A therapeutic Porphyromonas gingivalis gingipain vaccine induces neutralising IgG1 antibodies that protect against experimental periodontitis

Neil M O’Brien-Simpson1, James A Holden1, Jason C Lenzo1, Yan Tan1, Gail C Brammar1, Katrina A Walsh1, William Singleton1, Rebecca KH Orth1, Nada Slakeski1, Keith J Cross1, Ivan B Darby1, Dorit Becher2, Tony Rowe2, Adriana Baz Morelli2, Andrew Hammet2, Andrew Nash2, Anna Brown3, Bing Ma3, Didier Vingadassalom3, Jacqueline McCluskey3, Harold Kleanthous3 and Eric C Reynolds1

Porphyromonas gingivalis infected mice with an established P. gingivalis-specific inflammatory immune response were protected from developing alveolar bone resorption by therapeutic vaccination with a chimera (KAS2-A1) immunogen targeting the major virulence factors of the bacterium, the gingipain proteinases. Protection was characterised by an antigen-specific IgG1 isotype antibody and Th2 cell response. Adoptive transfer of KAS2-A1-specific IgG1 or IgG2 expressing B cells confirmed that IgG1-mediated protection. Furthermore, parenteral or intraoral administration of KAS2-A1-specific polyclonal antibodies protected against the development of P. gingivalis-induced bone resorption. The KAS2-A1-specific antibodies neutralised the gingipains by inhibiting: proteolytic activity, binding to host cells/proteins and co-aggregation with other periodontal bacteria. Combining key gingipain sequences into a chimera vaccine produced an effective therapeutic intervention that protected against P. gingivalis-induced periodontitis.

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INTRODUCTION

Chronic periodontitis is an inflammatory disease of the supporting tissues of the teeth that is associated with a polymicrobial biofilm (subgingival plaque) accreted to the tooth which results in destruction of the tooth’s supporting tissues including the alveolar bone.1 From epidemiological surveys moderate to severe forms of periodontitis affect one in three adults2 and the disease has been linked to an increased risk of cardiovascular diseases, certain cancers, preterm birth, rheumatoid arthritis and dementia related to the regular bacteremia and chronic inflammation associated with the disease.3–7 The global prevalence of severe periodontitis has been estimated from 2010 epidemiological data to be 10.5–12.0%6 and the global economic impact of dental diseases, of which periodontitis is a major component, has been estimated to be US$442 billion per year.9 The conventional therapy for periodontitis involves scaling and root planing to remove plaque microorganisms. Treatment can sometimes involve surgery to improve access and/or to reduce pocket depth and can also include the use of antibiotics and/or antimicrobials. However, treatment outcomes are variable and heavily dependent on patient compliance. Even in patients on a periodontal maintenance program involving regular professional intervention sites continue to progress and teeth are lost.10

Although chronic periodontitis is associated with a polymicrobial biofilm, specific bacterial species of the biofilm such as Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia as a complex or consortium have been closely associated with clinical measures of disease.11,12 Recently the level of P. gingivalis in subgingival plaque above threshold levels of 10–15% of the total bacterial load has been demonstrated to predict imminent clinical attachment loss (>2 mm in 3 months) in a prospective clinical trial of chronic periodontitis patients on a clinical maintenance program.10 These results are consistent with previous studies showing high levels of P. gingivalis at refractory sites, i.e., sites where disease continued to progress after conventional scaling and root planing therapy.13–18

P. gingivalis is found at the base of deep periodontal pockets as microcolony blooms in the superficial layers of subgingival plaque adjacent to the periodontal pocket epithelium, which helps explain the strong association with underlying tissue inflammation and bone resorption at relatively low proportions (10–15%) of the total bacterial cell load in the pocket.10,19–21 Furthermore, it has been shown from studies using the mouse periodontitis model that P. gingivalis is a keystone pathogen, which dysregulates the host immune response to favour the polymicrobial biofilm disrupting homeostasis with the host to cause dysbiosis and disease.22 The mouse periodontitis model has also been used to show that inflammation is essential to allow establishment of P. gingivalis at the levels in plaque (10–15% or greater of total bacterial cell load) necessary to produce dysbiosis and alveolar bone resorption,23 which makes the mouse model consistent with human disease.10,23

The extracellular Arg- and Lys-specific proteinases ‘gingipains’ (RgpA/B and Kgp) of P. gingivalis have been implicated as major virulence factors that are critical for colonisation, penetration into host tissue, dysregulation of the immune response, dysbiosis and disease.20,22,24–26 The gingipains, in particular the Lys-specific proteinase Kgp is essential for P. gingivalis to induce alveolar bone

1Oral Health Cooperative Research Centre, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Melbourne, VIC, Australia; 2CSL Ltd., Bio21 Institute, Parkville, VIC, Australia and 3Sanofi Pasteur, Cambridge, MA, USA.
Correspondence: EC Reynolds (e.reynolds@unimelb.edu.au)
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resorption in the mouse periodontitis model. The gingipains have also been found in gingival tissue at sites of severe periodontitis at high concentrations proximal to the subgingival plaque and at lower concentrations at distal sites deeper into the gingival tissue. This has led to the development of a cognate mechanism to explain the keystone role played by P. gingivalis in the development of chronic periodontitis.

The role of P. gingivalis as a keystone pathogen in the initiation and progression of chronic periodontitis suggests that a strategy of targeting the major virulence factors of the bacterium, the gingipains by vaccination may have utility in the prevention of P. gingivalis-induced periodontitis. Indeed, studies using the gingipains as a prophylactic vaccine that induces a high-titre antibody response in naive animals before superinfection with the pathogen have shown protection against alveolar bone resorption. However, patients with P. gingivalis-associated periodontitis harbour the pathogen at above threshold levels in subgingival plaque and exhibit an inflammatory immune response, hence it is possible that therapeutic vaccination could exacerbate inflammation and bone resorption in these patients. Here we show that therapeutic vaccination with a chimera antigen targeting the gingipains protects against alveolar bone resorption in P. gingivalis-associated experimental periodontitis and that this protection is mediated via a predominant Th2 anti-inflammatory response with the production of gingipain-neutralising IgG1 antibodies.

RESULTS

Recombinant protein chimeras of the KAS peptide and KgpA domain of the RgpA–Kgp proteinase-adhesin complex are effective as prophylactic vaccines in experimental periodontitis. We have previously identified antigens from the RgpA–Kgp complex that protect against P. gingivalis-induced lesions and alveolar bone resorption. We hypothesised that a recombinant chimera of the most effective antigens; a Lys-gingipain active site peptide (KAS) and a recombinant KgpA1 adhesin sequence may exhibit enhanced protection against P. gingivalis induced disease by neutralising both proteolytic and adhesin functions of the virulence factor (Supplementary Figure 1). On the basis of epitope mapping and structural modelling of the Kgp proteinase and adhesin domain, two chimera proteins were produced; KAS1-sA1 representing the minimal immunogenic sequences of KAS (Kgp-cat, 433–454) and KgpA1 (759–989) and KAS2-A1, which extended the minimal immunogenic sequences of KAS (Kgp-cat, 433–468) and KgpA1 (751–1056) (Supplementary Figure 2).

Immunogenicity studies showed that KAS1-sA1 and KAS2-A1 antisera recognised KAS2 peptide, KgpA1(751–1056) and FK-P. gingivalis W50 cells at comparable or higher levels when compared with antisera generated to KAS2-DT, KgpA1(759–989), KgpA1(751–1056) and FK-P. gingivalis W50 cells (Figure 1). Only the recombinant chimera proteins were found to recognise all three (KAS2, KgpA1 and P. gingivalis W50 cells) antigens (Figure 1a). Western blot analysis showed that the KAS1-sA1 and KAS2-A1 antisera recognised the Kgp proteinase and adhesin domains (Figures 1b–d). Epitope mapping of the lysine proteinase active site sequence (433–468) identified that the antigenic epitopes for both KAS1 and KAS2 were around the His(444) catalytic residue (Supplementary Figure 3). For KAS1 the major epitopes were HSETAWADPLL453, TGVSFANYTA463 and for KAS2 ETAWADPLL453, TGVSFANYTA463. These antigenic epitopes matched those induced by the KAS2-DT conjugate indicating that the KAS epitope in KAS1-sA1 and KAS2-A1 remained antigenic (Supplementary Figure 3).

The recombinant proteins KAS1-sA1, KAS2-A1, KgpA1(759–989), KgpA1(751–1056); FK-P. gingivalis W50 and the RgpA–Kgp complex were used to immunise BALB/c mice to determine their efficacy as prophylactic vaccines against P. gingivalis-induced alveolar bone resorption. Mice immunised with all of the recombinant antigens, RgpA–Kgp complex and FK-W50 cells exhibited significantly (P < 0.001) less bone loss compared with that of the PBS control group (Figure 2a). Both KAS2-A1 and KAS1-sA1 immunised mice exhibited significantly less alveolar bone resorption than the mice immunised with the RgpA–Kgp complex. KAS2-A1 provided a greater level of protection (P < 0.01) than KgpA1(759–989), KgpA1(751–1056) or FK-P. gingivalis W50.

Prior to intra-oral challenge with P. gingivalis each mouse group immunised with one of the immunogens had a high IgG antibody titre to FK-P. gingivalis W50 cells with a predominant IgG1 antibody subclass and only weakly immunoreactive IgG2a, IgG2b and IgG3 responses (Figure 2b). Post intra-oral challenge with P. gingivalis W50 each of the protective immunogens retained a predominant and significantly (P < 0.05) higher IgG1 antibody subclass response to P. gingivalis (Figure 2c). However, the PBS/IFA immunised group had a predominant and significantly (P < 0.05) higher IgG2b antibody subclass response to P. gingivalis (Figure 2c). Based on the above data the KAS2-A1 chimera was chosen as the lead vaccine candidate to be used in subsequent studies.
KAS2-A chimera prophylactic vaccine confers protection against P. gingivalis-induced bone resorption at low antigen doses

The KAS2-A chimera was formulated with aluminium hydroxide (alum) and BALB/c mice were immunised with 50 μg, 5.0 μg or 0.5 μg per dose (being adult human equivalent doses of 12, 1.2 and 0.12 mg, respectively) (Figure 3). The KAS2-A1 formulated in alun at all doses trialed conferred significant (P < 0.01) protection against P. gingivalis-induced bone loss (Figure 3a). There was no significant difference in the level of bone loss for the positive intervention (amoxicillin-treated), positive vaccination (RgpA–Kgp complex/IFA) and all of the KAS2-A1/alum immunised groups (Figure 3a).

Mice immunised with KAS2-A1/alum at each dose or the RgpA–Kgp complex/IFA had a high IgG titre to FK-P. gingivalis W50 cells with a predominant IgG1 subclass (Figure 3b). The PBS/alum immunised group and the infected control groups had a predominant and significantly (P < 0.05) higher IgG2b antibody subclass response to P. gingivalis (Figure 3b).

To determine the cellular interferon (IFN)-γ and IL-4 cytokine response for each group following P. gingivalis oral infection, submandibular lymph node (SMLN) T cells and gingival tissue lymphocytes were isolated and the number of P. gingivalis-specific cytokine producing cells determined by ELISPOT (Figure 3c). The PBS/alum treated and P. gingivalis-infected control groups had significantly (P < 0.05) higher numbers of IFNγ secreting SMLN T cells and gingival lymphocytes. Mice immunised with KAS2-A1/alum at all doses had significantly (P < 0.05) higher numbers of IL-4 secreting SMLN T cells and gingival lymphocytes, characteristic of a Th2 predominant response.

Protective efficacy of the KAS2-A1 chimera in a therapeutic vaccination periodontitis model

To develop a therapeutic vaccination model we first conducted a time course study to determine the timing of a distinct P. gingivalis-specific immune response post intra-oral challenge (infection). Figure 4a shows that only challenge with viable P. gingivalis W50 cells, and not formalin-killed (FK) P. gingivalis cells, induced significant (P < 0.05) bone loss. Infiltrating gingival CD4+ T cells were significantly (P < 0.05) higher in numbers on days 8, 17, 28 and 56 in the viable P. gingivalis infected group compared with the uninfected control and the FK-P. gingivalis challenged group (Figure 4b). A significantly (P < 0.05) higher number of P. gingivalis-specific IFNγ and IL-17 secreting T cells and P. gingivalis-specific serum antibody responses were found on days, 17, 28 and 56 for the viable P. gingivalis infected group compared with the uninfected control and the FK-P. gingivalis challenged group (Figures 4c–f). The time course study showed that only challenge with viable P. gingivalis cells induced bone loss and that an antigen-specific immune response was established from day 17 and onwards after the first oral challenge. Hence, for the therapeutic vaccination

Figure 2. Recombinant chimera proteins KAS1-sA1 and KAS2-A1 protected against P. gingivalis-induced bone loss in a mouse prophylactic vaccine periodontitis model. (a) Mice were immunised with KAS1-sA1, KAS2-A1, RgpA–Kgp complex, formalin killed P. gingivalis W50 cells (FK-W50), recombinant proteins rA1 (759–989), rA1 (751–1056) or adjuvant alone (PBS, IFA). After the second immunisation mice were pre-treated with antibiotics and orally challenged with a total of eight doses of 1.0 × 10^{10} P. gingivalis W50 or treated with PG buffer containing 2% CMC alone (non-challenged group as N-C). P. gingivalis-induced bone resorption was determined as described in the Materials and Methods section for each group (n = 12), and the data are expressed as the mean ± s.d. in mm² and were analysed using a one-way ANOVA and Dunnett's T3 post hoc test. * and ** indicates data that are significantly different (P < 0.01, P < 0.001, respectively) from the data for P. gingivalis challenged group. Serum antibody subclass responses of immunised mice in the periodontitis model, (b) post 1° and 2° immunisation and pre intra-oral challenge and (c) post intra-oral challenge with live P. gingivalis cells. Antisera was used to probe formalin killed P. gingivalis strain W50 as the absorbed antigen in an ELISA. Antibody responses IgG (black bars), IgG1 (grey bars), IgG2a (white bars), IgG2b (horizontally striped bars), IgG3 (diagonal stripped bars), are expressed as the ELISA titre (log 2) obtained minus double the background level, with each titre representing the mean ± s.d. of three values. * indicates IgG subclass significantly higher (P < 0.05) than other IgG subclasses in that group.
model we chose day 19 as an appropriate treatment/vaccination time point.

To test the KAS2-A1 chimera as a therapeutic vaccine BALB/c mice were immunised on day 19 and 40, post oral infection with P. gingivalis, using the same antigen doses and alum formulation as described above. KAS2-A1-induced significant (P < 0.01) protection against P. gingivalis-induced bone resorption in the therapeutic vaccination model (Figure 5a). There was no significant difference in the level of bone loss for the positive intervention (amoxicillin-treated), positive vaccination (RgpA–Kgp complex/IFA) and all of the KAS2-A1/alum immunised groups (Figure 5a).

Mice immunised with KAS2-A1/alum or the RgpA–Kgp complex/IFA had a high IgG antibody titre to FK-P. gingivalis cells with a predominant IgG1 antibody subclass response (Figure 5b). The PBS/alum immunised group and the infected control groups had a predominant and significantly (P < 0.05) higher IgG2b antibody subclass response to P. gingivalis (Figure 5b). Mice immunised with KAS2-A1/alum at 50 and 5.0 μg doses had significantly (P < 0.05) higher numbers of IL-4 secreting SMLN T cells and gingival lymphocytes, whereas, the PBS/alum immunised group and P. gingivalis infected control group had significantly (P < 0.05) higher numbers of IFN-γ secreting SMLN T cells (PBS/alum only) and gingival lymphocytes (Figure 5c).

DNA was extracted from half maxillae of each of the groups and the P. gingivalis level expressed as a percentage of total bacterial cell numbers recovered. All of the KAS2-A1 immunised and RgpA–Kgp complex groups had significantly (P < 0.05) lower levels of P. gingivalis as a percentage of total bacterial cells compared with the PBS/alum and infected control groups (Figure 5d).

KAS2-A1 chimera provides protection against alveolar bone resorption in mice orally challenged with P. gingivalis/T. denticola/T. forsythia

As P. gingivalis is found in subgingival plaque with T. denticola and T. forsythia we investigated whether the KAS2-A1 chimera would...
The antibody response expressed as spot forming cells/10^6 obtained minus the background level, with data representing the mean ± standard deviation and were analysed using Student’s t-test. * indicates data that are significantly different (P < 0.05) from the data for negative control group; (b) the number of gingival CD4^+ T cells (TCRβ^+CD4^+) in the lymphocyte infiltrate were determined using flow cytometry using lymphocytes isolated from gingival tissue. Data are expressed as the mean ± standard deviation and were analysed using Student’s t-test. * indicates data that are significantly different (P < 0.05) from the data for negative control group; (c) IFNy and IL-17-producing submandibular lymph nodes (SMLN) T-cells were determined by ELISPOT using FK-P. gingivalis W50 as the absorbed antigen. Cytokine response expressed as spot forming cells per 10^6 obtained minus the background level, with data representing the mean ± s.d. of three values. * indicates which cytokine is significantly higher (P < 0.05) from the data for negative control group; (e) P. gingivalis-specific serum antibody subclass responses were determined by ELISA using formalin killed P. gingivalis strain W50 as the absorbed antigen. * indicates IgG subclass significantly higher (P < 0.05) from other IgG subclasses in that group; (f) P. gingivalis-specific antibody producing submandibular lymph nodes (SMLN) B-cells were determined by ELISPOT on day 56 using FK-P. gingivalis W50 as the stimulating antigen. Antibody response expressed as spot forming cells/10^6 obtained minus the background level, with data representing the mean ± s.d. of three values. * indicates Ig subclass significantly higher (P < 0.05) than other Ig subclass in that group.

protect against challenge and superinfection with all three species (Figure 6). The P. gingivalis/T. denticola/T. forsythia challenged animals exhibited a slightly higher level of bone resorption when compared with the animals challenged with P. gingivalis alone at the higher dose confirming bacterial synergy in bone loss with these species.\(^4\) KAS2-A1 formulated in alum conferred significant (P < 0.01) protection against P. gingivalis/T. denticola/T. forsythia-induced bone loss (Figure 6a). KAS2-A1 immunised animals had a predominant P. gingivalis-specific IgG1 (Figure 6b) and IL-4 secreting T-cell response (Figure 6c). For both P. gingivalis/T. denticola/T. forsythia and P. gingivalis infected control groups, only a P. gingivalis-specific antibody, IgG2b dominant (Figure 6b), and IFNy secreting T-cell response (Figure 6c) was detected. No significant immune responses were observed towards T. forsythia or T. denticola.

Isolation and phenotyping of gingival CD69^+ (early activated) and CD25^+ (activated/antigen stimulated) CD4^+ T cells showed that KAS2-A1 immunised animals had a significantly higher population of CD25^+ but not CD69^+. CD4^+ gingival T cells compared with the non-infected (NC) animals. The P. gingivalis/T. denticola/T. forsythia and P. gingivalis infected control groups had a significantly higher population of CD25^+ and CD69^+,

KAS2-A1 chimera protection against P. gingivalis-induced bone loss is antibody mediated

To investigate whether KAS2-A1-specific T cells or B cells mediate protection in the mouse periodontitis model, donor mice (Ly5.1 C57Bl6 congenic mice) were immunised with KAS2-A1 chimera or ovalbumin (antigen control) and IgG1 and IgG2 expressing B cells and CD4^+ T cells harvested and adoptively transferred into recipient mice (Ly5.2 C57Bl6 congenic mice). The day following the adoptive transfer mice were orally infected with P. gingivalis and bone loss determined. Mice that received CD4^+ T cells, IgG2 expressing B cells from KAS2-A1 immunised mice or B cells from ovalbumin immunised mice developed P. gingivalis-induced bone loss at a similar level to that in the
infected control group and had a predominant IgG2a/b response to \textit{P. gingivalis} (Figures 7a and b). However, mice that received IgG1 expressing B cells from KAS2-A1 immunised mice did not develop \textit{P. gingivalis}-induced bone loss and had similar alveolar bone levels to that in the non-infected (NC) control group and a predominant IgG1 response to \textit{P. gingivalis}. Cellular phenotyping of the gingival and submandibular lymph nodes (SMLN) T cells and gingival tissue (gingiva) lymphocytes isolated from immunised mice in the periodontitis model. Cytokine-producing cells were determined by ELISPOT using FK\_P. gingivalis W50 as the stimulating antigen. Cytokine response expressed as cytokine-producing cells/106 obtained minus the background level, with data representing the mean ± s.d. of three values. * indicates cytokine is significantly higher (P < 0.05) than other cytokines in that group. (c) IFN\_γ and IL-4 producing submandibular lymph nodes (SMLN) T cells and gingival tissue (gingiva) lymphocytes isolated from immunised mice in the periodontitis model. Antiserum was used to probe formalin killed \textit{P. gingivalis} strain W50 as the absorbed antigen in an ELISA. Antibody responses are expressed as the ELISA titre (log 2) obtained minus double the background level, with each titre representing the mean ± s.d. of three values. * indicates IgG subclass significantly higher (P < 0.05) than other IgG subclasses in that group. (d) Serum antibody subclass responses of immunised mice in the periodontitis model. The antigenicity of the purified \textit{P. gingivalis} strain W50 as the absorbed antigen in an ELISA. Antibody responses are expressed as the log 2 antibody titre minus double the background level, with each titre representing the mean ± s.d. of three values. The data are expressed as the mean ± s.d. of three values. * indicates IgG subclass significantly different (P < 0.01) from the data for \textit{P. gingivalis} vaccine. ** indicates IgG subclass significantly different (P < 0.001) from the data for \textit{P. gingivalis} vaccine. *** indicates IgG subclass significantly different (P < 0.0001) from the data for \textit{P. gingivalis} vaccine.
KAS2-A1-pAb neutralisation of gingipain activities and binding to P. gingivalis

Several studies have shown that the Lys-X and Arg-X proteolytic activity, binding to oral epithelial cells, host matrix and blood proteins and co-aggregation with T. denticola are activities by which gingipains contribute to P. gingivalis virulence. We investigated the ability of purified KAS2-A1-pAb compared with a non-specific (NS, PBS/alum)-pAb to inhibit each of these gingipain activities associated with the bacterium’s pathogenicity (Figure 8). The KAS2-A1-pAb inhibited P. gingivalis whole-cell Lys-X proteolytic activity within 30 min and the level of inhibition slightly increased with incubation time (Figure 8a). Interestingly, the KAS2-A1-pAb also inhibited P. gingivalis whole-cell Arg-X proteolytic activity after 4 h of incubation and this increased at the 24 h time point. The ability to inhibit both Lys-X and Arg-X proteolytic activities can be attributed to the sequence similarity of the Kgp domain of the Lys-specific gingipain Kgp that gingipains contribute to P. gingivalis strains representing the major laboratory strains, serotypes A-D and clinical isolates (from different regions of the World) tested at a similar antibody titre when compared with RgpA–Kgp complex antibodies (Supplementary Figure 7). These data suggest that the KAS2-A1 chimera should produce antibodies that would cross-react with all currently known P. gingivalis strains.

DISCUSSION

The results of our study indicate that a chimera vaccine comprising the active site sequence (KAS) and the A1 adhesin domain of the Lys-specific gingipain Kgp when administered
therapeutically switched a predominant Th1/Th17 inflammatory response to a predominant Th2 response generating gingipain-neutralising IgG1 antibodies, which protected against P. gingivalis-induced alveolar bone resorption. The vaccination prevented the emergence of P. gingivalis in subgingival plaque above threshold levels (10–15%) that cause dysbiosis and disease. The neutralising ability of the chimera-generated antibodies was confirmed as the antibodies inhibited RgpA/B and Kgp proteolytic activities, inhibited binding of the RgpA–Kgp complex to host proteins, inhibited binding to oral epithelial cells and inhibited co-aggregation with T. denticola. The abilities of P. gingivalis to adhere to oral epithelial cells and co-aggregate with T. denticola have been identified as key processes in colonisation and pathogenicity; and the gingipains have been shown to have a major role in these processes.24,29,41–47 The neutralisation of gingipain activity would help explain the protective mechanism of the antibodies in terms of preventing alveolar bone resorption as the gingipains are essential for P. gingivalis virulence and have been shown to be critical for colonisation, penetration into host tissue, dysregulation of the immune response, chronic inflammation, dysbiosis and alveolar bone resorption.20,22,24–28 The protection conferred by the chimera vaccine was also observed in the mouse periodontitis model infected with P. gingivalis, T. denticola and T. forsythia confirming the key pathogenic role of P. gingivalis. Recently, the targeting of keystone bacterial pathogens in the diverse microbial communities of saliva and the gut has been shown to not only result in the reduction in the level of the keystone pathogen, but also in the level of synergistic accessory pathogens and the promotion and restoration of commensal species and homeostasis.49–51 The KAS-A1
chimera-specific antibodies recognised all of the *P. gingivalis* serotypes, laboratory-type strains and clinical isolates tested, indicating high conservation of the gingipain active site and A1 adhesin sequences.

The protection conferred by the chimera vaccine was mediated by the gingipain-neutralising IgG1 antibodies generated as shown by the adoptive transfer and passive administration (systemic and topical) experiments. An interesting result from these experiments was that the KAS2-A1 specific IgG2-expressing B cells were not protective and did not home to the SMLN or gingiva as effectively as the IgG1 expressing B cells. Also, the adoptive transfer of the KAS2-A1 stimulated CD4+ T cells did not protect mice from *P. gingivalis* induced bone resorption indicating that antigen-specific T helper cells alone are insufficient to stimulate production of specific gingipain antibodies and induce protection. Th cells are nevertheless important in periodontitis as a protective response to the chimera vaccine was characterised by a switch from a Th1/Th17- to a Th2-biased response. This is consistent with previous studies in mice and humans where periodontitis progression has been associated with a predominant Th1/Th17 response and health/stability has been associated with a predominant Th2 response.33-37,52 In fact, Moutsopoulos et al.34 have shown that *P. gingivalis*, facilitated by the gingipain proteases induced Th17 cell activation and that these cells...
may have a major role in orchestrating chronic inflammation. Our current data showing that Th17 cells are a major T-cell subset at the onset of *P. gingivalis*-induced disease in mice corroborates these findings. Hence, the ability of anti-KAS2A1 antibodies to inhibit *P. gingivalis* proteinase activity would help reduce the activation of Th17 cells; thus ameliorating the inflammatory immune response orchestrated by these cells.

T helper cells also have an important role as the Th immune bias does effect antibody class switching as antigen-stimulated B cells secrete IgG1 or IgG2a/b/G3 subclasses in the presence of IL-4 or IFNγ, respectively. Antibody subclasses are known to contribute to the progression of the immune response to a pathogen and have substantial impacts on immunotherapeutic or immunopathogenic outcomes. Phagocytosis of murine IgG1 opsonised pathogens induces anti-inflammatory cytokine/chemokine secretion, whereas, IgG2a/b/G3 opsonised pathogens stimulate an inflammatory cytokine/chemokine response upon phagocytosis. We have previously shown in humans with a high IgG4 (mouse IgG1 equivalent) response to *P. gingivalis* that they are periodontally stable and recognise epitopes in the KgpA1 adhesin domain that are not recognised by periodontitis patients with progressive disease who have a high IgG2 (mouse IgG2a/b equivalent) response to *P. gingivalis* LPS. These A1 adhesin epitopes (EP1–3 and ABM1–3, Supplementary Figure 1) have been shown to be protective and are incorporated into the chimaera vaccine.

The KAS2-A1 chimera formulated in a regulatory approved adjuvant ‘alum’ was an effective therapeutic vaccine against the induction of periodontal bone loss in the mouse model suggesting that it may have utility in the adjunctive treatment to scaling and root planing for patients with chronic periodontitis who have a pre-existing immune response to *P. gingivalis*. Several clinical studies in humans support the concept of vaccination with the gingipains and the generation of protective antibodies to prevent *P. gingivalis* emergence in plaque and progression of periodontitis. Booth et al. showed that subgingival application of an anti-gingipain A1 adhesin monoclonal antibody could prevent recolonisation of subgingival plaque by *P. gingivalis*. This result was confirmed by Yokokama et al. using anti-gingipain antibodies who showed a reduction in the plaque levels of *P. gingivalis* and also a significant reduction in pocket depth and bleeding on probing in the periodontitis patients who received the specific antibodies. O’Brien-Simpson et al. showed that subjects who had naturally developed a specific IgG4 response to the gingipains did not exhibit progressive disease and appeared stable compared with those subjects with predominant IgG2/IgG3 responses. These results are consistent with periodontitis in humans being associated with an inflammatory Th1/Th17 response (high IgG3/IgG2), whereas periodontal health/stability is more associated with an anti-inflammatory Th2 response (high IgG4/IgG1) as discussed above.

In conclusion, the results suggest that vaccination of humans with chronic periodontitis using the *P. gingivalis* gingipain chimera as an adjunct to scaling and root planing should induce a switch to a Th2 (less inflammatory) response generating gingipain neutralising antibodies that should help prevent re-emergence of *P. gingivalis* in subgingival plaque and thereby prevent dysbiosis and disease progression. As chronic periodontitis is a known risk factor for cardiovascular diseases, diabetes, spontaneous preterm birth and low-birth-weight infants, pancreatic cancer and rheumatoid arthritis the vaccine may also have broader health benefits.

**MATERIALS AND METHODS**

All Materials and Methods are provided in the Supplementary Information.
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