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Oral treatment with complement factor C5a receptor (CD88) antagonists inhibits experimental periodontitis in rats

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Background and Objective: The complement activation product 5a (C5a) is a potent mediator of the innate immune response to infection, and may thus also importantly determine the development of periodontitis. The present study was designed to explore the effect of several novel, potent and orally active C5a receptor (CD88) antagonists (C5aRAs) on the development of ligature-induced periodontitis in an animal model.

Material and Methods: Three different cyclic peptide C5aRAs, termed PMX205, PMX218 and PMX273, were investigated. Four groups of Wistar rats ($n = 10$ in each group) were used. Starting 3 d before induction of experimental periodontitis, rats either received one of the C5aRas (1–2 mg/kg) in the drinking water or received drinking water only. Periodontitis was assessed when the ligatures had been in place for 14 d.

Results: Compared with control rats, PMX205- and PMX218-treated rats had significantly reduced periodontal bone loss.

Conclusion: The findings suggest that complement activation, and particularly C5a generation, may play a significant role in the development and progression of periodontitis. Blockade of the major C5a receptor, CD88, with specific inhibitors such as PMX205, may offer novel treatment options for periodontitis.

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Periodontitis is a tissue-destructive inflammatory condition of the tooth-supporting tissues that may lead to tooth loss in the most severe cases. Severe periodontitis affects about 10% of most populations, and the disease is associated with bacterial, immunological, genetic and environmental factors such as smoking, diabetes and stress-related diseases (1–5). Exactly how these multifarious disorders influence the development and progression of periodontitis has been poorly understood. However, the disease is considered to be initiated by increased colonization of pathogenic microorganisms, including some gram-negative bacteria species, in subgingival dental plaque biofilms (6). This may indicate that immune system responses vital for clearing these pathogens are inappropriately regulated.

We have previously studied the impact of different immune aberrations on periodontal disease (7,8). In the present study, we wanted to investigate the role of complement system blockade. The complement system is a biochemical cascade that helps clear pathogens and is a decisive element of

the innate immune response to infection (9). The co-operative interaction between complement and microbial molecular recognition receptors on innate immune cells, including Toll-like receptors (TLRs), links innate and adaptive immunity, and an effective interplay between these two systems is essential to control and eliminate pathogens (10). A major effector of all complement activation products is the anaphylatoxin C5a, a split product of complement factor C5 (9,11). Local or systemic administration of lipopolysaccharide (LPS), a biologically active molecular structure of the outer cell membrane of all gram-negative bacteria, activates the complement system, generating C5a (11,12). Complement 5a binds to two specific receptors [C5aRs, otherwise known as CD88, and C5a-receptor-like 2 (C5L2)] located on polymorphonuclear leukocytes (PMNs), monocytes/macrophages, dendritic, endothelial and epithelial cells (13). The C5a–C5aR interaction modulates a number of immune cell responses, including release of cytokines and chemokines (9,11).

To elucidate how complement function may influence the development of periodontitis, we used recently developed potent and selective orally active antagonists of the C5a receptor CD88 (C5aRAs). This class of antagonists has been shown to be effective in reducing C5a-mediated pathology in various immunoinflammatory disease models in rodents, and is currently undergoing clinical evaluation for use in humans (14–17). The aim of the present experiments was to test the hypothesis that the major complement cascade activation fragment, C5a, may play a role in periodontal breakdown. We also wanted to compare the effectiveness of different antagonists in this respect.

Material and methods

Animals

Wistar rats were obtained from Möllegaard Breeding Center (Ejby, Denmark), and used after 2 wk of acclimatization. Standard rat chow pellets and tap water were available *ad libitum*. The animals were housed in groups of five under a 12 h–24 h light–dark cycle (light on from 07.00 to 19.00 h) with a temperature of 22°C and humidity at 40–60%. The experiments were registered and approved by the Norwegian Experimental Animal Board (NEAB).

Complement 5a receptor antagonists

Three C5aRAs were used in this study. These compounds are orally active cyclic hexapeptides, which are potent and selective noncompetitive inhibitors of the major C5aR, CD88. This class of drugs has been found to be efficacious in various animal models of inflammatory disease (14–17), and some of these drugs have undergone clinical evaluation for use in humans (18). The compounds are coded PMX205 {hydrocinnamate (HC)-[OP(dCha)WR]}, PMX273 {AcF-[OP(dPhe)WR]} and PMX218 {HC-[OP(dPhe)WR]}, and all display similar receptor binding affinity and antagonistic potency (19). However, importantly, PMX205 and PMX218 are more lipophilic analogues than PMX273, due to the substitution of the extracyclic phenylalanine residue with a hydrocinnamate residue, which may confer increased *in vivo* potency (19). The compounds were synthesized as previously described (18) and purified by reverse-phase HPLC.

Experimental design

This experiment was designed to test the efficacy of three C5aRAs (PMX205, PMX218 and PMX273) on the progression of experimental periodontitis. The rats were randomly assigned to four groups, each consisting of 10 rats. Each of the C5aRAs was administered in the drinking water to one of the three experimental groups starting 3 d before induction of ligature-induced periodontitis and during the entire experiment (20 mg/L, or approximately 1–2 mg/kg/d). Control animals received drinking water only. Three days thereafter, periodontitis was induced in both experimental and control rats. All animals were killed by decapitation 14 d after induction of the ligature-induced periodontitis.

Experimental periodontitis

Under general anaesthesia with Hypnorm–Dormicum (fentanyl/fluanizone, midazolam, Janssen and Cilag, Saunderton, UK; 0.2 mL/100 g body weight subcutaneously) the animals had a sterile silk ligature (Ethicon Permahand® Seide 3/0, Johnson & Johson Company, Norderstedt, Germany) tied around the neck of the maxillary right second molar tooth in the gingival sulcus. The procedure was performed on the third day after beginning C5aRA treatment. The ligatures serve as a retention device for oral micro-organisms, and change the microflora in the gingival pocket. Twelve days after application of the ligatures, all animals were killed by decapitation. The maxillae were excised and fixed in 4% buffered formaldehyde. The same procedure was performed in all animals.

Radiographic examination of alveolar bone loss

The specimens were stabilized with dental wax on a Sidexis digital X-ray sensor, orientated with the axis of the teeth parallel to the sensor surface by using ×4 magnification loupe glasses (Zeiss, Norstedt, Germany). The distance between the cemento-enamel junction and bone on the mesial surfaces of the second molars was displayed digitally. The examination was performed in a blinded fashion. Each X-ray was read three times, and the mean of the three readings calculated. Radiographs were taken from the animals in all three experiments. Digital X-rays of alveolar bone loss were used as an indicator for periodontal breakdown because our previous studies have shown that bone loss measured with X-rays is more valid than other frequently applied methods, such as periodontal fibre and bone loss measured on histological sections (7,8). Bone loss is also commonly used for diagnosis of the severity of periodontitis in humans.

Statistical analysis

Data are presented as means ± SD. Differences between values were tested

with one-way analysis of variance (ANOVA) followed by Dunnett's method. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of C5a receptor antagonist treatment on body weight

Body weights were measured over the study period, to assess any potential toxicological adverse effect of antagonists on body weight gain. The administration of C5aRA to rats had no significant effect on weight gain during the study period. At induction of the C5aRAs, the PMX205-treated animals weighed 287.0 ± 4.4 g, the PMX218-treated animals 285.3 ± 7.9 g, the PMX273-treated animals 285.0 ± 7.4 g and the control animals 289.0 ± 6.5 g ($p > 0.05$). At point of killing, the weight was 316.9 ± 10.8 g in the PMX205-treated animals, 324.0 ± 17.8 g in the PMX218-treated animals, 322.8 ± 12.4 g in the PMX273-treated animals and 328.1 ± 17.0 g in the control animals ($p > 0.05$ between the groups).

Effect of C5a receptor antagonist treatment on periodontal tissue destruction

Alveolar bone loss following experimental periodontal disease was assessed in all experimental groups following completion of the study using digital X-rays. Whilst PMX273 showed no effect on reducing bone loss in this model, both PMX205 and PMX218 treatment significantly reduced the periodontal bone loss compared with untreated control animals (Figs 1 and 2).

Discussion

In this study, we have demonstrated that chronic oral administration of the selective C5aR (CD88) antagonists PMX205 and PMX218 significantly attenuated the development of experimental periodontitis in Wistar rats. These data contribute to recent findings demonstrating a pathogenic involvement of complement activation and C5a generation in the pathogenesis of periodontitis (20,21).

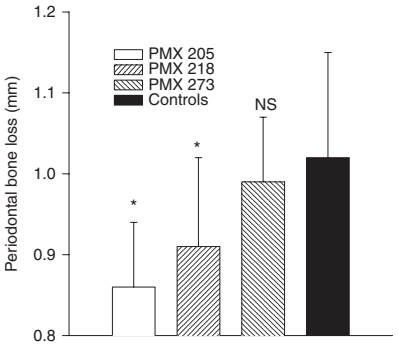

Fig. 1. Alveolar bone loss in periodontal disease after treatment with the complement 5a receptor antagonists PMX205, PMX218 or PMX273 as measured on digital radiographs (means ± SD). *$p < 0.05$ vs. controls; NS, nonsignificant vs. controls (one-way ANOVA with Dunnett's method).

Several tentative explanations for these effects may be put forward. In the first place, blockade of C5a–CD88 signalling may reduce the release of reactive oxygen species (ROS) and the formation of MMPs in response to the ligature-induced colonization of pathogenic micro-organisms in the subgingival pocket. Excessive recruitment and activation of PMNs, with increased release of potent effector molecules, plays a significant role in the development of inflammatory diseases (11), including periodontitis (22,23). It is well documented that C5a stimulates the migration of PMNs into inflammatory sites (11,24), elicits ROS release (25,26) and enhances the formation of MMPs in response to LPS and gram-negative bacteria (11,19). Furthermore, treatment with anti-C5a antibodies and C5aRAs inhibits PMN recruitment, ROS and MMP release after exposure to gram-negative bacteria and LPS, and reduces tissue damage (11,19).

Secondly, the reduced periodontal breakdown may, in part, be caused by C5aRA-induced alteration of other immune mediators. In addition to being a strong chemoattractant for PMNs, monocytes/macrophages, eosinophils and T cells (11,27,28), C5a can induce the release of secondary chemoattractants that can amplify the accumulation of immune cells. Complement 5a exerts this effect through the high-affinity C5a receptors (CD88 and C5L2). Complement 5a–CD88 signalling on macrophages may, for example, increase the chemokine subfamily interleukin (IL)-8/cytokine-induced neutrophil chemoattractant (CINC-1) (28–31). Interleukin-8 (in humans) has a high degree of homology with CINC-1 (in rodents, which do not produce IL-8), and both CINC-1 and IL-8 bind to the same CXC2 receptor (CXC2R; 32). Thus, both C5a and CINC-1 may direct PMNs to the site of inflammation. It is believed that C5a is involved in the initial recruitment of PMNs, and that IL-8/CINC-1 is responsible for the more prolonged influx (32,33). Production of CINC-1, mainly from macrophages, is induced by the proinflammatory cytokines tumour necrosis factor-α and IL-Iβ, as well as by bacterial components such as LPS (34). The C5aRA-induced inhibition of complement activation may also have changed other branches of the inflammatory network, such as

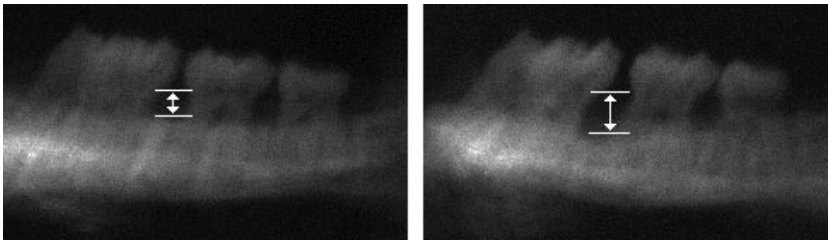

Fig. 2. Digital X-rays showing maxillary right molar teeth in a Wistar rat given the complement 5a receptor antagonist PMX205 in the drinking water (left) and a control animal given drinking water only (right). Experimental periodontitis was induced by tying a silk ligature around the maxillary right second molar tooth 3 d before PMX205 was administered. A difference in loss of alveolar bone on the mesial surfaces of the second molars can be observed. The mean bone loss was 0.86 ± 0.08 mm in the PMX205-treated animals and 1.02 ± 0.13 mm in the controls.

the production and release of pro- and anti-inflammatory cytokines.

Recent studies indicate that the gram-negative periodontal pathogen *Porphyromonas gingivalis* can control C5aR activation by generating C5a through its own C5 convertase-like enzymatic activity, and that *P. gingivalis* may use this mechanism to proactively and selectively inhibit TLR2-induced IL-12p70 release. In addition, C5aR-deficient or TLR2-deficient mice have been found to be resistant to periodontal bone loss, and C5RA-treated mice showed enhanced clearance of *P. gingivalis* relative to control mice (20,21).

Complement 5a has also been found to increase the sensitivity to inflammatory pain. For example, blockade of C5aRs with a chemically similar C5aRA (PMX53) inhibits inflammatory pain in humans, and the effect was dependent on the presence of PMNs at the inflammatory site (35). In line with this, we have recently shown that systemic blockade or desensitization of primary sensory peptidergic neurons induces weaker hypothalamic–pituitary–adrenal axis responses and stronger proinflammatory tumour necrosis factor-α cytokine to systemic LPS stimulation *in vivo*, and inhibits experimental periodontitis (36).

The final outcome of the adaptive immune response depends on the interactions of regulatory systems, including those controlled by the brain, such as the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system, which together comprise the stress response system, as well as the parasympathetic and peptidergic sensory nervous systems (37–39). For example, our previous experiments in rats have revealed that the reactivity of these overarching regulatory systems is an important determinant of the susceptibility and progression of experimental periodontitis (40–46). Furthermore, we have shown that stress-related diseases, such as severe anxiety and depression of the melancholic type, as well as nicotine abuse, may influence the severity of the disease by dysregulating these overarching immunoregulatory pathways (45,46). Thus, brain-controlled regulatory systems may be dysregulated, either genetically, as we have demonstrated in stress high-responding Fischer 344 rats and stress low-responding Lewis rats (40–42), and/or by changes in the environment, as demonstrated by stress-related diseases and nicotine abuse (45,46).

Based on these data, we have suggested that periodontitis may be the result of a dysregulated or misguided brain–neuroendocrine balance that reduces the ability of adaptive immunity to respond optimally to pathogens, and thus to clear the colonization of subgingival pathogenic dental plaque bacteria (45,46). Chronic overactivity of the innate immune system may be a compensatory response that protects the gingival connective tissues, along with the entire organism, from being infected by these pathogens. Accordingly, we hypothesize that periodontitis is the result of a hidden benefit that protects against infection in individuals with reduced ability to respond with an optimal adaptive immune response to pathogens. In contrast to this, other investigators have hypothesized that periodontitis is due to insufficient removal of PMNs and their products from the inflammatory sites (47), or that periodontitis is due to pathogens that have 'learned' to escape host defence mechanisms, including complement- and TLR-mediated immunity (19,20,48,49).

In conclusion, we have used a well-established animal model of periodontitis to provide new information on mechanisms involved in periodontal tissue destruction. We have shown that blockade of the major complement cascade activation fragment, C5a, by selective C5aRAs, potently inhibits periodontal breakdown. Overactivation of the complement system, which is one of the key components of the innate immune response to infection, may thus play an important role in periodontitis. As discussed above, there are several biological mechanisms by which the C5aRA treatment may impact upon the development of experimental periodontitis. Further studies are therefore required to investigate whether immunotherapeutic strategies with C5aRAs can switch the immune system, not only away from immunopathology, e.g. periodontal tissue destruction as demonstrated in the present study, but also whether the ability to clear the colonization of pathogenic dental plaque bacteria is affected.

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References

1. Genco RJ. Current view of risk factors for periodontal diseases. *J Periodontol* 1996; **67:**1041–1049.
2. Preshaw PM, Seymour RA, Heasman PA. Current concepts in periodontal pathogenesis. *Dent Update* 2004;**31:**570–574.
3. Tatakis DN, Kumar PS. Etiology and pathogenesis of periodontal diseases. *Dent Clin North Am* 2005;**49:**491–516.
4. Kornman KS. Mapping the pathogenesis of periodontitis: a new look. *J Periodontol* 2008;**79:**1560–1568.
5. Peruzzo DC, Benatti BB, Ambrosano GM *et al*. A systematic review of stress and psychological factors as possible risk factors for periodontal disease. *J Periodontol* 2007;**78:**1491–1504.
6. Socransky SS, Haffajee AD. Periodontal microbial ecology. *Periodontol 2000* 2005;**38:**135–187.
7. Breivik T, Rook GA. Oral treatment with SR299 (killed Mycobacterium vaccae) inhibits experimental periodontal disease in Wistar rats. *J Clin Periodontal* 2003;**30:**931–936.
8. Breivik T, Opstad PK, Engstad R, Gundersen G, Gjermo P, Preus H. Soluble β-1,3/1,6-glucan from yeast inhibits experimental periodontal disease in Wistar rats. *J Clin Periodontal* 2005;**32:**347–352.
9. Köhl J. Anaphylatoxins and infectious and non-infectious inflammatory diseases. *Mol Immunol* 2001;**38:**175–187.
10. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature* 2007;**449:**819–826.
11. Guo RF, Ward PA. Role of C5a in inflammatory responses. *Annu Rev Immunol* 2005;**23:**821–852.

12. Freudenberg MA, Galanos C. Interaction of lipopolysaccharides and lipid A with complement in rats and its relation to endotoxicity. *Infect Immun* 1978;**19:**875–882.
13. Ward PA. Functions of C5a receptors. *J Mol Med* 2009;**87:**375–378.
14. Arumugam TV, Shiels IA, Woodruff TM, Reid RC, Fairlie DP, Taylor SM. Protective effect of a new C5a receptor antagonist against ischemia-reperfusion injury in the rat small intestine. *J Surg Res* 2002;**103:**260–267.
15. Proctor LM, Arumugam TV, Shiels I, Reid RC, Fairlie DP, Taylor SM. Comparative anti-inflammatory activities of antagonists to C3a and C5a receptors in a rat model of intestinal ischaemia/reper fusion injury. *Br J Pharmacol* 2004;**142:** 756–764.
16. Woodruff TM, Arumugam TV, Shiels IA, Reid RC, Fairlie DP, Taylor SM. A potent human C5a receptor antagonist protects against disease pathology in a rat model of inflammatory bowel disease. *J Immunol* 2003;**171:**5514–5520.
17. Woodruff TM, Arumugam TV, Shiels IA, Reid RC, Fairlie DP, Taylor SM. Protective effects of a potent C5a receptor antagonist on experimental acute limb ischemia-reperfusion in rats. *J Surg Res* 2004;**116:**81–90.
18. Woodruff TM, Proctor LP, Taylor SM. Complement factor C5a as a therapeutic target. *Drugs Future* 2006;**31:**325–334.
19. Woodruff TM, Pollitt S, Proctor LM *et al*. Increased potency of a novel complement factor 5a receptor antagonist in a rat model of inflammatory bowel disease. *J Pharmacol Exp Ther* 2005;**314:**811–817.
20. Liang S, Krauss JL, Domon H *et al*.The C5a Receptor Impairs IL-12-Dependent Clearance of *Porphyromonas gingivalis* and Is Required for Induction of Periodontal Bone Loss. *J Immunol* 2010;**186:**869–877.
21. Wang M, Krauss JL, Domon H *et al*. Microbial hijacking of complement-toll-like receptor crosstalk. *Sci Signal* 2010;**3:** ra11.
22. Gustafsson A, Ito H, Asman B, Bergström K. Hyper-reactive mononuclear cells and neutrophils in chronic periodontitis. *J Clin Periodontol* 2006;**33:**126–129.
23. Guentsch A, Puklo M, Preshaw PM *et al*. Neutrophils in chronic and aggressive periodontitis in interaction with *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. *J Periodontal Res* 2009;**44:**368–377.
24. Allegretti M, Moriconi A, Beccari AR *et al*. Targeting C5a: recent advances in drug discovery. *Curr Med Chem* 2005;**12:**217–236.
25. Marder SR, Chenoweth DE, Goldstein IM, Perez HD. Chemotactic responses of human peripheral blood monocytes to the complement-derived peptides C5a and C5a des Arg. *J Immunol* 1985;**134:**3325–3331.
26. Czermak BJ, Breckwoldt M, Ravage ZB *et al*. Mechanisms of enhanced lung injury during sepsis. *Am J Pathol* 1999;**154:**1057–1065.
27. Fittschen C, Sandhaus RA, Worthen GS, Henson PM. Bacterial lipopolysaccharide enhances chemoattractant-induced elastase secretion by human neutrophils. *J Leukoc Biol* 1988;**43:**547–556.
28. March DR, Proctor LM, Stoermer MJ *et al*. Potent cyclic antagonists of the complement C5a receptor on human polymorphonuclear leukocytes. Relationships between structures and activit. *Mol Pharmacol* 2004;**65:**868–879.
29. Soruri A, Riggert J, Schlott T, Kiafard Z, Dettmer C, Zwirner J. Anaphylatoxin C5a induces monocyte recruitment and differentiation into dendritic cells by TNF-alpha and prostaglandin E2-dependent mechanisms. *J Immunol* 2003;**171:**2631–2636.
30. Morgan EL. Modulation of the immune response by anaphylatoxins. *Complement* 1986;**3:**128–136.
31. Bhandari V, Elias JA. Cytokines in tolerance to hyperoxia-induced injury in the developing and adult lung. *Free Radic Biol Med* 2006;**41:**4–18.
32. Lorenzetti BB, Veiga FH, Canetti CA, Poole S, Cunha FQ, Ferreira SH. Cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediates the sympathetic component of inflammatory mechanical hypersensitivitiy in rats. *Eur Cytokine Netw* 2002;**13:**456–461.
33. Collins PD, Jose PJ, Williams TJ. The sequential generation of neutrophil chemoattractant proteins in acute inflammation in the rabbit in vivo. Relationship between C5a and proteins with the characteristics of IL-8/neutrophil-activating protein 1. *J Immunol* 1991;**146:**677–684.
34. Watanabe K, Koizumi F, Kurashige Y, Tsurufuji S, Nakagawa H. Rat CINC, a member of the interleukin-8 family, is a neutrophil-specific chemoattractant in vivo. *Exp Mol Pathol* 1991;**55:**30–37.
35. Ting E, Guerrero AT, Cunha TM *et al*. Role of complement C5a in mechanical inflammatory hypernociception: potential use of C5a receptor antagonists to control inflammatory pain. *Br J Pharmacol* 2008;**153:**1043–1053.
36. Breivik T, Gundersen Y, Gjermo P, von Hörsten S, Fristad I, Opstad PK. Systemic chemical desensitization of peptidergic sensory neurons with resiniferatoxin inhibits experimental periodontitis. *Open Dent J* 2011;**5:**1–6.
37. Ader R, Cohen N, Felten D. Psychoneuroimmunology: interactions between the nervous system and the immune system. *Lancet* 1995;**345:**99–103.
38. Eskandari F, Sternberg EM. Neural immune interaction in health and disease. *Ann N Y Acad Sci* 2002;**966:**20–27.
39. Elenkov IJ. Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. *Neurochem Int* 2008;**52:**40–51.
40. Breivik T, Opstad PK, Gjermo P, Thrane PS. Effects of hypothalamic-pituitary-adrenal axis reactivity on periodontal tissue destruction in rats. *Eur J Oral Sci* 2000;**108:**115–122.
41. Breivik T, Thrane PS, Gjermo P, Opstad PK. Glucocorticoid receptor antagonist RU-486 treatment reduces periodontitis in Fischer 344 rats. *J Periodont Res* 2000;**35:** 285–290.
42. Breivik T, Thrane PS, Gjermo P, Opstad PK, Pabst R, von Horsten S. Hypothalamic-pituitary-adrenal axis activation by experimental periodontal disease in rats. *J Periodont Res* 2001;**36:**295–300.
43. Breivik T, Gundersen Y, Opstad PK, Fonnum F. Chemical sympathectomy inhibits periodontal disease in Fischer 344 rats. *J Periodont Res* 2005;**40:**325–330.
44. Breivik T, Gundersen Y, Osmundsen H, Opstad PK, Fonnum F. Chronic treatment with the glutamate receptor antagonist MK-801 alters periodontal disease susceptibility. *J Periodont Res* 2005;**40:** 28–35.
45. Breivik T, Gundersen Y, Myhrer T *et al*. Enhanced susceptibility to periodontitis in an animal model of depression: reversed by chronic treatment with the antidepressant tianeptine. *J Clin Periodontol* 2006; **33:**469–477.
46. Breivik T, Gundersen Y, Gjermo P, Von Hörsten S, Opstad PK. Nicotinic acetylcholine receptor activation mediates nicotine-induced enhancement of experimental periodontitis. *J Periodont Res* 2009;**44:** 297–303.
47. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003;**82:**82–90.
48. Krauss JL, Potempa J, Lambris JD, Hajishengallis G. Complementary Tolls in the periodontium: how periodontal bacteria modify complement and Toll-like receptor responses to prevail in the host. *Periodontol 2000* 2010;**52:**141–162.
49. Hajishengallis G. Complement and periodontitis. *Biochem Pharmacol* 2010;**15:** 1999–2001.