Review Article

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Antigens in Periodontal Disease: A Review

Sidharth Shrikant Joshi[1*] Atul Ashok Pandharbale[2] Parth Pratim Roy[3] Swati Vishwakarma[4]

[1]Senior Lecturer, Department of Periodontology, Aditya Dental College and Hospital, Beed, Maharashtra, India.
[2]Senior Lecturer, Department of Oral Medicine and Radiology, Aditya Dental College and Hospital, Beed, Maharashtra, India.
[3]Senior Lecturer, Department of Periodontology, Shri Rajiv Gandhi Dental College and Hospital, Bangalore, Karnataka, India.
[4]Senior Lecturer, Department of Periodontology, Shri Rajiv Gandhi Dental College and Hospital, Bangalore, Karnataka, India.

ABSTRACT

Background: An antigen has been defined as any substance which, when introduced parenterally into the body, stimulates the production of an antibody with which it reacts specifically and in an observable manner. Each of the periodontal pathogens discussed in this review produces an array of antigens that stimulate epithelial cells and fibroblasts to produce pro-inflammatory cytokines. Furthermore, these antigens stimulate pro-inflammatory cells such as macrophages, monocytes and neutrophils and these in turn produce a wide variety of cytokines that maintain a pro-inflammatory response and induce a Th1 (inflammatory) cytokine response. The development of a multispecies vaccine that is able to target all four bacterial species that have been implicated in the development of periodontitis may be more successful than a vaccine against a single species.

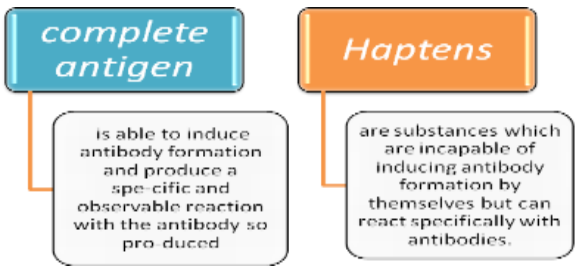
Keywords: Antigens, Cytokines, Periodontitis, Vaccine.

INTRODUCTION

An antigen has been defined as any substance which, when introduced parenterally into the body, stimulates the production of an antibody with which it reacts specifically and in an observable manner.[1] This traditional description of an antigen is no longer comprehensive enough in the light of current concepts about the immune response. Some antigens may not induce antibodies but may sensitize specific lymphocytes leading to cell mediated immunity or may cause immunological tolerance.[2]

The word 'parenteral' (meaning, outside the intestinal tract) is used in the definition because orally administered antigens are usually hydrolyzed by digestive enzymes and their antigenicity destroyed, so that no antibody formation takes place. When given parenterally, antigens do not undergo any such inactivation and can induce antibody production. However, there are exceptions and some antigens can be immunogenic when given orally, such as oral vaccines.[3]

The two attributes of antigenicity are 1) induction of an immune response (immunogenicity), and 2) specific reaction with antibodies or sensitized cells (immunological reactivity). Based on the ability of antigens to carry out these two functions, they may be classified into two different types:



Haptens become immunogenic (capable of inducing antibodies) on combining with a larger molecule *carrier.* Haptens may be complex or simple; while *complex haptens* can precipitate with specific antibodies, *simple haptens* are nonprecipitating.[4]

The smallest unit of antigenicity is known as the *antigenic determinant* or *epitope.* The epitope is

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***Correspondence Dr. Sidharth Shrikant Joshi.**
Department of Periodontology, Aditya Dental College and Hospital, Beed, Maharashtra, India.
Email: sidharthjsh@rediffmail.com

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capable of sensitising an immunocyte and of reacting with its complementary site on the specific antibody or T cell receptor. Epitopes may be' present as a single linear segment of the primary sequence (sequential or linear epitope) or formed by bringing together on the surface residues from different sites of the peptide chain during its folding into the tertiary structure (conformational epitope).[4]

Determinants of antigenicity

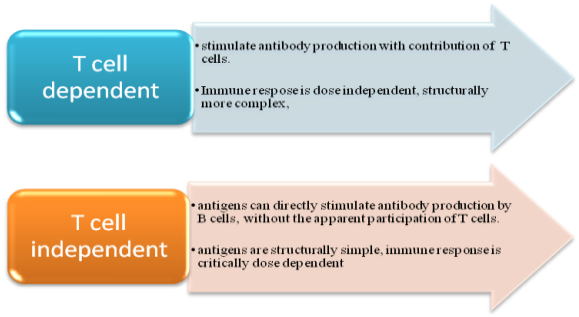
A number of properties have been identified which make a substance antigenic but the exact basis of antigenicity is still not clear.[3,4]

Sr No	DETERMINANTS OF ANTIGENICITY	PROPERTIES
1	Molecular Size	Antigenicity Is Related To Molecular Size. Very Large Molecules, Such As Hemocyanins (Mw 6.75 Million), Are Highly Antigenic And Particles With Low Molecular Weight (Less Than 50001 Are Nonantigenic Or Feebly.So.[3]
2	Chemical Nature	Most Naturally Occurring Antigens Are Proteins And Polysaccharides. Lipids And Nucleic Acids Are Less Antigenic. Their Antigenicity Is Enhanced By Combination With Proteins.[4]
3	Determinants	Phagocytosis And Intracellular Enzymes Appear To Play An Essential Role In Breaking Down Antigens Into Immunogenic Fragments. Substances Unsusceptible To Tissue Enzymes Such As Polystyrene Latex Are Not-Antigenic.[4]
4	*Foreignness*	Only Antigens Which Are 'Foreign' To The Individual (Nonself) Induce An Immune Response This Was First Recognized By Ehrlich Who Proposed The Concept Of 'Horror Auto-Toxicus' (Which Means Fear Of Self Poisoning).[3]

In general, the antigenicity of a substance is related to the degree of its foreignness. Antigens from other individuals of the same species are less antigenic than those from other species. Antigens from related species are less antigenic than those from distant species.[4]

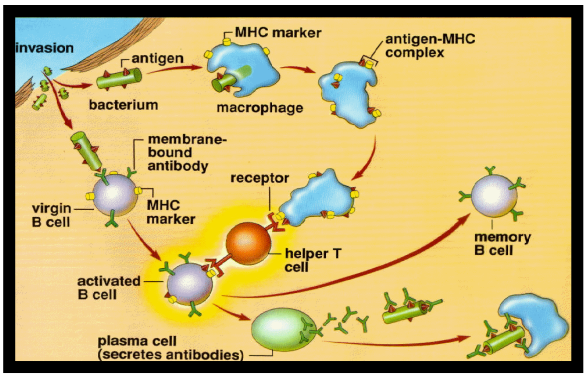
Biological classes of antigens

Depending on their ability to induce antibody formation, antigens are classified as [4]



Antigen Processing and Presentation

The *major histocompatability complex* (MHC) is a locus on the short arm of chromosome *b* that encodes a number of molecules, including MHC classes I, II, and III molecules, which ace involved with antigen uptake, processing, and presentation. All cells process and present self-derived antigens (intracellular antigens) in association with MHC class 1 molecule. MHC class I molecules are used to present intracellular antigens to CD8+ r cells and NK cells.[5]



***Actinobacillus actinomycetemcomitans* antigens**

Polysaccharide and lipopolysaccharide

A. actinomycetemcomitans is a gram-negative, non-spore forming, non-motile, capnophilic, facultative anaerobic coccobacillus. *A. actinomycetemcomitans* can be classified into six distinct serotypes (a–f) based on surface polysaccharides located on the O side chains of lipopolysaccharide. The presence of *A. actinomycetemcomitans* in subgingival plaque has been associated with aggressive periodontitis with serotype b twice as prevalent as serotype a These data are supported by a number of other studies

that have shown serotype b to be the predominant *A. actinomycetemcomitans* serotype associated with periodontitis Animal studies have shown that serotype b induces the pro-inflammatory cytokine interleukin-6 (IL-6) from murine splenocytes.[6] Furthermore, serotype b induces more IL-1 (another pro-inflammatory cytokine) from thymocytes than serotype a or c. Ebersole et al. have reported that the order of virulence for *A. actinomycetemcomitans* serotypes in the murine lesion model was serotype b > a > c[7] The serotype-specific polysaccharides are reported to be major antigenic targets of the immune response of aggressive periodontitis patients infected with *A. actinomycetemcomitans* with the predominant antibody subclass being IgG2 followed by IgG1 and IgG3.[8]

A. actinomycetemcomitans lipopolysaccharide also stimulated the release of IL-1β from polymorphonuclear leukocytes as well as other pro-inflammatory cytokines, such as, tumor necrosis factor-α (TNF-α) and IL-8 . Murine bone marrow cells stimulated with lipopolysaccharide from *A. actinomycetemcomitans* have also been reported to release IL-1α and prostaglandin E_2 (PGE_2).[8]

Leukotoxin

A. actinomycetemcomitans produces a 116 kDa immunomodulating protein antigen, termed leukotoxin. The leukotoxin is a pore-forming protein and is a member of the repeats-in-toxin (RTX) family of bacterial toxins.[9] In a study by Zambon et al. fifty-five percent of localized aggressive periodontitis patients were shown to be infected with *A. actinomycetemcomitans* strains that produced a leukotoxin that was able to lyse human peripheral blood polymorphonuclear leukocytes and HL-60 cells (a promyelocytic cell line) *in vitro*. The leukotoxin is not released by *A. actinomycetemcomitans* but is anchored by a C-terminal hydrophobic domain to the cellular outer membrane or membranous vesicles. A number of studies have shown that the killing of T-cells, neutrophils, natural killer (NK) cells by leukotoxin is by the formation of pores in the cell membrane resulting in osmotic lysis and necrosis. *A. actinomycetemcomitans* leukotoxin at low doses induces apoptosis in HL-60 cells, which supported earlier observations that cell death of T-cells and NK cells caused by leukotoxin was by an apoptotic mechanism. As at high doses, leukotoxin has been reported to cause cell death by necrosis, then it has been suggested that *A. actinomycetemcomitans* leukotoxin behaves in a dose-dependent manner.[10]

Extracellular proteolytic enzymes

Proteolytic activity in subgingival plaque, in particular trypsin-like proteolytic activity, has been highly correlated with clinical measurements of periodontitis. Culture supernatants of *A. actinomycetemcomitans* serotype b have been shown to have high trypsin-like proteolytic activity[11]. Wang et al. purified a 50-kDa trypsin-like protease from the culture supernatant of *A. actinomycetemcomitans* b that hydrolysed the synthetic substrates as well as the protein substrates, fibrinogen and collagen type I. Protease inhibition assays indicated that this enzyme is a serine- or metallo-protease. Furthermore, the tyrpsin-like protease reduced the proliferation of human gingival epithelial cells and cell surface levels of fibronectin.[12] As increased proteolytic activity of other periodontal pathogens, especially *P. gingivalis,* has been linked to enhanced virulence, the greater proteolytic activity, especially trypsin-like activity, of *A. actinomycetemcomitans* serotype b compared with serotype c is consistent therefore with the greater virulence of this serotype. As well as degrading major components of connective tissue, proteolytic enzymes in *A. actinomycetemcomitans* culture supernatants have been reported to degrade IgG (all four subclasses), serum IgA and IgM but not IgD or IgE *in vitro.* Thus the proteolytic activity of *A. actinomycetemcomitans* may be an important virulence factor involved in dysregulation of the host's immune response.[12]

GroEL heat shock protein

A. actinomycetemcomitans produces an antigenic 64 kDa GroEL protein that is equally expressed on the cell surface of all serotypes a–e . *A. actinomycetemcomitans* GroEL has 95% DNA sequence identity with *E. coli* GroEL and 57% and 42% sequence identity with *M. tuberculosis* heat shock protein (hsp) 65 and human hsp60, respectively In a study by Koga et al. antibodies directed to *A. actinomycetemcomitans* GroEL were detected in 50% of sera taken from aggressive periodontitis patients whereas no antibodies

directed to GroEL were detected in healthy patient sera. Heat shock proteins have been suggested to be associated with the etiology and pathogenesis of both experimental and naturally occurring autoimmune diseases such as juvenile chronic arthritis, rheumatoid arthritis and atherosclerosis. Microbial heat shock proteins, despite the high homology between prokaryotic and eukaryotic cells, are highly immunogenic and can lead to antimicrobial immunity. It has been speculated, therefore that the long term infection associated with chronic inflammatory diseases, such as periodontitis, results in the constant exposure of microbial heat shock antigens to the immune system, which may promote autoimmune disease.[13]

Fimbriae

A. actinomycetemcomitans produces highly antigenic bundle-forming fimbriae that are 5 nm in diameter and several μm in length. Using liquid chromatography–electrospray ionization mass spectrometry (LC–MS). Inoue et al. Identified three fimbrial sub-unit (fimbrillin) molecular mass species (6,226, 6,366, and 6,513 Da) which were higher than the predicted molecular mass for the fimbrillin (5,118 Da) derived from the nucleotide sequence of the fimbrillin gene *flp* . The discrepancies in the predicted and observed molecular masses of the fimbrillin were attributed to post-translational glycosylations. The fimbriae are suggested to play an important role in colonization as fimbriated *A. actinomycetemcomitans* strains have greater affinity for epithelial cells and saliva-coated hydroxyapatite than non-fimbriated strains. Fimbriae may also have a role in *A. actinomycetemcomitans* invasion of epithelial cells by receptor-mediated endocytosis. Harano et al. have immunized rabbits with synthetic oligopeptides representing a part of the fimbrial amino acid sequence. This immunization produced high titer antibodies that recognized purified fimbrial protein. Antisera raised to one of the fimbrial peptides were reported to inhibit the attachment of fimbriated *A. actinomycetemcomitans* to saliva-coated hydroxyapatite beads, buccal epithelial cells and fibroblasts.[13]

***Treponema denticola* antigens**

Lipopolysaccharide

T. denticola is a gram-negative, motile, asaccharolytic, anaerobic spirochete with typical helical morphology. Unlike lipopolysaccharide of other Gram-negative bacteria the outer membrane sheath of *T. denticola* lacks 3-deoxy-D-*manno*-octulosonic acids, heptoses and β-hydroxy fatty acids and the cytoplasmic membrane and outer membrane sheath have similar fatty acyl chain composition (anteisopentadecanoic acid, palmitic acid and iso-palmitic acid) indicating that the membrane fluidity of *T. denticola* is similar to the membranes containing lipoteichoic acid of gram-positive bacteria.[14] However, *T. denticola* lipid does form normal bilayer membranes with a distinct phase transition typical of lipopolysaccharide, but the phase transition of the outer sheath lipid is broader and the temperature lower (22°C) than for the phase transition of lipopolysaccharide (35.5°C). These data indicate that the ultrastructure of the outer membrane sheath of *T. denticola* is similar to the outer membrane of other gram-negative bacteria, however, the lipid composition of the outer sheath is similar to lipoteichoic acid of the cell surface of gram-positive bacteria. *T. denticola* have been reported to induce LPS, in a dose dependent manner, the inflammatory mediators, nitric oxide, TNF-α and IL-1 from murine macrophages. In this study the outer membrane lipids and lipoproteins also induced the activation of the inflammatory cytokines, nitric oxide and TNF-α from lipopolysaccharide-unresponsive macrophages.[15] Gopalsami et al. have reported that the outer membrane lipid of *T. denticola* was able to induce bone resorption as measured by a Ca-release assay. *T. denticola* induce bone resorption and activation of inflammatory cytokines by a separate mechanism to lipopolysaccharide.[16,]

Major sheath protein

A major surface protein and antigen of *T. denticola* is the major sheath protein (Msp), which has been reported to have different molecular masses in different *T. denticola* strains. The protein exists as a 53-kDa protein in strains ATCC 35405 and 33520 and a 64-kDa protein in strain OKTI. In its native form the Msp aggregates to form oligomers with reported molecular masses of 116–200 and 130–300 kDa. Although earlier studies indicated that Msp formed a hexagonal array structure in the outer sheath, Caimano et al. has more recently

suggested that Msp is predominately a periplasmic protein that has limited surface exposure.[17]

Flagellum

A major distinction between *T. denticola* and the other three periodontal pathogens discussed here is that *T. denticola* is motile. The axial helical-flagella and the helically-shaped cell cylinder of *T. denticola,* like all spirochetes, result in a cork screw-like locomotion, which aids movement in highly viscous environments like gingival crevicular fluid and the penetration of gingival epithelial and connective. The flagellum of *T. denticola* is composed of a basal body, rod, hook and a filament. The flagellar filament consists of three core proteins FlaB1 (35 kDa), FlaB2 (35 kDa) and FlaB3 (34 kDa) and a major sheath protein FlaA (38 kDa). Although each of the filament proteins are antigenic, FlaA and FlaB3 have been shown to be the immunodominant antigens. FlaA has been suggested to have a role in adherence of *T. denticola* to host cells as it binds fibronectin.[18]

Extracellular proteolytic enzymes

Important extracellular protein antigens of *T. denticola* are its proteolytic enzymes, reviewed in Potempa et al. The prolyl-phenylalanine specific, chymotrypsin-like, serine proteinase known as dentilisin or repulsion is the best characterized *T. denticola* protease. This enzyme occurs on the cell surface and has been purified as a complex of three proteins, a 72-kDa proteinase encoded by the *prtP* gene and two smaller proteins that have been reported in a number of studies with different molecular masses, possibly due to variable proteolytic processing. The proteinase had been shown to degrade bradykinin, substance P and angiotensin I and II as well as host protease inhibitors α_1-antitrypsin, antichymotrypsin, α_2-macroglobulin, antithrombin III, antiplasmin and cystatin C. Dentilisin has also been suggested to play a role in the degradation of pro-IL1β into its bioactive forms and thus stimulate the inflammatory response. The presence of *T. denticola* in subgingival plaque samples has been correlated with trypsin-like proteolytic activity, which in turn has been correlated with clinical parameters of periodontitis. [18]

***Tannerella forsythia* (formerly *Bacteroides forsythus*) antigens**

T. forsythia is a gram-negative, anaerobic, saccharolytic fusiform bacterium . Very little is known about the antigens and virulence factors of *T. forsythia* compared with the other major periodontal pathogens, *P. gingivalis, A. actinomycetemcomitans* and *T. denticola.* This has mainly been the result of the difficulty in reliably cultivating *T. forsythia.* However, the finding that *T. forsythia* could be grown together with another bacterium such as *F. nucleatum* or *Streptococcus sanguis* revealed that it requires exogenous *N*-acetylmuramic acid for growth. Inclusion of *N*-acetylmuramic acid in conventional media now allows routine cultivation of *T. forsythia* in batch culture.[18]

Lipopolysaccharide

Very little is known about the structure and chemical composition of *T. forsythia* lipopolysaccharide, however a study by Vasel et al indicated that *T. forsythia* lipopolysaccharide may be similar to *P. gingivalis* lipopolysaccharide. Antibodies from rabbits immunized with lipopolysaccharide from *T. forsythia* or *P. gingivalis* cross-reacted in an ELISA, Western and dot blot analyses to epitopes in lipid A and core lipopolysaccharide carbohydrates, indicating antigenic and potentially structural and chemical similarity. Firoozkoohi et al. analysed *T. forsythia* lipopolysaccharide using tricine–SDS–PAGE and showed that the lipopolysaccharide was a typical S-form exhibiting long ladders with multiple steps. The same authors also showed that the *O*-polysaccharide side chains of *T. forsythia* lipopolysaccharide are of similar length to those of *Bacteroides* species.[18]

Extracellular proteolytic enzymes

Several studies have shown that *T. forsythia* produces cell surface proteolytic enzymes. These include the trypsin-like serine proteases with molecular masses 70 and 81 kDa and a 48-kDa hemolytic cysteine–protease designated PrtH . *T. forsythia* trypsin-like activity, together with trypsin-like activities of *T. denticola* and *P. gingivalis* in subgingival plaque samples have been correlated with clinical parameters of periodontitis .

Interestingly, unlike *P. gingivalis* and *T. denticola, T. forsythia* has been reported to be unable to proteolytically degrade a variety of host protease inhibitors and lactoferrin. The trypsin-like activity has been suggested to play a role in binding of *T. forsythia* to erythrocytes, polymorphonucleocytes and fibroblasts.[18] Using PCR Tan et al. showed that *T. forsythia* occurred in 91% of subgingival plaque samples from chronic periodontitis patients and that 85% of these were colonized by *T. forsythia* with the *prtH* genotype, whereas only 9% of subgingival plaque samples from healthy patients contained this genotype.[18]

Porphyromonas gingivalis antigens

P. gingivalis is a gram-negative, non-spore forming, non-motile, asaccharolytic, obligate anaerobic coccobacillus. A number of studies have attempted to classify *P. gingivalis* into serotypic groups based on antisera binding to either the capsule, fimbriae or outer membrane antigens of *P. gingivalis*.[19]

Capsule

One major surface antigen of *P. gingivalis* is the capsule, a polysaccharide heteropolymer up to 15 nm thick,that surrounds the outer membrane. Based on the antigenicity of the polysaccharide K antigens of the capsule, six K antigen serotypes have been reported.[20] The capsule of *P. gingivalis* has been shown to be immunochemically distinct from lipopolysaccharide, to contain galactosamine, glucosamine, galactosaminuronic acid and glucose and to have high amino acid content. The capsule of *P. gingivalis* is suggested to be an important virulence factor as strains representing the six K-antigen positive serotypes.*P. gingivalis* capsule also inhibits the attachment of periodontal ligament fibroblasts to the tooth root surface and long-term exposure to capsular-polysaccharide alters the properties of the tooth root surface decreasing the ability of fibroblasts to attach.[18]

Lipopolysaccharide

Several studies have suggested that lipopolysaccharide of *P. gingivalis* is an important antigen in the host immune response in periodontitis. Lipopolysaccharide has been shown to be a major *P. gingivalis* antigen recognized by sera from periodontitis patients with the antibody being of the IgG2 subclass. *P. gingivalis* lipopolysaccharide lacks heptose and β-hydroxydecanoic acid typically found in classical enterobacterial lipopolysaccharide, but exhibits typical S-form and smooth lipopolysaccharide chemotype.[21] Reddi et al. have shown that the lipid A component is more biologically active than whole lipopolysaccharide in inducing IL-6 in human gingival fibroblasts and a macrophage cell line.[22] The mechanism by which *P. gingivalis* lipopolysaccharide stimulates IL-6 secretion in fibroblasts is suggested to be not via CD14, the lipopolysaccharide receptor, but via Toll-like receptors (TLRs) as antibodies to TLR-4 inhibited IL-6 expression. Jotwani et al. have shown that dendritic cells pulsed with *P. gingivalis* lipopolysaccharide undergo maturation, up-regulate accessory molecules and secrete IL-1β, PGE_2, IL-10 and IL-12.[23] The ability of *P. gingivalis* lipopolysaccharide to stimulate counter regulatory cytokines from non-myeloid, myeloid and lymphoid cells using different cellular receptors may aid in the dysregulation of the host immune response and thus exacerbate disease.[24]

Fimbriae

The fimbriae of *P. gingivalis* are highly antigenic with chronic and aggressive periodontitis patients exhibiting high serum IgA and IgG antibody responses, predominantly an IgG3 isotype, to fimbrial antigen compared with healthy subjects. The fimbriae are curly, single-stranded filaments, 5 nm in diameter and are composed of fimbrillin (fimbrial sub-unit), a 43-kDa protein encoded by the *fimA* gene.[25] Currently five *P. gingivalis* fimbrial types (types I–V) have been described based on their antigenicity. *P. gingivalis* fimbriae are thought to be important for the adhesion of the bacterium to host tissues.[26] In this study by Nakagawa et al. the adherence and invasion of epithelial cells was suggested to be mediated by fimbrial interaction with the surface receptor a α5β1 integrin.[27] Sojar et al. have reported that *P. gingivalis* fimbriae bind to cytokeratin on the surface of epithelial cells and suggested that this interaction may be important for colonization of the oral mucosa by *P. gingivalis*.[28] Active invasion of endothelial cells by *P. gingivalis* mediated via fimbriae stimulates the expression of the surface cell adhesion molecules ICAM-1, VCAM-1, P- and E-selectins, thus recruiting leukocytes to the site and enhancing the inflammatory response. *P. gingivalis* fimbriae have also been shown to

stimulate the release of IL-1α, IL-1β TNF-α, neutrophil chemotactic factor KC, and MCP-1 from macrophages and fibroblasts and TNF-α, IL-6 and IL-8 from polymorphonuclear cells.[29]

Extracellular proteolytic enzymes

P. gingivalis produces a variety of proteinases and their ability to degrade host proteins and modulate the immune response has been recently reviewed by Potempa et al., therefore only those proteinases found to be highly antigenic will be reviewed here. The Arg-X and Lys-X specific extracellular cysteine proteinases and their associated adhesins are highly antigenic. Three genes encode the major extracellular Arg-X and Lys-X specific cysteine proteinases of *P. gingivalis*. These are *rgpA*, *rgpB* and *kgp*. *rgpA* encodes an Arg-X proteinase.[18]

In a recent study the RgpA-Kgp proteinase–adhesin complexes were shown to be highly antigenic as 92% of patients with chronic periodontitis had a serum IgG response to these complexes. Furthermore, an increase in IgG antibody response to RgpA-Kgp was shown to be positively correlated with clinical parameters of disease and the percentage of periodontal pockets infected with *P. gingivalis*.[18]

GroEL heat shock protein

The *P. gingivalis* heat shock protein GroEL is highly antigenic and anti-*P. gingivalis* GroEL antibodies have been detected in gingival tissue extracts.[30] Furthermore, Tabeta et al. have shown that there was a higher proportion of sera from periodontitis patients that recognized *P. gingivalis* GroEL than from healthy subjects. Maeda et al. suggested that an antibody response to *P. gingivalis* GroEL may lead to human hsp60 cross reactivity and an autoimmune reaction.[18]Unlike human hsp60, *P. gingivalis* GroEL was unable to stimulate peripheral blood monocytes to produce Th1 or Th2 cytokines or to stimulate the release of pro-inflammatory cytokines from macrophages.[30]

Haemagglutinins

The cell surface haemagglutinating adhesin, HA-Ag2, is a surface antigen of *P. gingivalis* as all of the sera from 8 chronic periodontitis patients reacted with two HA-Ag2 proteins with masses of 43 and 49 kDa.[31] Furthermore HA-Ag2 was a common antigen to all the *P. gingivalis* strains tested. The two HA-Ag2 proteins have been described as having masses of either 33 and 38 kDa or 43 and 49 kDa. These discrepancies have been attributed to different extraction techniques. The 33 and 38 kDa HA-Ag2 proteins were extracted using an EDTA containing buffer whereas the 43 and 49 kDa forms were isolated from extracellular vesicles.[18] Specific antibodies to HA-Ag2 have been shown to inhibit haemagglutination and binding of *P. gingivalis* to epithelial cells . A number of studies have identified four *P. gingivalis* genes that encode haemagglutinin proteins HagA, B, C, and D . Research into these four Hag proteins has focused on HagA and B with particular interest in HagB as a potential vaccine candidate.

CONCLUSION

Each of the periodontal pathogens discussed in this review produces an array of antigens that stimulate epithelial cells and fibroblasts to produce pro-inflammatory cytokines. Furthermore, these antigens stimulate pro-inflammatory cells such as macrophages, monocytes and neutrophils and these in turn produce a wide variety of cytokines that maintain a pro-inflammatory response and induce a Th1 (inflammatory) cytokine response.

Proteolytic activity, in particular 'trypsin-like' proteolytic activity of subgingival plaque has been positively correlated with clinical parameters of periodontitis. The four pathogens discussed in this review that have been highly associated with periodontitis all produce extracellular proteolytic activity including 'trypsin-like' protelytic activity. These enzymes are likely to be involved directly or indirectly in destruction of host tissue and dysregulation of host defences.

The current treatment of periodontitis is non-specific and is centered on the removal of subgingival plaque by mechanical debridement often involving surgical procedures. The elucidation of the specific bacterial aetiology of periodontitis suggests that the development of a specific treatment modality to target site colonization or virulence of *P. gingivalis, T. forsythia, T. denticola* and *A. actinomycetemcomitans* is now a more rational approach to treat the disease.

The development of a multispecies vaccine that is able to target all four bacterial species that have been implicated in the development of periodontitis may be more successful than a vaccine against a single species.

Vaccination may also have a therapeutic benefit as although the bacteria may be more resistant to the adaptive immune response when present in subgingival plaque as an undisturbed biofilm, specific antibodies may still restrict the progression of disease by blocking the penetration of the major virulence factors into the gingival tissues. Thus it is important to use a combined proteomic, genomic and immunological strategy to identify bacterial antigens of periodontal pathogens and evaluate their potential as vaccine candidates for the development of a multispecies vaccine for periodontitis as an adjunct to current periodontal therapies.[32]

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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