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(54) **Title**: METHOD OF TREATING PERIODONTAL DISEASE BY ADMINISTERING ANTAGONISTS OF PAR-2

(57) **Abstract**: The present invention relates to methods of and compositions for preventing and treating periodontal disease, including the inflammation associated with such periodontal, by administering to a subject an effective amount of an antagonist of PAR-2. In particular, the invention relates to reducing oral pathological inflammation associated with Porphyromonas gingivalis infection. The PAR-2 antagonist used in the methods and compositions is selected from the group consisting of peptides, antibodies, nucleic acids and small molecules.

METHOD OF TREATING PERIODONTAL DISEASE BY ADMINISTERING ANTAGONISTS OF PAR-2

Field of the invention

The present invention relates to methods of preventing and treating periodontal disease including the associated inflammation. Particular application of the invention relates to reducing oral pathological inflammation associated with *Porphyromonas gingivalis* infection. The present invention also relates to uses of compounds in the prevention and treatment of such periodontal conditions.

Background of the invention

Protease activated receptors (PARs) are G-protein coupled, seven transmembrane proteins present on many cell types, including epithelial, endothelial and neuronal cells, T-cells and osteoblasts. Rather than being activated solely by ligand occupancy, these receptors are activated by proteolysis of their N-terminus to reveal a tethered ligand that interacts with the extracellular loops of the receptor. There are four known PARs, with PARs-1, -3 and -4 having thrombin as their usual physiological activator, while PAR-2 is activated by a variety of proteases, including trypsin, mast cell tryptase and neutrophil protease-3.

Chronic periodontitis is an inflammatory condition involving a host response to bacterial components that have diffused into the subjacent gingival tissue from the subgingival plaque biofilm. The inability of the host immune system to remove the biofilm (as it is external to the tissue and accreted on a non-shedding tooth root surface) results in continual external stimulation, leading to a chronic inflammatory state. This chronic inflammation leads to periodontal tissue damage, including bone resorption caused by the cells and molecules of the host system response. The disease is a major public health problem in all societies and is estimated to affect up to 15% of the adult population with severe forms affecting 5-6%. Chronic periodontitis has been linked with certain systemic diseases such as diabetes, cardiovascular diseases and certain cancers.

Periodontal diseases range from simple gum inflammation to serious disease that results in major damage to the soft tissue and bone that support the teeth. Periodontal

disease includes gingivitis and periodontitis. Bacteria, such as *P. gingivalis* causes inflammation of the gums that is called "gingivitis." In gingivitis, the gums become red, swollen and can bleed easily. When gingivitis is not treated, it can advance to "periodontitis". In periodontitis, gums pull away from the teeth and form "pockets" that are infected. The body's immune system fights the bacteria as the plaque spreads and grows below the gum line. If not treated, the bones, gums, and connective tissue that support the teeth are destroyed. The teeth may eventually become loose and have to be removed. The method of the invention is contemplated to be applicable at any stage of periodontal disease initiation or progression. The development and progression of chronic periodontitis has been associated with specific Gram-negative bacteria in subgingival plaque. The presence of three Gram-negative bacterial species *Tannerella forsythia (T.forsythia), Treponema denticola (T. denticola)* and *Porphyromonas gingivalis (P. gingivalis)* in subgingival plaque has been associated with periodontal disease.

One option for the treatment or prevention of periodontal disease has involved targeting the pathogenic bacteria causing the disease. Targeting bacteria has a number of problems including that the bacteria can develop resistance to the agent used in the treatment or prevention. In addition, there are a variety of pathogenic bacterial species that may cause periodontal disease and targeting one species may have little effect on the virulence of any of the other bacterial species. Non-pathogenic bacteria that are beneficial for normal functioning of the oral cavity may also be adversely affected by any agent intended to target the periodontal disease causing pathogenic bacteria. There exists a need for a better or alternative treatment or prevention of periodontal disease.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

Summary of the invention

According to the present invention there is provided a method of treating or preventing periodontal disease comprising administering to a subject an effective amount of an

antagonist of PAR-2. The antagonist is selected from the group consisting of peptides, antibodies, nucleic acids and small molecules. An effective amount is a therapeutically effective amount.

In another embodiment there is provided a method for treating or preventing diseases characterised by pathological elevation of T cell proliferation, T cell activation or T cell mediated inflammatory cytokine expression comprising administering to a subject an effective amount of an antagonist of PAR-2.

In another embodiment there is provided a method of reducing or inhibiting the production of IL-6 by gingival epithelial cells comprising administering to a subject an effective amount of an antagonist of PAR-2.

In another embodiment there is provided a method of reducing or inhibiting mast cell infiltration into periodontal tissue comprising administering to a subject an effective amount of an antagonist of PAR-2.

In another embodiment there is provided a method of reducing or inhibiting neutrophil recruitment into periodontal tissue comprising administering to a subject an effective amount of an antagonist of PAR-2.

In another embodiment there is provided a method for treating or preventing diseases characterised by pathological elevation of a T cell helper 1 (Th1) type immune response comprising administering to a subject an effective amount of an antagonist of PAR-2.

In one embodiment, there is provided a method for treating or preventing one or more of the conditions described above comprising administering to a subject an effective amount of an antagonist of an activator of PAR-2, such as the activators gingipain, tryptase or proteinase-3. It is believed that such an antagonist downregulates PAR-2 with therapeutic benefits similar to antagonising PAR-2 itself.

In one embodiment, the antagonist is administered directly to the gums of the subject. The antagonist may be a part of a composition applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs.

The subject in need of treatment or at risk of developing periodontal disease is an animal. Preferably, the subject is a human or a dog.

In another embodiment the invention further comprises also administering an agent selected from the group consisting of anti-inflammatory agents, antibiotics and antibiofilm agents. The antibiotic may be selected from the group consisting of amoxicillin, doxycycline and metronidazole. Anti-inflammatory agents include Nonsteroidal Anti-inflammatory Drugs (NSAIDs). Examples of NSAIDs include compounds than inhibit a cyclooxygenase. Specific examples of NSAIDs include aspirin, ibuprofen and naproxen.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for the treatment or prevention of periodontal disease.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for treating or preventing diseases characterised by pathological elevation of T cell proliferation, T cell activation or T cell mediated inflammatory cytokine expression.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for reducing or inhibiting the production of IL-6 by gingival epithelial cells.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for reducing or inhibiting mast cell infiltration into periodontal tissue.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for reducing or inhibiting neutrophil recruitment into periodontal tissue.

In another embodiment the invention provides a use of an effective amount of an antagonist of PAR-2 in the preparation of a medicament for treating or preventing diseases characterised by pathological elevation of a T cell helper 1 (Th1) type immune response.

In another aspect of the invention, there is provided an antagonist of PAR-2 for the treatment or prevention of periodontal disease.

In another embodiment there is provided an antagonist of PAR-2 for treating or preventing diseases characterised by pathological elevation of T cell proliferation, T cell activation or T cell mediated inflammatory cytokine expression.

In another embodiment there is provided an antagonist of PAR-2 for reducing or inhibiting the production of IL-6 by gingival epithelial cells.

In another embodiment there is provided an antagonist of PAR-2 for reducing or inhibiting mast cell infiltration into periodontal tissue.

In another embodiment there is provided an antagonist of PAR-2 for reducing or inhibiting neutrophil recruitment into periodontal tissue.

In another embodiment there is provided an antagonist of PAR-2 for treating or preventing diseases characterised by pathological elevation of a T cell helper 1 (Th1) type immune response.

The present invention also provides a composition for the treatment or prevention of periodontal disease (and/or the other conditions identified above as suitable for treatment) comprising an effective amount of an antagonist of PAR-2 and a pharmaceutically acceptable carrier. The antagonist in a composition of the invention may be selected from the group consisting of peptides, antibodies, nucleic acids and small molecules. The composition may further include an agent selected from the group consisting of anti-inflammatory agents, antibiotics and antibiofilm agents. The antibiotic may be selected from the group consisting of amoxicillin, doxycycline and metronidazole.

In another embodiment the invention provides a composition for the treatment or prevention of periodontal disease (and/or the other conditions identified above as suitable for treatment) comprising as an active ingredient an antagonist of PAR-2.

In another embodiment the invention provides a pharmaceutical composition comprising an effective amount of an antagonist of PAR-2 as a main ingredient. The composition may be used, for example, for the treatment or prevention of periodontal disease and/or the other conditions identified above as suitable for treatment.

In another embodiment the invention provides a composition comprising an antagonist of PAR-2 for use in the treatment or prevention of periodontal disease.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgement or any form of suggestion that this prior art forms part of the common general knowledge.

Brief description of the figures

Figure 1: Regions used for histomorphometry in sections of mouse maxillae. a) Section of a maxilla from a PAR-2+/+ mouse following challenge with *P. gingivalis*. The arrows indicate the fields in which the mast cells were counted. E = Gingival epithelium, T = tooth, A = alveolar bone. b) Section showing mast cells (arrows); insert shows mast cells at higher magnification. c) Section of a maxilla showing the field taken for measuring the eroded surface of the alveolar bone. The bold line indicates the region of interest, which is 450 μm in length. C = alveolar bone crest; T = tooth.

Figure 2: Quantification of periodontal bone loss of PAR-2+/+ and PAR-2-/- mice in experimental periodontitis. Half-maxilla stained with methylene blue from a) a PAR-2-/- mouse and b) a PAR-2+/+ mouse that were orally infected with *P. gingivalis* W50. The area of exposed root surface [as outlined by the white lines in (b)] was measured and counted blind by one evaluator and the results (Mean ± SEM, n=12, * = $p<0.05$ for comparison between infected and sham-infected mice, and ** = $p<0.01$ for comparison between PAR-2-/- and PAR-2+/+ infected mice) are shown in (c). S-Inf = sham-infected mice, Inf = infected mice.

Figure 3: Quantification of the number of mast cells in the periodontal tissue of mice orally challenged with *P. gingivalis* W50. Results are presented as the mean ± SEM (n=3). * = $P < 0.05$, ** = $P < 0.01$ for comparisons indicated by lines above bars. S-Inf = sham-infected mice, Inf = infected mice.

Figure 4: Eroded bone surface measurements in sections of mouse maxillae (Figure 1c). Results are presented as mean ± SEM (n=3); * = $P < 0.05$ for comparison between infected and sham-infected mice. S-Inf = sham-infected mice, Inf = infected mice.

Figure 5: The expression of CD90 from T cells of PAR-2+/+ and PAR-2-/- mice immunized with *P. gingivalis* FKW50. Lymphocytes were isolated from inguinal and popliteal lymph nodes of mice (PAR-2+/+ and PAR-2-/-) seven days after immunisation in the hind hock. Expression of CD90 on T cell subsets was detected by flow cytometry using a Beckman Coulter FC500 flow cytometer and staining the lymphocyte population with anti-CD4-PE, anti-CD8-PE, anti-TCRβ-APC and anti-CD90-FITC antibodies. The data are expressed as cytometry histograms and are representative of three independent experiments. CD90 expression of T cell (TCRβ+) subsets (CD4+ and CD8+) is represented by a solid line for PBS immunized mice (control) and a dashed line for antigen (*P. gingivalis* FKW50) immunized mice for the PAR-2+/+ isolated lymphocytes (a and b) and for the PAR-2-/- isolated lymphocytes (c and d). A total of 10,000 events were measured.

Figure 6: T cell proliferation assay. T cells isolated from inguinal and popliteal lymph nodes of mice (PAR-2+/+ and PAR-2-/-) seven days after immunisation in the hind hock were incubated with serially diluted *P. gingivalis* FKW50 antigen in the presence of γ-irradiated syngeneic antigen presenting cells *in vitro*. The T cell proliferation was measured by [^{3}H]thymidine incorporation, with the results presented as the stimulatory index (SI), calculated by dividing the mean cpm obtained after antigen stimulation by the mean cpm detected in control, unstimulated wells. This graph is a representation of four independent experiments with similar results. S-Im = sham-immunised mice, Im = immunised mice.

Figure 7: Cytokine responses of *P. gingivalis* FKW50-primed T cells isolated from the popliteal and inguinal lymph nodes of PAR-2+/+ and PAR-2-/- mice to stimulation with *P. gingivalis* FKW50 antigen. T cells isolated from inguinal and popliteal lymph nodes of mice (PAR-2+/+ and PAR-2-/-) seven days after immunisation in the hind hock were stimulated with *P. gingivalis* FKW50 (1 μg/mL) in the presence of γ-irradiated syngeneic antigen presenting cells *in vitro*. After two days the ELISPOT was stopped and

developed. Data is expressed as spot-forming cells (SFC) per million ± SD minus the background and are the average of triplicate assays.

Figure 8: Cytokine responses of T cells isolated from the submandibular lymph nodes of PAR-2+/+ and PAR-2-/- mice after stimulation with *P. gingivalis* FKW50 antigen. T cells isolated from submandibular lymph nodes of mice (PAR-2+/+ and PAR-2-/-) were stimulated with *P. gingivalis* FKW50 (1 µg/mL) in the presence of γ-irradiated syngeneic antigen presenting cells *in vitro*. After two days the ELISPOT was stopped and developed. Data are expressed as spot-forming cells (SFC) per million ± SD minus the background and are the average of duplicate assays.

Figure 9: Cytokine secretion responses of T cells isolated from the popliteal and inguinal lymph nodes of PAR-2+/+ and PAR-2-/- mice to stimulation with *P. gingivalis* FKW50 antigen. T cells isolated from inguinal and popliteal lymph nodes of mice (PAR-2+/+ and PAR-2-/-) seven days after immunisation in the hind hock were stimulated with *P. gingivalis* FKW50 in the presence of γ-irradiated syngeneic antigen presenting cells *in vitro*. Supernatants from the T cell proliferation assays (Fig 6) were collected and those supernatants corresponding to maximal T cell proliferation in response to *P. gingivalis* FKW50 antigen (0.78 µg/mL, Fig 6) were analysed for IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17, GM-CSF, IFNγ and TNFα using the Bio-plex cytokine array system. Data are expressed as pg/mL minus no antigen control and are representative of three independent T cell proliferation assays. Sample size: n = 3.

Detailed description of the embodiments

The inventors propose that PAR-2 plays a pivotal role in the progression of the inflammatory events that underpin the pathogenesis of periodontitis. *P. gingivalis* has been implicated as a major causative agent of chronic periodontitis and a live virulent strain of *P. gingivalis* (W50) was orally inoculated into PAR-2+/+ and PAR-2-/- mice to induce disease. It was clearly shown that less alveolar bone resorption occurred in mice lacking the PAR-2 gene. Further histological, cellular and molecular analysis was carried out to investigate the immunological mechanisms involved in the reduction of bone loss in the PAR-2 null mice in response to oral infection with *P. gingivalis*.

Antagonists of PAR-2 are compounds that inhibit, reduce, or block at least one function of PAR-2 or the expression of PAR-2. Examples of functions of PAR-2 that may be

inhibited, blocked or reduced by an antagonist of PAR-2 include ligand binding and down stream signalling.

Suitable antagonists include, but are not limited to, antibodies and antibody fragments that bind PAR-2, other polypeptides that bind to PAR-2 and inhibit its activity, other compounds that inhibit PAR-2 activity or expression including small organic compounds and inhibitory nucleic acids that interact with PAR-2 encoding nucleic acids. Exemplary antagonists that may block or displace an endogenous ligand from binding PAR-2 and/or signalling via PAR-2 include those described in WO 2004/002418 and WO 2006/023844 (e.g. peptides having the amino acid sequence LIGK or LIGKV). Antagonists that bind to PAR-2 and prevent proteolytic cleavage of the region of PAR-2 that acts as a tethered ligand are exemplified in WO 2007/092640.

Antagonists that inhibit, reduce or block expression of PAR-2 include inhibitory nucleic acids, including, but not limited to, ribozymes, triplex-forming oligonucleotides (TFOs), external guide sequences (EGSs) that promote cleavage by RNase P, peptide nucleic acids, antisense DNA, siRNA, and microRNA specific for nucleic acids encoding PAR-2.

Antagonists that directly bind to protein, DNA or RNA that encode PAR-2 termed "direct antagonists".

PAR-2 may be inhibited indirectly by "indirect antagonists" that antagonise the activity of proteases which under normal circumstances cleave PAR-2 resulting in its activation. Proteases which can cleave PAR-2 include gingipains, trypsins, tryptases and neutrophil proteinase-3. Examples of indirect antagonists that are useful in a method of the invention or that can be used in a composition of the invention include trypsin inhibitors disclosed in WO 93/14779 and tryptase inhibitors disclosed in WO 02/47762.

The antagonist or composition of the invention may be administered directly to the gums of the subject in need of treatment or prevention of periodontal disease. Topical administration of the antagonist or composition of the invention is preferred, however it will be appreciated by a person skilled in the art that a PAR-2 antagonist or composition may also be administered parenterally, e. g, by injection intravenously, intraperitoneally, intramuscularly, intrathecally or subcutaneously.

Alternatively, the antagonist may be formulated as a composition for oral administration (including sublingual and buccal), pulmonary administration (intranasal and inhalation), transdermal administration, and rectal administration.

The route of administration may depend on a number of factors including the nature of the antagonist or composition to be administered and the severity of the subject's condition.

It is understood that the frequency of administration of an antagonist PAR-2 and the amount of antagonist of PAR-2 administered may be varied from subject to subject depending on, amongst other things, the stage of periodontal disease initiation or progression in the subject. The frequency of administration may be determined by a clinician.

It is also contemplated that chronic inflammatory diseases other than periodontal disease may also be treated or prevented in a subject by administering an effective amount of a PAR-2 antagonist. Such chronic inflammatory diseases include those described above. Other diseases that may also be treated or prevented by a method of the invention include those characterised by pathological elevation of T cell activation or T cell mediated inflammatory cytokine expression.

Although the invention finds application in humans, the invention is also useful for veterinary purposes. The invention is useful for domestic animals such as cattle, sheep, horses and poultry; for companion animals such as cats and dogs; and for zoo animals.

An oral composition of this invention which contains the above-mentioned pharmaceutical composition may be prepared and used in various forms applicable to the mouth such as dentifrice including toothpastes, toothpowders and liquid dentifrices, mouthwashes, troches, chewing gums, dental pastes, gingival massage creams, gargle tablets, dairy products and other foodstuffs. An oral composition according to this invention may further include additional well known ingredients depending on the type and form of a particular oral composition.

Optionally, the composition may further include one or more antibiotics that are toxic to or inhibit the growth of Gram negative anaerobic bacteria. Potentially any bacteriostatic or bactericidal antibiotic may be used in a composition of the invention. Preferably, suitable antibiotics include amoxicillin, doxycycline or metronidazole.

In certain preferred forms of the invention the oral composition may be substantially liquid in character, such as a mouthwash or rinse. In such a preparation the vehicle is typically a water-alcohol mixture desirably including a humectant as described below. Generally, the weight ratio of water to alcohol is in the range of from about 1:1 to about 20:1. The total amount of water-alcohol mixture in this type of preparation is typically in the range of from about 70 to about 99.9% by weight of the preparation. The alcohol is typically ethanol or isopropanol. Ethanol is preferred.

The pH of such liquid and other preparations of the invention is generally in the range of from about 5 to about 9 and typically from about 5.0 to 7.0. The pH can be controlled with acid (e.g. citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).

In other desirable forms of this invention, the composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material.

In a toothpaste, the liquid vehicle may comprise water and humectant typically in an amount ranging from about 10% to about 80% by weight of the preparation. Glycerine, propylene glycol, sorbitol and polypropylene glycol exemplify suitable humectants/carriers. Also advantageous are liquid mixtures of water, glycerine and sorbitol. In clear gels where the refractive index is an important consideration, about 2.5 - 30% w/w of water, 0 to about 70% w/w of glycerine and about 20-80% w/w of sorbitol are preferably employed.

Toothpaste, creams and gels typically contain a natural or synthetic thickener or gelling agent in proportions of about 0.1 to about 10, preferably about 0.5 to about 5% w/w. A suitable thickener is synthetic hectorite, a synthetic colloidal magnesium alkali metal silicate complex clay available for example as Laponite (e.g. CP, SP 2002, D) marketed by Laporte Industries Limited. Laponite D is, approximately by weight 58.00% SiO_2, 25.40% MgO, 3.05% Na_2O, 0.98% Li_2O, and some water and trace metals. Its true specific gravity is 2.53 and it has an apparent bulk density of 1.0 g/ml at 8% moisture.

Other suitable thickeners include Irish moss, iota carrageenan, gum tragacanth, starch, polyvinylpyrrolidone, hydroxyethylpropylcellulose, hydroxybutyl methyl cellulose,

hydroxypropyl methyl cellulose, hydroxyethyl cellulose (e.g. available as Natrosol), sodium carboxymethyl cellulose, and colloidal silica such as finely ground Syloid (e.g. 244). Solubilizing agents may also be included such as humectant polyols such propylene glycol, dipropylene glycol and hexylene glycol, cellosolves such as methyl cellosolve and ethyl cellosolve, vegetable oils and waxes containing at least about 12 carbons in a straight chain such as olive oil, castor oil and petrolatum and esters such as amyl acetate, ethyl acetate and benzyl benzoate.

It will be understood that, as is conventional, the oral preparations will usually be sold or otherwise distributed in suitable labelled packages. Thus, a bottle of mouth rinse will have a label describing it, in substance, as a mouth rinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for metering out the contents, having a label describing it, in substance, as a toothpaste, gel or dental cream.

Organic surface-active agents may be used in the compositions of the present invention to achieve increased therapeutic or prophylactic action, assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant compositions more cosmetically acceptable. The organic surface-active material is preferably anionic, non-ionic or ampholytic in nature and preferably does not interact with the active agent. It is preferred to employ as the surface-active agent a detersive material which imparts to the composition detersive and foaming properties. Suitable examples of anionic surfactants are water-soluble salts of higher fatty acid monoglyceride monosulfates, such as the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, higher alkyl sulfates such as sodium lauryl sulfate, alkyl aryl sulfonates such as sodium dodecyl benzene sulfonate, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, and the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds, such as those having 12 to 16 carbons in the fatty acid, alkyl or acyl radicals, and the like. Examples of the last mentioned amides are N-lauroyl sarcosine, and the sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine which should be substantially free from soap or similar higher fatty acid material. The use of these sarconite compounds in the oral compositions of

the present invention is particularly advantageous since these materials exhibit a prolonged marked effect in the inhibition of acid formation in the oral cavity due to carbohydrates breakdown in addition to exerting some reduction in the solubility of tooth enamel in acid solutions. Examples of water-soluble non-ionic surfactants suitable for use are condensation products of ethylene oxide with various reactive hydrogen-containing compounds reactive therewith having long hydrophobic chains (e.g. aliphatic chains of about 12 to 20 carbon atoms), which condensation products ("ethoxamers") contain hydrophilic polyoxyethylene moieties, such as condensation products of poly (ethylene oxide) with fatty acids, fatty alcohols, fatty amides, polyhydric alcohols (e.g. sorbitan monostearate) and polypropyleneoxide (e.g. Pluronic materials).

The surface active agent is typically present in amount of about 0.1-5% by weight. It is noteworthy, that the surface active agent may assist in the dissolving of the active agent of the invention and thereby diminish the amount of solubilizing humectant needed.

Various other materials may be incorporated in the oral preparations of this invention such as whitening agents, preservatives, silicones, chlorophyll compounds and/or ammoniated material such as urea, diammonium phosphate, and mixtures thereof. These adjuvants, where present, are incorporated in the preparations in amounts which do not substantially adversely affect the properties and characteristics desired.

Any suitable flavouring or sweetening material may also be employed. Examples of suitable flavouring constituents are flavouring oils, e.g. oil of spearmint, peppermint, wintergreen, sassafras, clove, sage, eucalyptus, marjoram, cinnamon, lemon, and orange, and methyl salicylate. Suitable sweetening agents include sucrose, lactose, maltose, sorbitol, xylitol, sodium cyclamate, perillartine, AMP (aspartyl phenyl alanine, methyl ester), saccharine, and the like. Suitably, flavour and sweetening agents may each or together comprise from about 0.1% to 5% more of the preparation.

The PAR-2 antagonists or compositions of the invention can also be incorporated in lozenges, or in chewing gum or other products, e.g. by stirring into a warm gum base or coating the outer surface of a gum base, illustrative of which are jelutong, rubber latex, vinylite resins, etc., desirably with conventional plasticizers or softeners, sugar or other sweeteners or such as glucose, sorbitol and the like.

In a further aspect, the present invention provides a kit of parts including (a) a PAR-2 antagonist or composition and (b) a pharmaceutically acceptable carrier. Desirably, the

kit further includes instructions for their use for the treatment or prevention of periodontal disease in a patent in need of such treatment.

Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents; for example sodium carboxymethylcellulose, methylcellulose, hydropropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene

sorbitan monooleate.

The aqueous suspensions may also contain one or more preservatives, for example benzoates, such as ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The present invention also provides interfering RNA molecules which are targeted against the mRNA molecules encoding PAR-2. Accordingly, in a further aspect of the present invention there is provided an interfering RNA molecule, the molecule comprising a double stranded region of at least 19 base pairs in each strand wherein one of the strands of the double stranded region is complementary to or capable of hybridizing under normal intracellular conditions with a region of an mRNA molecule encoding PAR-2. So called RNA interference or RNAi is known and further information regarding RNAi is provided in Hannon (2002) *Nature* 418: 244-251, and McManus & Sharp (2002) *Nature Reviews: Genetics* 3(10): 737-747, the disclosures of which are incorporated herein by reference. The present invention also contemplates chemical modification(s) of siRNAs that enhance siRNA stability and support their use *in vivo* (see for example, Shen *et al.* (2006) *Gene Therapy* 13: 225-234). These modifications might include inverted abasic moieties at the 5' and 3' end of the sense strand oligonucleotide, and a single phosphorthioate linkage between the last two nucleotides at the 3' end of the antisense strand.

It is preferred that the double stranded region of the interfering RNA comprises at least 20, preferably at least 25, and most preferably at least 30 base pairs in each strand of the double stranded region. The present invention also provides a method of treating or preventing periodontal disease in a subject comprising administering to the subject at least one of the interfering RNA molecules of the invention.

To describe the invention in more detail, the following examples are described to illustrate some aspects and embodiments of the invention.

Animals and Materials

In these experiments, PAR-2 null mice were generated on a 129Sv background. For experiments utilizing PAR-2-/- mice, PAR-2+/+ littermates were used as controls. The mouse colony was maintained by PAR-2+/- x PAR-2+/- matings and all mice were genotyped using a PCR-based approach. Experiments making use of animal tissues were approved by the Animal Care and Use Committee of the Department of Biochemistry and Molecular Biology, Monash University (Ethics approval: BAM/B/23/2003). All primers for PCR and Q-PCR were synthesized by Geneworks (Adelaide, Australia). Polymerase chain reaction (PCR) reagents, such as deoxynucleoside triphosphates (dNTPs), 50 base pairs (bp) DNA ladder, PCR buffer, 25 mM $MgCl_2$, and *GoTaq* DNA polymerase were purchased from Promega (Madison, USA). *P. gingivalis* strain W50 was grown and harvested as described previously[1,2] and used as a live inoculum for the mouse periodontitis model. For immunisation and T-cell assays *P. gingivalis* cultures were harvested and the cell pellets were re-suspended in 40 mL 0.5% (v/v) formalin overnight at room temperature. Fresh formalin was added the next day, following which the bacteria [formalin-killed W50 (FK-W50)] were ready for use.

Mouse Periodontitis Model

The mouse periodontitis model was performed as described previously[3] and involved orally inoculating mice with live *P. gingivalis* cells (strain W50). Overall 24 PAR-2+/+ and 24 PAR-2-/- mice at 8 weeks of age were used for the experiment, with the two groups being equally further subdivided into groups which were infected with *P. gingivalis* or sham-infected. The infection regimen for the periodontitis model was as follows: four doses of 10^{10} bacterial cells 2 days apart, followed by 10 days break before another four doses of 10^{10} bacterial cells two days apart. Mice were killed 4 weeks after the last inoculation, then the maxillae were removed and dissected into left-half and right-half maxillae for analyses as previously described[3].

Histomorphometric analysis of maxillae

For one half maxilla from each animal, a digital image of the buccal aspect was

captured with an Olympus DP12 digital camera mounted on a dissecting microscope, using OLYSIA BioReport software version 3.2 (Olympus Australia Pty Ltd., NSW, Australia) to assess horizontal bone loss, defined as loss occurring in a horizontal plane, perpendicular to the alveolar bone crest (ABC) that results in a reduction of the crest height. Each half maxilla was aligned so that the molar buccal and lingual cusps of each image were superimposed, and the image was captured with a micrometer scale in frame, so that measurements could be standardized for each image. The area of exposed root surface, between the cementoenamel junction and the ABC for each molar tooth was measured using the software. These measurements were determined twice by a single blinded examiner using a randomized and coded protocol.

The other half maxillae from three mice of each group were processed for histomorphometry as previously described[4]. Briefly, the specimens were fixed, decalcified and embedded in Spurr's resin. Semi-thin sections (2-5 μm) were stained in 0.5% (w/v) methylene blue. Mast cell counts and analysis of alveolar bone resorption (eroded surface) were conducted on images of sections captured with a digital camera (Spot, Diagnostic Instrument Inc.) linked to an Olympus BX60 microscope. Measurements were made with Image-Pro Plus image analysis software (Media Cybernectics, Silver Spring, MD, USA).

Mast cells were identified as large cells containing numerous intensely stained granules in their cytoplasm (Fig. 1b insert). Mast cells were counted in a specific field located at the base of the gingival sulcus (buccal and lingual aspects) and in between the cementum and alveolar bone (illustrated in Fig. 1a and b). The eroded surface (ES) of the alveolar bone on the buccal aspect was measured in a region extending 450 μm from the ABC towards the apex of the tooth (illustrated in Fig. 1c), and expressed as percentage of bone surface (BS), that is ES/BS x 100. For the mast cell counts and percentage of alveolar surface erosion, results from three sections were averaged to give the result for each animal. Results for mast cell counts and alveolar surface erosion were obtained from three animals per genotype and treatment group. All histomorphometric analyses were performed by a single blinded examiner using a randomized and coded protocol.

Flow cytometric analysis

Mice were immunised (25 µg/mouse with formalin-killed *P. gingivalis* W50 in incomplete Freund's adjuvant administered subcutaneously to the hind limb) and 7 days later the popliteal and inguinal lymph nodes were removed and the lymph nodes were pooled into their respective groups. Lymphocytes were isolated from the four treatment groups (PAR-2+/+ sham-immunised, PAR-2+/+ immunised, PAR-2-/- sham-immunised and PAR-2-/- immunised) after processing the lymph nodes through a sieve, following which the separated tissue was collected and gently layered onto 5 mL Lympholyte M and centrifuged (800 x g, 20 min). The lymphocyte layer was collected and washed twice with Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) FCS, penicillin (100 U/mL), streptomycin sulphate (100 µg/mL) and glutamate. The lymphocytes were counted using a Coulter particle counter (Beckman Coulter, Fullerton, USA), following which 1 x 10^6 cells were added per well for each experimental group on a 96 well plate. The plate was centrifuged at 800 x g for 5 min and supernatant was removed. For each treatment group, four wells were used, with two wells for antibody mixture 1 (anti-CD90/Thy-1.2-FITC, anti-CD8-PE and anti-TCRβ-APC; BD Biosciences, NSW, Australia), and two wells for antibody mixture 2 (anti-CD90/Thy-1.2-FITC, anti-CD4-PE and anti-TCRβ-APC). Both antibody mixtures, containing 1% (v/v) FC blocker (BD Biosciences, NSW, Australia) were incubated with shaking for 20 min at room temperature in the dark. Cells alone (PAR-2+/+ non-immunised) and single colour controls (where PAR-2 +/+ non-immunised lymphocytes were incubated with one fluorescent antibody) were used as background controls. A Cytomics FC500 flow cytometer (Beckman-Coulter, NSW, Australia) was used to detect the stained lymphocytes.

T-cell Proliferation and ELISPOT assays

The remaining isolated lymphocytes from the immunised and sham-immunised mice were further purified using mouse PAN T cell MAC beads and an AUTOMAC bead cell sorter (Miltenyi Biotech, NSW, Australia). Spleens were also removed from the sham-immunised mice and pooled into PAR-2+/+ and PAR-2-/- groups. Spleens were processed through a sieve and the single cell suspension washed twice (800 x g) in DMEM and the red blood cells lysed using red cell lysis buffer (Sigma-Aldrich, NSW, Australia). After washing three times (800 x g) in DMEM the splenic cells were irradiated

(2200 rads) and used as a source of syngeneic antigen presenting cells in the T-cell and ELISPOT assays.

A serial dilution of *P. gingivalis* FK-W50 cells (starting from 25 μg protein/100 mL) was added to a 96 well tissue culture plate. T cells from the four experimental treatment groups were added in triplicate to the wells containing *P. gingivalis* FK-W50 at a concentration of 1 x 10^5 cells/well. PAR-2+/+ sham-immunised antigen presenting cells at a concentration of 1 x 10^5 cells were added to the wells containing T cells from PAR-2+/+ sham-immunised and immunised mice. PAR-2-/- sham-immunised antigen presenting cells were added to the wells containing T cells from PAR-2-/- sham-immunised and immunised mice. For each group a negative control was employed where wells only contained T cells and antigen presenting cells. Serially diluted concanavalin A (conA) was used as a positive control by adding it to wells that contained T cells and antigen presenting cells.

The plates were incubated at 37°C in 5% CO_2 for three days, following which 1 μCi of [^{3}H] thymidine (Amersham Biosciences, Buckinghamshire, UK) was added to the cells and incubated for a further 18 hours. After incubation, 40 μL of mammalian cell lysis buffer (Sigma-Aldrich, NSW, Australia) was added to the wells for 20 min. Cells were subsequently harvested onto glass fibre filter mats using the MACHIII cell harvester (Tomtec, Hamden, USA) and the filter was air-dried and sealed into sample bags containing 5 mL of scintillant fluid. A Wallac Microbeta β-scintillation counter instrument (Perkin-Elmer, NSW, Australia) was used to count the radioactivity emitted from the glass fibre filter mats and the counts per minute (cpm) in each well were determined. The mean cpm values for either FK-W50-stimulated or control cells were calculated by averaging the counts in the respective triplicate wells. The stimulatory index (SI) was calculated by dividing the mean cpm obtained after antigen stimulation by the mean cpm detected in control, unstimulated wells.

For the ELISPOT assays, Millipore Multiscreen 96-well filtration plates (MAHAS450, Millipore, N.S.W, Australia) were coated with anti-mouse cytokine capture antibodies (eBiosciences, San Diego, CA, USA), specific for IL-4 and interferon-γ (IFN-γ), at a concentration of 4 μg/mL in 0.1 M sodium bicarbonate buffer (pH 9.5) and incubated overnight at 4°C. The ELISPOT plates were washed with Dulbecco's PBS and blocked

with enriched DMEM for 1 h at 37°C. Lymph node T-cells from each group and spleens were prepared as above. Lymph node cells (1 x 10^5/well) from FK-W50 immunised PAR-2+/+ or PAR-2-/- mice were incubated with γ-radiated (2200 Rads) syngeneic spleen cells as a source of antigen presenting cells (PAR-2 +/+ or PAR-2 -/-, respectively, 1 x 10^5cells/well) and *P. gingivalis* FK-W50 cells (1.0 μg/mL). Plates were incubated at 37°C in an atmosphere of 5% CO_2 in air for 48 h in a humidified incubator, following which they were washed with PBS containing 0.05% (v/v) Tween 20 (PBST) three times and once with deionised water. Cytokine-specific biotinylated antibodies (eBiosciences, San Diego, CA, USA) specific for IL-4 and IFN-γ were added at a concentration of 2 μg/mL in Dulbecco's PBS/enriched DMEM (1:1, v/v) and incubated at room temperature for 2 h. Plates were washed six times with PBST and streptavidin-alkaline phosphatase conjugate (Roche, Castle Hill, NSW, Australia) was added to the plates at a 1:1000 dilution in Dulbecco's PBS/enriched DMEM (1:1, v/v) and incubated for 1 h at room temperature. The plates were washed with PBST and PBS, following which substrate (5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium; BCIP/NBT, [Sigma-Aldrich, St Louis, MO, USA]) was added to allow spots to develop for 20–30 mins, which were counted using EliSpot Reader Lite (version 2.9, Autoimmun Diagnostika GmbH, Strassberg, Germany). As previously described above, ELISPOT studies were also undertaken to analyse the T cell response in the mouse periodontitis model, where T cells were isolated from the submandibular lymph nodes (SMLN), which are the gingival tissue draining lymph nodes.

Bioplex cytokine array

For the simultaneous quantitation of multiple secreted cytokines, undiluted supernatants were collected from three separate T cell proliferation assays where T cells obtained from FK-W50-immunised PAR-2+/+ and PAR-2-/- mice were exposed to 0.78 μg/ml *P. gingivalis* FK-W50 (see Fig. 6) and were analysed using a mouse cytokine kit containing IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p70), IL-13, IL-17, GM-CSF, IFN-γ, and TNF-α beads on the Bio-Plex suspension array system (Bio-Rad, Hercules, USA) according to the manufacturer's instructions. Standard curves were prepared and samples were analysed using the Bio-Plex Manager software on the Bio-Plex 2200 instrument. Concentrations of the above cytokines in the supernatants from each T cell assay were analysed in triplicate and the results were averaged.

Statistical analysis

Bone loss (mm2) data were statistically analysed using a one-way ANOVA, Dunnett's 3T test and Cohen's effect size d. Effect sizes, represented as Cohen's[5], were calculated using an effect size calculator provided online (http://cem.dur.ac.uk/ebeuk/researc/effectsize/). According to Cohen[5], a small effect size is $d \geq 0.2$ and < 0.5, a moderate effect size is $d \geq 0.5$ and < 0.8, and a large effect size is $d \geq 0.8$. All other data were statistically analysed using the student's t-test and Cohen's effect size, and results are expressed as the mean ± SEM. The significance of differences between experimental groups was determined with Student's t-test assuming unequal variances; P values <0.05 were considered significant.

The role of PAR-2 in P. gingivalis-induced alveolar bone resorption

To determine whether PAR-2 has a role in *P. gingivalis*-induced periodontal bone loss, PAR-2-/- and PAR-2+/+ littermates were orally infected with *P. gingivalis* strain W50 and the alveolar bone loss induced was analysed. The maxillae of PAR-2+/+ mice orally infected with *P. gingivalis* W50 had a significantly ($p<0.05$) greater area of exposed root surface than the PAR-2+/+ sham-infected group, indicating that the infection had resulted in alveolar bone loss (Fig. 2c). However, the PAR-2-/- mice orally infected with *P. gingivalis* W50 were found to have significantly ($p<0.01$) less exposed root surface than their respective PAR-2+/+ counterparts (Fig. 2c). Moreover, there was no significant difference in the values between the PAR-2-/- mice that were orally infected with *P. gingivalis* and the control (sham-infected) PAR-2-/- or PAR-2+/+ mice.

Histological examination of mast cells in the periodontal tissue

In the histological analysis, mast cells were found in higher numbers in the periodontal tissue of PAR-2+/+ mice orally infected with *P. gingivalis* compared with those of sham-infected PAR-2+/+ mice. In contrast, *P. gingivalis* oral infection had no effect on mast cell numbers in PAR-2-/- mice. It is noteworthy that sham-infected PAR-2-/- mice were also found to have significantly fewer mast cells in their periodontal tissue than PAR-2+/+ unchallenged mice (Fig. 3).

Eroded surface, as a histomorphometric parameter of recent bone resorption, was greater in alveolar bone from PAR-2+/+ mice orally infected with *P. gingivalis* than in alveolar bone from their sham-infected counterparts (Figure 4). There was no difference

in the alveolar bone eroded surface between *P. gingivalis*-infected and sham-infected PAR-2-/- mice.

PAR-2-/- mice display impaired T-cell immune responses compared to PAR-2+/+ mice.

Initially, T-cells from PAR-2+/+ and PAR-2-/- mice that had been immunised with *P. gingivalis* FK-W50 cells were isolated using CD90 (Thy1.2) microbeads to positively select for T-cells. However, poor yields (around 1 x 10^5 total cells) of T-cells from the PAR-2-/- immunised mice were consistently obtained, whereas for the PAR-2+/+ immunised mice a typical yield ranging from 3 - 7 x 10^7 T cells in total was obtained . Preliminary phenotyping of the lymphocyte populations from the PAR-2+/+ and PAR-2-/- mice indicated that there was no significant difference in the numbers of TCRβ+, CD4+ or CD8+ T-cells between the strains of mice (data not shown). With these data in mind, T-cells were isolated from both strains of mice by negative sorting using the PAN T-cell microbeads (which deplete all lymphocytes, except T-cells), and obtained cell numbers in the typical (higher) yield range above for both the PAR-2-/- and PAR-2+/+ genotypes using this strategy. Phenotyping analysis by flow cytometry of the lymphocyte populations from *P. gingivalis* FK-W50 immunised and sham-immunised (PBS) PAR-2+/+ and PAR-2-/- mice indicated differing expression levels of CD90/Thy-1.2 (Fig. 5). In the PAR-2+/+ mice there was no significant difference between the non-immunised and immunised animals in the CD90/Thy-1.2 expression in TCRβ+, CD4+ T-cells or TCRβ+, CD8+ T-cells (Fig. 5a and 5b). However, in the PAR-2-/- mice there was a significant ($p<0.001$) decrease in CD90/Thy-1.2 expression in both TCRβ+, CD4+ T-cells and TCRβ+, CD8+ T-cells from immunised PAR-2-/- mice compared to the sham-immunised PAR-2-/- mice (Fig. 5c and 5d). Furthermore, in PAR-2-/- *P. gingivalis* FK-W50 immunised mice, 59% of TCRβ+, CD4+ T-cell and 55% TCRβ+, CD8+ T-cell populations were determined to have no CD90/Thy-1.2 expression. In comparing the CD90/Thy-1.2 expression in sham-immunised mice, the PAR-2-/- mice had significantly ($p<0.01$) less CD90/Thy-1.2+ in CD4+ and CD8+ T cells than their PAR-2+/+ counterparts. Interestingly, in the PAR-2+/+ mice, although there was no significant difference in the CD4+ or CD8+ T cell populations positive for CD90/Thy-1.2, there was an observed decrease in CD90/Thy-1.2 expression (as determined by mean fluorescence intensity) in both T cell populations in the *P. gingivalis* FK-W50 immunised groups compared to the sham-immunised groups.

To further examine differences in the T cell populations, cells isolated (using PAN-T microbeads) from PAR-2+/+ and PAR-2-/- mice immunised with *P. gingivalis* FK-W50 were subjected to proliferation and cytokine analysis in response to *P. gingivalis* W50 cells. Fig. 6 shows that the *P. gingivalis* FK-W50-primed T-cells from PAR-2-/- mice had significantly ($p<0.05$) lower maximal proliferation in response to *P. gingivalis* than their PAR-2+/+ counterparts. Furthermore, the numbers of IL-4 and IFN-γ secreting T-cells from *P. gingivalis* FK-W50 immunised PAR-2-/- mice were significantly ($p<0.05$) less than their PAR-2+/+ counterparts (Fig. 7), with a large difference in the IFN-γ (d = 7.49, 95.0, CI: 12.88, 3.22) response compared with the IL-4 (d = 5.25, 95.0, CI: 7.42, 1.46) response. On comparing the IL-4 and IFN-γ response in each strain, the PAR-2+/+ mice had a significantly ($p<0.05$) higher IFN-γ response compared with the IL-4 response. In contrast, the number of IFN-γ secreting T cells was not significantly higher than the number of IL-4 secreting T cells in PAR-2-/- mice, in fact the mean number of IL-4 secreting T cells was slightly higher (d = 0.91, 95.0, CI: -0.86, 2.46).

In the mouse periodontitis model, T cells were also isolated and used in an ELISPOT assay to determine the numbers of IL-4 and IFNγ secreting T cells in response to *P. gingivalis* stimulation. No significant difference was found in the numbers of IL-4 secreting T cells between PAR-2+/+ and PAR-2-/- mice orally infected with *P. gingivalis* (Fig. 8). However, there was a significantly ($p<0.05$, d = 19.58) higher number of IFN-γ secreting PAR-2+/+ T cells compared with PAR-2-/- T cells. Furthermore, in PAR-2+/+ mice orally infected with *P. gingivalis* there were significantly ($p<0.05$, d = 8.34) higher numbers of IFN-γ secreting T cells than IL-4 secreting T cells. However, in the PAR-2-/- mice orally infected with *P. gingivalis*, there was no significant difference between the number of IL-4 secreting T cells and the number of IFN-γ secreting T cells, in fact the mean number of Il-4 secreting T-cells was slightly higher (d = 0.97, 95%, CI: -0.77, 2.59).

To further characterise the T cell cytokine response, the supernatants from the T cell proliferation assays were analysed by Bioplex cytokine array for the presence of the following cytokines: IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p70), IL-13, IL-17, GM-CSF, IFN-γ, and TNF-α. From results shown in Fig. 9, it may be seen that T cells from *P. gingivalis* immunised PAR-2+/+ mice secreted significantly ($p<0.05$) higher amounts of IL-2, IL-3, IL-12 (p70), IL-17, GM-CSF, IFN-γ and TNF-α than their PAR-2-/-

counterparts in response to *P. gingivalis* FK-W50. Interestingly the strongest response, at least 4-fold higher than other cytokines secreted, in both PAR-2+/+ and PAR-2-/- T cells, was the level of IL-17 (145.8±69.4 pg/mL and 14.4±4.9 pg/mL, PAR-2+/+ and PAR-2-/-, respectively). Although there was no significant difference in the levels of the other cytokines analysed, there were, however, higher numbers (as determined by effect size) of PAR-2+/+ T cells secreting IL-5, IL-6, IL-9, IL-10, and IL-13, but not IL-4, than PAR-2-/- T cells (d = 0.87, 1.24, 0.67, 0.89 and 0.97, respectively). Furthermore, although analysis of the levels of secreted cytokines found that no significant difference was observed (Students' t-test), PAR-2+/+ T cells did secrete higher concentrations of IL-5, IL-6, IL-9, IL-10, and IL-13 than secreted by PAR-2-/- T cells (d = 0.87, 1.24, 0.67, 0.89 and 0.97, respectively, as analysed by Cohen's effect size).

PAR-2 can be activated by several proteases, including trypsin, mast cell tryptase and bacterial proteases, such as the gingipains from *P. gingivalis* .

P. gingivalis has been implicated as a major causative agent of chronic periodontitis and in the experiments described herein a live virulent strain of P, gingivalis (W50) was orally inoculated into PAR-2+/+ and PAR-2-/- mice to induce disease. The results in this model showed that less alveolar bone resorption occurred in mice lacking the PAR-2 gene, other models have also suggest reduced bone loss under certain conditions.[6] Furthermore, this data appears consistent with a report that suggested, in another model, topical addition of a PAR-2 agonist to the lower right first molar in rats may increase alveolar bone destruction when compared to saline treatments.[7]

The data obtained from the histological analyses indicated that the increase in mast cell numbers seen in the maxillary tissue of the infected PAR-2 +/+ mice were not seen in infected PAR-2-/- mice. It must be noted that in sham-infected animals, there were also significantly less mast cells in the tissue of PAR-2-/- mice compared with the PAR-2+/+ mice, indicating that the lack of PAR-2 may also play role/s in mast cell differentiation or infiltration into tissues. There is evidence for PAR-2 expression by mast cells, thus activation of this receptor may play a role in the recruitment of these cells.

Mast cells have been shown to be activated by bacterial antigens, following which inflammatory mediators released by these cells have been found to be pivotal to the innate immune response by activating surrounding cells, the immune response and immune clearance mechanisms. It is possible that these cells may therefore play a

pivotal role in the early inflammatory response to *P. gingivalis* in the context of chronic periodontitis. This may result from activation of PAR-2 on their surface by the arginine-specific gingipains or other tissue proteases, as well as other mechanisms of activation. Activation of PAR-2 on mast cells leads to degranulation by these cells, causing the release of pro-inflammatory compounds aimed at killing pathogens and upregulating the host immune response. Mast cell tryptase, which is released from the granules of these cells when activated, is a known activator of PAR-2 and therefore it may be inferred that these cells could also play a primary role in periodontitis by causing the activation of the receptor on cells in the periodontal tissues.

The above results illustrate that PAR-2 has a role in the innate immune response to *P. gingivalis*, so the next question was whether PAR-2 was involved in the adaptive immune response. T cells obtained from the lymph nodes of PAR-2-/- mice that were immunised with formalin-killed *P. gingivalis* W50 (FK-W50) proliferated significantly less in response to antigen than T cells obtained from PAR-2+/+ immunised mice. The decreased proliferation of T cells is another plausible reason for the reduction in bone loss seen in the PAR-2-/- mice in the mouse periodontitis model, since impairment in T cell activation would lead to a less effective immune response, thereby also decreasing the tissue damage associated with the inflammatory response.

It was noted in both periodontitis model and immunisation experiments, that use of the pan-T cell marker, CD90/Thy-1, for isolation of T cells led to poor recovery of T cells in antigen immunised PAR-2-/- mice; therefore it was hypothesised that these T cells may have a decreased expression of CD90/Thy-1 compared with wild type controls. This hypothesis was confirmed using FACS analysis of whole lymph node lymphocyte isolates. It was found that expression of CD90/Thy-1 was markedly down-regulated following immunisation in PAR-2-/- mice, with the majority of CD4+ and CD8+ T cells being CD90/Thy-1 negative. CD90/Thy-1 has been implicated as playing important roles in T cell activation and maturation, possibly due to its action as co-receptor for the T cell receptor, which may act as a *cis* ligand for Thy-1. Thy-1 is also a regulator of cell-cell and cell-matrix adhesion and migration, thus down-regulation or absence of this receptor on T cells would have a significantly detrimental effect on their migration to the site of infection and thus their contribution to inflammation. Thus the absence of CD90/Thy-1 or its marked down-regulation of expression in the T cells from immunised

PAR-2-/- mice might contribute to the reduced proliferation of the T cells from PAR-2-/- mice. It is also possible that the reduction or abolition of expression of Thy-1 might influence the profile of cytokines expressed by T cells from the PAR-2-/- mice.

ELISPOT analysis of T cells from both wild type immunised mice and those from the mouse periodontitis model showed similar results in terms of a strong increase in the number of IFNγ-positive T cell colonies compared with IL-4-positive colonies upon stimulation by the bacterium, either through immunisation or infection in the model. The pronounced increase in IFNγ-positive colonies upon immunisation or infection was not seen for the T cells from PAR-2-/- mice. The increase in IFNγ-secreting T cells from wild type mice would be expected to induce a strong T cell helper 1 (Th1) type of immune response in these animals, which has been found to contribute strongly to a tissue destructive type of response, consistent with the tissue destruction observed in periodontitis. In the PAR-2-/- mice, the majority of the T cells were Thy-1 negative after antigen stimulation and this reduction in Thy-1 expression may affect the T cell activation state and thus the production of cytokines. The data presented here suggest that the antigen-induced down regulation of Thy-1 resulting in CD4+ and CD8+ Thy-1 negative T cells does not result in functional Thy-1 T cells but rather a "deactivated" T cell population. The findings from this study therefore suggest that antigen stimulation of PAR-2-/- T cells results in a down regulation of CD90/Thy-1, leading to a deactivated state. Consequently, these cells are unable to contribute to an inflammatory response and thus the induction of a tissue destructive response, consistent with the results showing a pronounced decrease in bone resorption in the receptor null mice. These findings also point to the potential of a PAR-2 antagonist being used to restrict the contribution of T cells to inflammation by inhibiting their activation via the receptor.

Supernatants from the T cell proliferation experiments were tested for a range of cytokine levels in a protein array in a further attempt to understand how the absence of PAR-2 might be affecting the behaviour of these cells. It was immediately notable that levels of IL-2 were markedly down-regulated in the supernatants of T cells from PAR-2-/- mice. As this cytokine plays a vital role in T cell proliferative responses, this finding provides a major explanation for the lack of proliferation by the T cells from the receptor null mice. The levels of a number of other secreted cytokines were also affected, including the reduction of IFNγ expression, consistent with the results of the ELISPOT

assays. IL-17 protein levels were highly upregulated in the supernatants of T cells from PAR-2+/+ mice, and were approximately ten-fold higher compared to the level in supernatants of T cells from PAR-2-/- immunised mice. Also of interest is that there was no difference between the supernatants from PAR-2+/+ versus PAR-2-/- derived T cells in the sham (PBS) immunised mice, suggesting that the differences were likely a reflection of events occurring during an immune response to antigen. The cells responsible for the expression of IL-17 have been identified as a subset of T cells (Th_{17}). IL-17 is a potent inducer of inflammation and studies have shown it to be involved in autoimmune diseases such as rheumatoid arthritis (RA) in which IL-17 expression is upregulated. Administration of IL-17 into joints causes cartilage damage, and cells over expressing IL-17 cause bone erosion and cartilage damage in a mouse model of RA. Recent studies have reported the IL-17 levels in gingival tissue and gingival crevicular fluid are higher in periodontitis patients compared to healthy patients and patients with gingivitis, indicating that Th17 cells may play a significant role in the inflammatory response associated with chronic periodontitis. Since the identification of the Th_{17} cells, it has been found that IL-6 is a potent inducer of their production. Since PAR-2 activation causes expression of IL-6 in a variety of cells, this pathway could be involved in the upregulation of IL-17 levels seen in the T cell supernatants from PAR-2+/+ mice and thus the lower levels of IL-17 seen in the PAR2-/- mice maybe a consequence of the lower levels of IL-6 observed in these animals.

In addition to the highly altered cytokine levels already noted above, it is worth noting that almost every cytokine tested showed a difference between the PAR-2-/- and the PAR-2+/+ derived cells, with the levels of IL-3, IL-12p70, GM-CSF and TNF-α all showing statistically significant depressed levels in the PAR-2-/- mice. These data suggest that although the CD4 and CD8 T cell population numbers in PAR-2-/- mice are similar to PAR-2+/+ mice, upon antigen activation, potential inflammatory T cells become less active, resulting in reduced proliferation, cytokine secretion and down regulation of CD90/Thy-1. Furthermore, proliferation in the PAR-2-/- mice was not completely prevented and this may be due to a response by CD90/Thy-1 negative T cells. Th2 and/or CD90/Thy-1 negative cells may be stimulated by antigen, resulting in proliferation and IL-4 secretion that is not regulated by the inflammatory/Th1 T cells that would normally be the major responsive T cell population to bacterial infection.

The role of PAR-2 in periodontal disease progression needs to be considered at several levels. Firstly, *P. gingivalis* releases arginine-specific gingipains, which may penetrate subjacent gingival tissue and activate PAR-2 on epithelial, endothelial and connective tissue cells, thus causing an inflammatory response. Without being bound by any theory, the following possible outcomes of the activation of PAR-2 are believed to occur. First, the production of IL-6 by gingival epithelial cells, IL-6 being a pro-inflammatory cytokine that can directly promote the resorption of bone through the induction of osteoclast formation and it is possible that this pathway operates to cause bone loss in periodontal disease. Secondly, mast cells attracted to the site of inflammation may also be involved in recognising *P. gingivalis*, causing them to release TNF-α, which would recruit neutrophils to the site of infection, and mast cell tryptase which would activate PAR-2 on surrounding cells. Thirdly, the recruitment of neutrophils would add more potential activators of PAR-2 as neutrophils produce proteinase-3, which has been shown to activate of PAR-2. The activation of PAR-2 by the gingipains, tryptase and proteinase-3 may all lead to activation and proliferation of T cells, strongly upregulating a tissue destructive immune response. Due to the multiple potential levels of activation of the receptor during the inflammatory process, in its absence, the inflammatory response to the bacterium is markedly reduced, thereby decreasing host tissue degradation and bone resorption.

Compositions and Formulations

To help illustrate compositions embodying aspects of the invention directed to treatment or prevention, the following sample formulations are provided.

The following is an example of a toothpaste formulation.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Glycerol	20.0
Sodium carboxymethyl cellulose	1.0
Sodium lauryl sulphate	1.5
Sodium lauroyl sarconisate	0.5
Flavour	1.0
Sodium saccharin	0.1

Chlorhexidine gluconate	0.01
Dextranase	0.01
PAR-2 antagonist	0.2
Water	balance

The following is an example of a further toothpaste formulation.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Lauroyl diethanolamide	1.0
Sucrose monolaurate	2.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Dextranase	0.01
PAR-2 antagonist	0.1
Water	balance

The following is an example of a further toothpaste formulation.

Ingredient	% w/w
Sorbitol	22.0
Irish moss	1.0
Sodium Hydroxide (50%)	1.0
Gantrez	19.0
Water (deionised)	2.69
Sodium Monofluorophosphate	0.76
Sodium saccharine	0.3
Pyrophosphate	2.0
Hydrated alumina	48.0
Flavour oil	0.95

PAR-2 antagonist	0.3
sodium lauryl sulphate	2.00

The following is an example of a liquid toothpaste formulation.

Ingredient	% w/w
Sodium polyacrylate	50.0
Sorbitol	10.0
Glycerol	20.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Ethanol	3.0
PAR-2 antagonist	0.2
Linolic acid	0.05
Water	balance

The following is an example of a mouthwash formulation.

Ingredient	% w/w
Ethanol	20.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.3
PAR-2 antagonist	0.2
Water	balance

The following is an example of a further mouthwash formulation.

Ingredient	% w/w
Gantrez® S-97	2.5
Glycerine	10.0
Flavour oil	0.4

Sodium monofluorophosphate	0.05
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.2
PAR-2 antagonist	0.3
Water	balance

The following is an example of a lozenge formulation.

Ingredient	% w/w
Sugar	75-80
Corn syrup	1-20
Flavour oil	1-2
NaF	0.01-0.05
PAR-2 antagonist	0.3
Mg stearate	1-5
Water	balance

The following is an example of a gingival massage cream formulation.

Ingredient	% w/w
White petrolatum	8.0
Propylene glycol	4.0
Stearyl alcohol	8.0
Polyethylene Glycol 4000	25.0
Polyethylene Glycol 400	37.0
Sucrose monostearate	0.5
Chlorhexidine gluconate	0.1
PAR-2 antagonist	0.3
Water	balance

The following is an example of a periodontal gel formulation.

Ingredient	% w/w
Pluronic F127 (from BASF)	20.0
Stearyl alcohol	8.0
PAR-2 antagonist	3.0

Colloidal silicon dioxide (such as Aerosil® 200™)	1.0
Chlorhexidine gluconate	0.1
Water	balance

The following is an example of a chewing gum formulation.

Ingredient	% w/w
Gum base	30.0
Calcium carbonate	2.0
Crystalline sorbitol	53.0
Glycerine	0.5
Flavour oil	0.1
PAR-2 antagonist	0.3
Water	balance

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

References

1. O'Brien-Simpson NM, Paolini RA, Hoffmann B, Slakeski N, Dashper SG, Reynolds EC: Role of RgpA, RgpB, and Kgp proteinases in virulence of *Porphyromonas gingivalis* W50 in a murine lesion model, Infect Immun 2001, 69:7527-7534

2. O'Brien-Simpson NM, Paolini RA, Reynolds EC: RgpA-Kgp peptide-based immunogens provide protection against *Porphyromonas gingivalis* challenge in a murine lesion model, Infect Immun 2000, 68:4055-4063

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6. Holzhausen M, Spolidorio LC, Ellen RP, Jobin MC, Steinhoff M, Andrade - Gordon P, Vergnolle N: Protease-activated receptor-2 activation: a major role in the pathogenesis of *Porphyromonas gingivalis* infection, Am J Pathol 2006, 168:1189-1199.

7. Holzhausen M, Spolidorio LC, Vergnolle N: Proteinase-activated receptor-2 (PAR2) agonist causes periodontitis in rats, J Dent Res 2005, 84:154-159

The claims defining the invention are as follows:

1. A method of treating or preventing periodontal disease comprising administering to a subject an effective amount of an antagonist of PAR-2.

2. A method according to claim 1, wherein the antagonist is selected from the group consisting of peptides, antibodies, nucleic acids and small molecules.

3. A method according to claims 1 or 2 wherein the antagonist is administered directly to the gums of the subject.

4. A method according to any one of claims 1 to 3, further comprising administering an anti-inflammatory agent.

5. A method according to any one of the preceding claims, further comprising administering an antibiotic.

6. A method according to any one of the preceding claims, further comprising administering an antibiofilm agent.

7. A composition for the treatment or prevention of periodontal disease comprising an effective amount of an antagonist of PAR-2 and a pharmaceutically acceptable carrier.

8. A composition according to claim 7 wherein the antagonist is selected from the group consisting of peptides, antibodies, nucleic acids and small molecules.

9. A composition according to claim 7 or 8 further including an agent selected from the group consisting of anti-inflammatory agents, antibiotics and antibiofilm agents.

10. A method of reducing or inhibiting mast cell infiltration into periodontal tissue comprising administering to a subject an effective amount of an antagonist of PAR-2.

11. A method of reducing or inhibiting neutrophil recruitment into periodontal tissue comprising administering to a subject an effective amount of an antagonist of PAR-2.

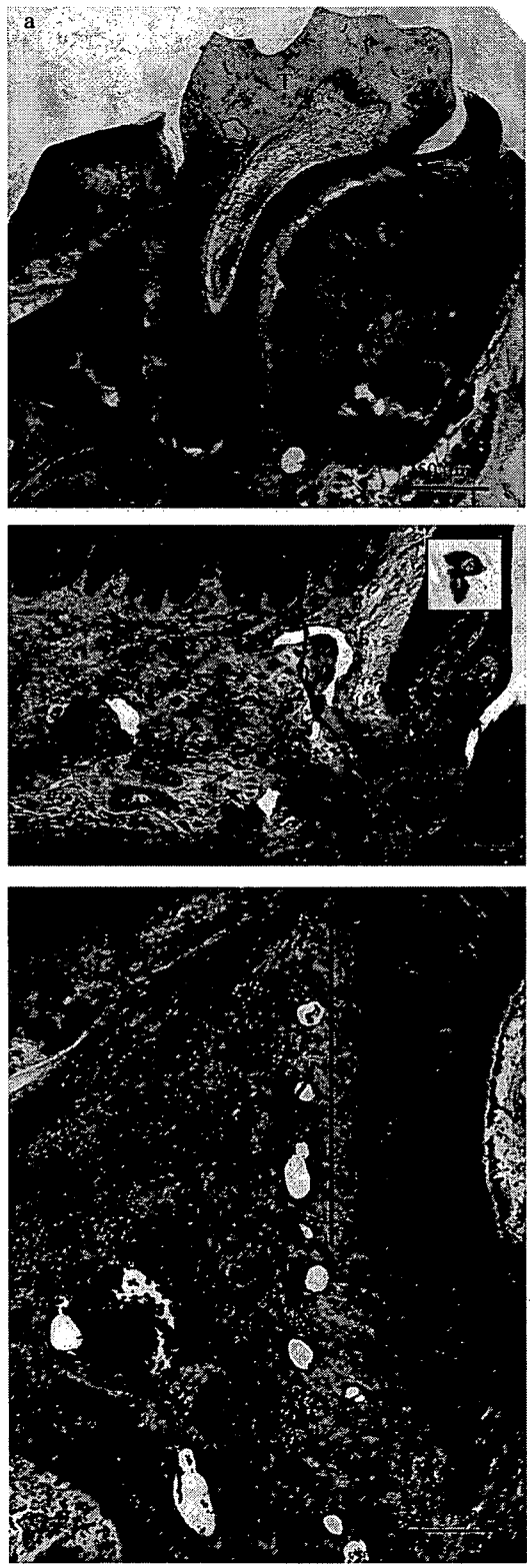

Figure 1

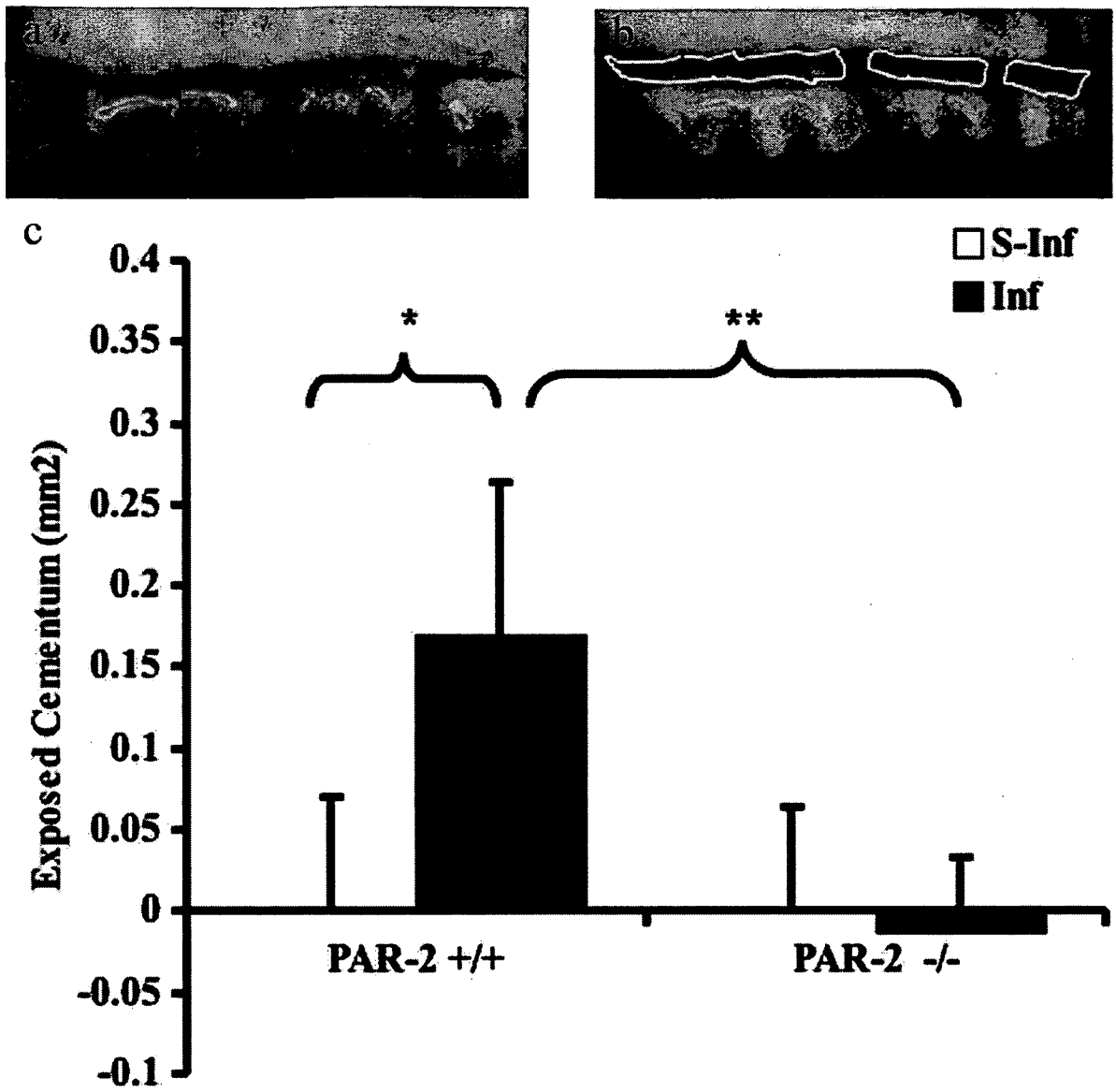
a
b
c
S-Inf
Inf
*
**
Exposed Cementum (mm2)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
PAR-2 +/+
PAR-2 -/-

Figure 2

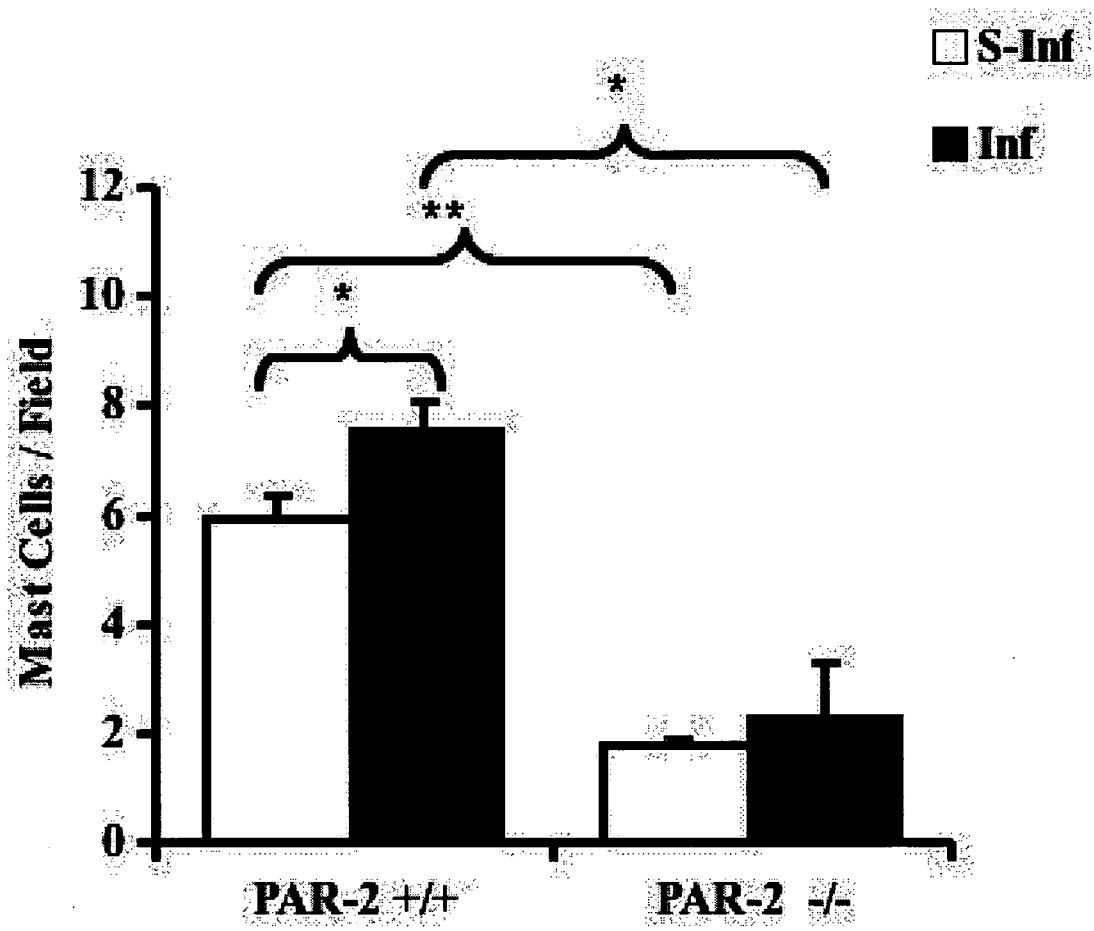

Figure 3

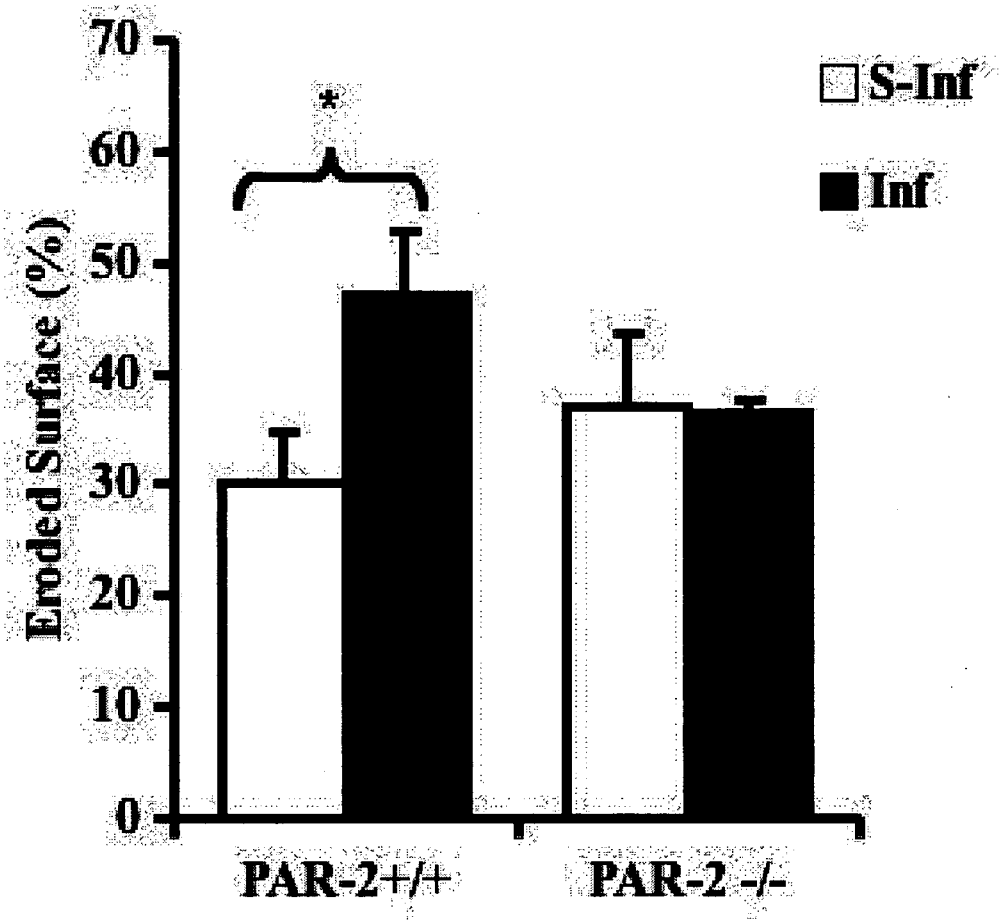

Figure 4

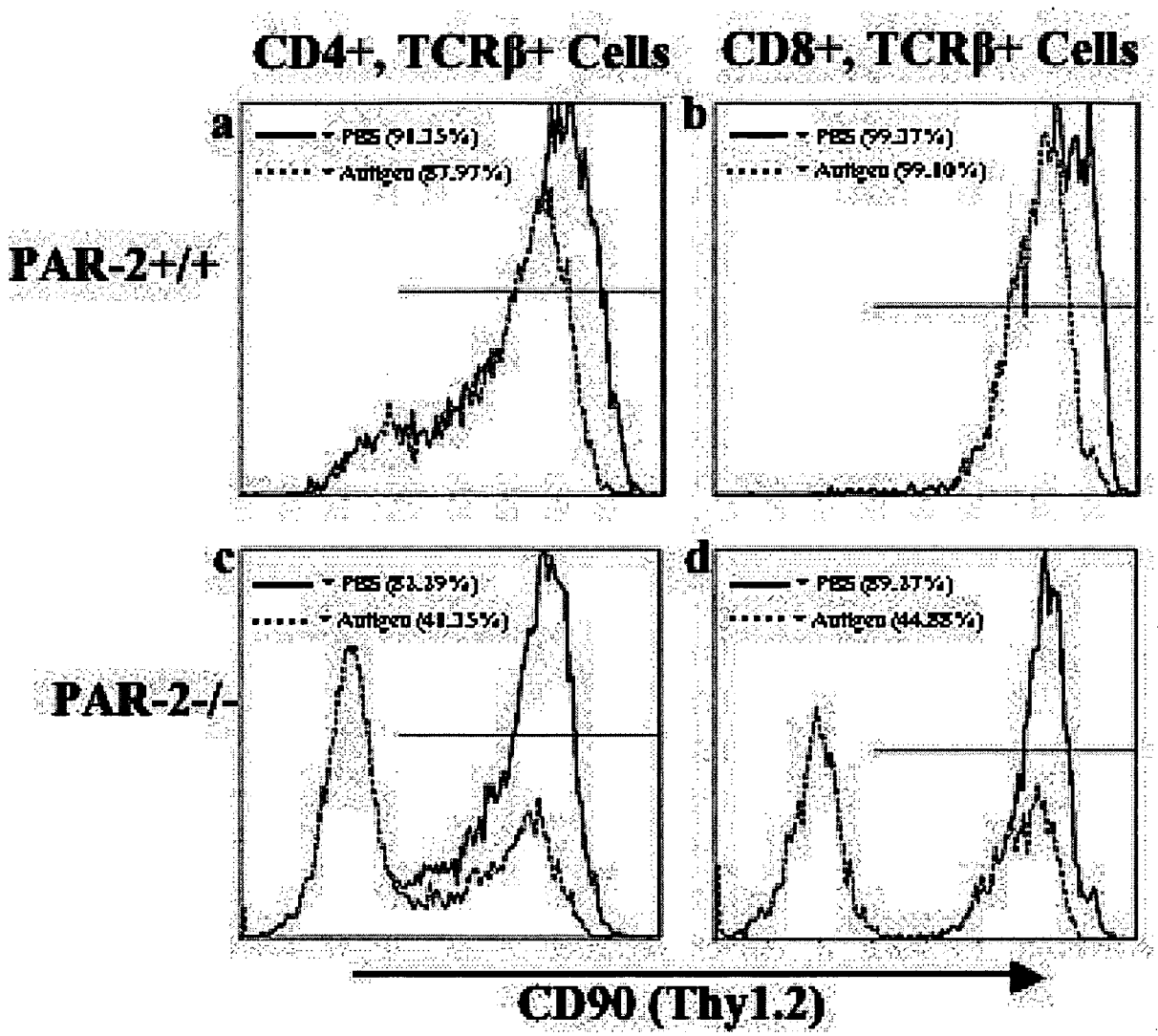
CD4+, TCRβ+ Cells
CD8+, TCRβ+ Cells
a
b
c
d
PAR-2+/+
PAR-2-/-
PBS (91.35%)
Antigen (87.97%)
PBS (99.37%)
Antigen (99.10%)
PBS (61.39%)
Antigen (41.35%)
PBS (89.37%)
Antigen (44.85%)
CD90 (Thy1.2)

Figure 5

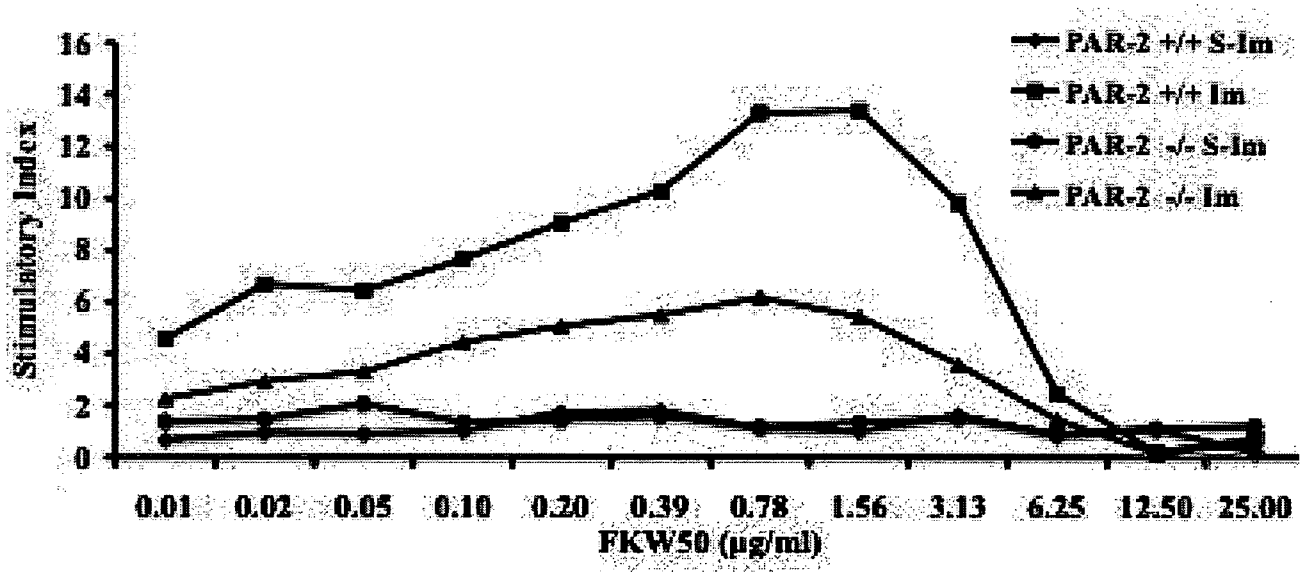
Stimulatory Index
16
14
12
10
8
6
4
2
0
0.01
0.02
0.05
0.10
0.20
0.39
0.78
1.56
3.13
6.25
12.50
25.00
FKW50 (µg/ml)
PAR-2 +/+ S-Im
PAR-2 +/+ Im
PAR-2 -/- S-Im
PAR-2 -/- Im

Figure 6

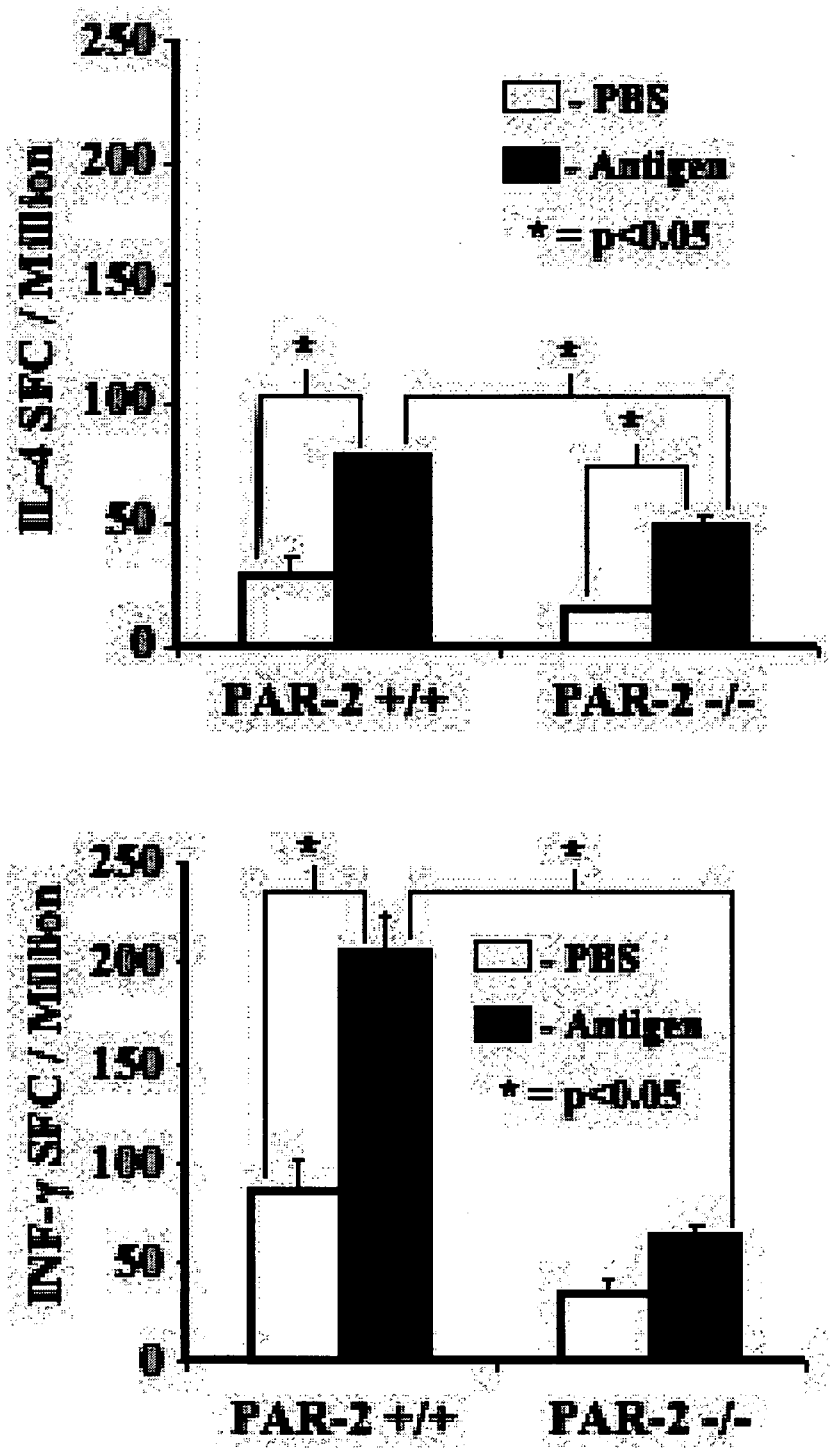
IL-4 SFC / Million
250
200
150
100
50
0
- PBS
- Antigen
* = p<0.05
PAR-2 +/+
PAR-2 -/-
INF-γ SFC / Million
250
200
150
100
50
0
- PBS
- Antigen
* = p<0.05
PAR-2 +/+
PAR-2 -/-

Figure 7

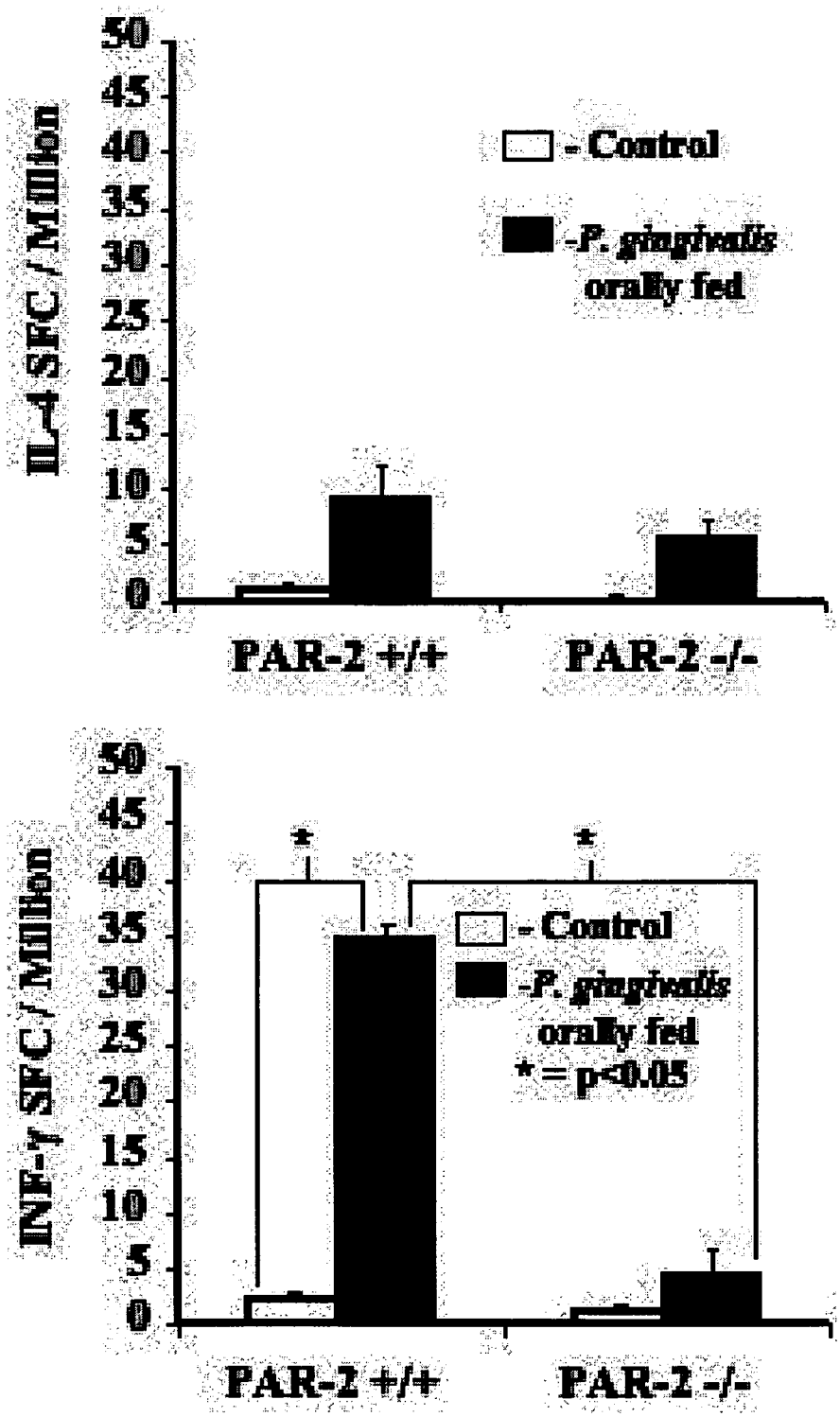
IL-4 SFC / Million
50
45
40
35
30
25
20
15
10
5
0
- Control
-P. gingivalis orally fed
PAR-2 +/+
PAR-2 -/-
INF-γ SFC / Million
50
45
40
35
30
25
20
15
10
5
0
- Control
-P. gingivalis orally fed
* = p<0.05
PAR-2 +/+
PAR-2 -/-

Figure 8

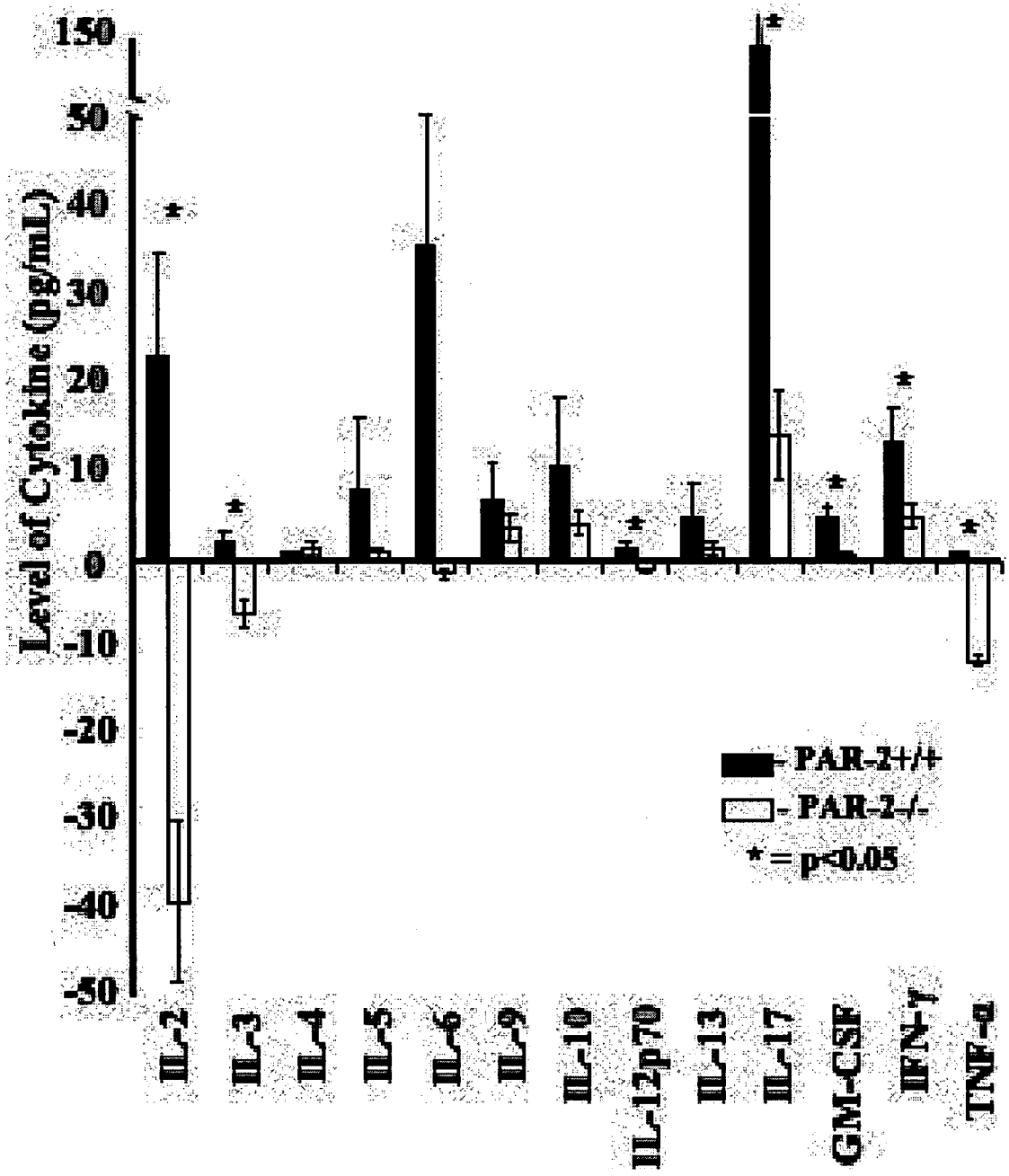
Level of Cytokine (pg/mL)
150
50
40
30
20
10
0
-10
-20
-30
-40
-50
IL-2
IL-3
IL-4
IL-5
IL-6
IL-9
IL-10
IL-12p70
IL-13
IL-17
GM-CSF
IFN-γ
TNF-α
PAR-2+/+
PAR-2-/-
* = p<0.05

Figure 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2010/000615

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 38/07 (2006.01) ***A61K 38/08*** (2006.01) ***A61P 43/00*** (2006.01)
A61K 38/00 (2006.01) ***A61K 39/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI, EPODOC, Medline, CAplus and Biosis; Keywords used: PAR()2, antagonists, inhibitors, periodont?, gingiv? and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0197244 A1 (SEIBERG, MIRI. ET AL.,) 26 December 2002 See [0068 lines 8-10], [0072], [0023], [0075] and [0009]	1 and 7
X	WO 2006/052723 A2 (NEW ENGLAND MEDICAL CENTER HOSPITALS, INC.) 18 May 2006 See page 2 lines 15-25, see page 20, lines 15-20, SEQ ID 17, page 22 Table 1 first row, claim 10, page 5 lines 10-15 and claim 28.	1, 2, 7 & 8

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2010	09 SEP 2010

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2010/000615

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Holzhausen, M. et al., 'Proteinase-activated Receptor-2 (PAR2) Agonist Causes Periodontitis in Rats' J Dent Res, 2005, Vol. 84, No. 2, pages 154-159 See page 156 Results paragraph 4, page 157 lines 1-6 & paragraph 6, page 159 lines 1-5, page 155 paragraph 5, page 154 lines 3-8 and page 158 3rd paragraph last six lines	1-6 and 9-11 1
X Y	Holzhausen, Marinella. et al., 'Protease-Activated Receptor-2 Activation', American Journal of Pathology, April 2006, Vol. 168, No. 4, pages 1189-1199 See page 1190 second paragraph-last four lines, Figure 4A, page 1196 first paragraph, Figure 4c, Abstract and page 1196 lines 10-17	1-4 and 6 1
X Y	EP 1 806 141 A1 (KOWA COMPANY, LTD. 11 July 2007 See pages 13-15 and 18-19, claim 2, [0038], [0069], [0071] and [0070]	7 and 8 1
X	WO 2007/092640 A2 (AMGEN INC.) 16 August 2007 See Abstract, page 2 lines 30-40, Figure 3 and claim 7.	7-9
X	WO 2004/002418 A2 (ENTREMED, INC) 8 January 2004 See SEQ ID 1, page 12 lines 5-11, page 29 lines 25-35, claims 5, 7 and 18.	7-8
X	WO 1993/014779 A1 (CORVAS INTERNATIONAL, INC.) 5 August 1993 See page 3, lines 28-38, page 27, page 25, page 42, Table 1, example B, page 28, pages 49-50 and example C.	7-8
P,X	Wong, David. M. et al., 'Protease-Activated Receptor 2 Has Pivotal Roles in Cellular Mechanisms Involved in Experimental Periodontitis', Infection and Immunity, Feburary 2010, Vol. 78, No. 2, pages 629-638 See Abstract; page 632 lines 1-6 and page 635 lines 22-24.	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2010/000615

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	2002197244	NONE					
WO	2006052723	AU	2005304963	AU	2010202328	BR	PI0517058
		CA	2586344	CN	101094866	EP	1814911
		US	2008214451				
EP	1806141	EP	1806355	US	2009012006	US	2009012263
		WO	2006035936	WO	2006035937		
WO	2007092640	AU	2007212195	CA	2638849	EP	1981913
		MX	2008009886	US	2007237759		
WO	2004002418	AU	2003247754	CA	2490129	EP	1536813
		US	2004266687	US	2006142203		
WO	9314779	CA	2128711	EP	0627925	US	5534498
		US	5714580				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX