December 2013, using keywords like endodontic infection, lipopolysaccharide, lipoteichoic acid and cytokine. Search criteria

AND was mostly used to search for more relevant article. Around 58 articles were obtained, with a mixture of journals and books. Inclusion eligibility for the review was determined by specific criteria that are, the paper must be a research paper, published within the past 15 years, with virulence factors (LPS or LTA) and cytokines identified, and must be an endodontic-related infection. Only those that fulfill all these criteria were used in this review. The author, year, virulence factors, cytokines and conclusion of the selected studies are summarized in Table 1. According to the papers selected, TNF- $\alpha$  is the most abundant cytokine that has been detected in endodontic infection, 53% of the studies detected this cytokine. Generally, both LPS and LTA cause up-regulation of TNF- $\alpha$ . Gram negative species tend to induce higher ratio of TNF- $\alpha$  to IL-8. Interleukins especially IL-1 $\beta$ , IL-6, IL-8, and IL-10 are also increased during the course of endodontic infection. Chemokines such as CXCL2 and CXCL10 are also detected in endodontic infections. The chemokines activate inflammatory cells and also influence angiogenesis. Other than chemokines, LTA is also shown to induce VEGF (Vascular Endothelial Growth Factor) in macrophages up to nine folds.

Cytokines are small signaling molecules that mediate host responses to infection, inflammation, and trauma. Pro-inflammatory cytokines initiate or enhance systemic inflammation while anti-inflammatory cytokines reduce inflammation and promote healing. Examples of pro-inflammatory cytokines include IL-1 and TNF- $\alpha$  whereas IL-10 is an important anti-inflammatory cytokine. Some cytokines can have both pro-inflammatory and anti-inflammatory properties such as the IL-6 as it can inhibit TNF- $\alpha$  and IL-1, at the same time activates IL-10. Cytokines work with each other in a homeostatic network regulation to prevent the constant state of inflammation. In endodontic infection; gram-negative bacteria contribute a high percentage of LPS, a primary virulence factor [29-39]. Murakami *et al.* [29] detected *P.endodontalis* LPS in 90% of the samples obtained from patients with acute abscesses. LPS is also known as endotoxins, it forms an integral part of cell wall of gram-negative bacteria [40]. The LPS molecule consists of O-antigen, core oligosaccharide, and a glycolipid component named lipid A [30]. These endotoxins are released during bacteria multiplication and after cell death [40]. Similarly, LTA is the main virulence factor of gram positive bacteria. It is an amphiphilic molecule consisting of a poly-glycerolphosphate with a complex glycolipids group attached to it [17]. LPS activates mammalian cells by first binding with Lipopolysaccharide Binding Protein (LBP) in the serum. Subsequently, LBP presents LPS to the CD14/TLR4/MD2 receptor complex. This receptor complex is present on cells like-neutrophils, macrophages, dendritic cells and endothelial cells [18,22]. This interaction activates an intracellular signal transduction cascade, resulting in secretion of inflammatory cytokines. Gram positive bacteria trigger similar cascade by mediating ligands through TLR2 instead of TLR4 [5,23]. According to Tietze *et al.* [31], Gram negative species tend to induce higher levels of TNF- $\alpha$  than IL-8. However, evidences have shown that *P. gingivalis* is an exception for this presumption as it has similar activity with the gram-positives. This is due to the heterogeneity of the species' lipid A molecule

**Table 1:** Summary of the studies selected.

Ref	Year	Author	Title	Virulence factor and cytokines	Conclusion
29	2001	Murakami et al.	A possible mechanism of maxillofacial abscess formation: involvement of <i>Porphyromonasendodontalis</i> lipopolysaccharide via the expression of inflammatory cytokines	<i>Porphyromonasendodontalis</i> LPS TNF- $\alpha$ , IL-1 $\beta$	LPS of the bacteria is present in the bacterially infected root canal fluid of about 90% of the patients. The ability of LPS to induce cytokines was reduced by incubating the macrophages with antibodies against the bacterial LPS. ELISA showed a significantly high level of TNF- $\alpha$ to be present in the infectious materials.
30	2001	Gutsmann et al.	Dual Role of LBP in Neutralization of LPS and Enhancement of LPS-Induced Activation of Mononuclear Cells (MNC)	LPS IL-1,IL-6, TNF- $\alpha$	LBP level rises after acute-phase response due to the activation by IL-1 and IL-6. LPSs activate monocytes and macrophages to secrete TNF- $\alpha$ and IL-1, etc. LBP present as a soluble protein and also as a transmembrane protein of MNC. Low concentrations of LBP enhance the LPS-induced activation of MNC, whereas the acute-phase rise in LBP concentrations inhibits LPS-induced cellular stimulation.
25	2003	Telles et al.	Lipoteichoic Acid Up-regulates VEGF Expression in Macrophages and Pulp Cells	LTA VEGF	LTA induced up to a 9-fold increase in VEGF protein expression in macrophages. LTA is sufficient to induce expression of a pro-angiogenic factor.
31	2006	Tietze et al.	Differences in innate immune responses upon stimulation with gram positive and gram-negative bacteria	LTA, LPS, TNF- $\alpha$ , IL-8	Gram negative species produced higher amounts of TNF- $\alpha$ while gram positive species produced higher amount of IL-8 due to the activation of different TLRs. Purified gram-negative and gram-positive products may not mimic the response to whole pathogen.
32	2006	Durand et al.	Lipoteichoic Acid Increases TLR and Functional Chemokine Expression while Reducing Dentin Formation in Odontoblasts	LTA Chemokines CXCL2	TLR trigger secretion of pro-inflammatory chemokines and cytokines that activate blood borne inflammatory cells. CCL2 is the key chemokine produced during microbial infection. Chemokines also influence angiogenesis.
33	2007	Adachi et al.	Caries-related Bacteria and Cytokines Induce CXCL10 in Dental Pulp	LTA, CXCL10	HDPF stimulated with PGN, but not LTA, were able to produce CXCL10.
34	2008	Staquet et al.	Different Roles of Odontoblasts and Fibroblasts in Immunity	LTA CXCL2 and CXCL10	<i>CXCL2</i> and <i>CXCL10</i> were thus increased by LTA only in odontoblast-like cells
35	2008	Baik JE et al.	Lipoteichoic Acid Partially Contributes to the Inflammatory Responses to <i>Enterococcus faecalis</i>	LTA, TNF- $\alpha$	LTA of <i>E. faecalis</i> stimulate macrophage to produce significant high level of TNF- $\alpha$ and nitric oxide. LTA also stimulate production of inflammatory mediators. LTA stimulate TLR2 but not TLR4.
36	2009	Soden et al.	Angiogenic Signaling Triggered by Cariogenic Bacteria in Pulp Cells	LTA, VEGF	LTA induces VEGF upregulation in pulp cells via P13K-Akt signaling
37	2012	Kraus et al.	Interactions of Adiponectin and Lipopolysaccharide from <i>Porphyromonas gingivalis</i>	LPS, IL-1 $\beta$ , IL-6, IL-8, IL-10	LPS significantly up-regulate IL-1 $\beta$ , IL-6, IL-8, IL-10 expressions.
38	2012	Maciel et al.	Cytokine expression in response to root canal infection in gnotobiotic mice	<i>Fusobacteriumnucleatum</i> , <i>Peptostreptococcus prevotii</i> IFN-g, TNF- $\alpha$ , IL-10, IL-4, TGF-b	Different bacteria have different effect on cytokines levels. <i>F. nucleatum</i> causes increase in IFN-g, TNF- $\alpha$ , IL-4. IL-10 is induced by either one of the two bacteria. TGF-b is induced by combination of the two bacteria.

which can activate both TLR 2 and 4 [9,10]. Therefore, the types of cytokines released should depend on the TLR activated instead of the Gram stain of the bacterial species. Studies revealed that the cytokines induced by LPS and LTA shares a wide range of pathophysiological effects, depending on the type of cytokine. However, the potency of LTA in inducing cytokine release is 100- to 1000- folds lower compared to LPS [19]. These cytokines are associated with pulp and peri-apical inflammation as well as activation of complement system to eradicate the pathogens. Specifically, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 are important mediators during acute phase of inflammation. However, if the homeostasis of cytokines is not achieved, there will be fibrosis and necrosis of the pulp. This is because cytokines can activate neutrophils and macrophages to produce reactive oxygen, nitrogen species, and cytotoxic cytokines that may cause cell damage [24]. Besides, cytokines like Vascular Endothelial Growth Factor (VEGF) will cause angiogenesis and swelling in pulp cavity [2,25]. Eventually, the vessels will be damaged. Osteomyelitis and periapical bone destruction can also take place. Although LPS is known to induce the production of cytokines, the amount of cytokines can be lowered to control inflammation by conferring 'LPS tolerance' to the immune cells. This can be done by exposing the immune cells to low doses of LPS [26]. The ability to develop tolerance is also better in young compared to older adults [4].

## Conclusion

The present work provides insight about the role of LPS and LTA in the initiation of immune responses that occur in human dental cavity. By analyzing the evidences and studies carried out, a positive association was found between LPS and LTA with cytokines. Results from the various studies showed that certain inflammatory cytokines especially TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8 are up-regulated in endodontic infections. Although the cellular responses caused by cytokines are usually beneficial, they might also harm the host through septic shock due to overproduction of cytokines.

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