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Chemical sympathectomy inhibits periodontal disease in Fischer 344 rats

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Objective: The responsiveness of the sympathetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis plays a major role in immune regulation and for the outcome of infections and inflammatory disorders. This study was designed to investigate whether chemical SNS denervation with the noradrenaline-selective neurotoxic drug 6-hydroxydopamine (6-OHDA), which destroys peripheral noradrenaline terminals, would influence immune responses to Gram-negative bacterial lipopolysaccharide (LPS) stimulation, and the progression of ligature-induced periodontal disease in Fischer 344 rats.

Material and methods: 6-OHDA (40–60 μg/kg) or vehicle was injected intraperitoneally (i.p.) on days 1, 3 and 5, 10 days before application of the ligatures, and thereafter weekly in doses of 80 μg/kg. Periodontal disease was assessed when the ligatures had been in place for 49 days. At 24 and 2 h before decapitation, all rats received LPS (150 μg/kg i.p.) to induce a robust immune and HPA axis response.

Results: The 6-OHDA-treated rats showed significantly reduced bone loss as measured by digital X-rays ($p < 0.01$), and enhanced levels of the cytokines transforming growth factor-β ($p = 0.05$) and interleukin-6 ($p = 0.05$), as well as the HPA axis derived hormone corticosterone ($p = 0.01$), induced by LPS stimulation.

Conclusions: 6-OHDA-induced chemical sympathectomy inhibits ligature-induced periodontal disease in this model. This effect may be attributable to the well-documented ability of the SNS to regulate immune system function primarily via the adrenergic neurotransmitter noradrenaline released at sympathetic nerve terminals. The enhanced HPA axis activation may be a compensatory response that reduces the T helper (Th)2 to Th1 skewing effect of treatment with 6-OHDA.

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It is well established that the immune system and the brain communicate during immune and inflammatory responses to microorganisms or other antigenic challenges, and that this bi-directional communication plays a significant role to avert, eliminate and cope with dangerous pathogens (1–6). Invading micoorganisms activate the immune system, which in turn stimulates specific brain areas such as the paraventicular nucleus of the anterior hypothalamus. The hypothalamus again regulate immune system responses and the homeostasis of the body via the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS). Furthermore, several lines of research have established that the way by which this cross-talk is controlled by the brain plays an important role for the outcome of immune responses, health, and disease (7, 8). Dysregulation or inappropriate brain control of immune responses at any level may increase the susceptibility to infections and inflammatory disorders, including periodontal disease (9).

The HPA axis regulates immune and inflammatory responses by releasing glucocorticoid hormones, predominantly cortisol in humans and corticosterone in rodents (10). It is now well documented that misguided HPA axis over-responsiveness of miscellaneous

origin, leading to excessive amounts of circulating glucocorticoid hormones, can predispose to periodontal disease, whereas under-responsiveness is associated with resistance (9, 11–13).

The SNS communicates with the immune system primarily via the neurotransmitter noradrenaline released from sympathetic nerve endings (14, 15). In addition, the SNS can regulate immune responses systemically by releasing noradrenaline and adrenaline from the adrenal medulla. The SNS (as well as the HPA axis) are activated by cytokines that originate from immune cells recognizing pathogenic antigens such as lipopolysaccharide (LPS). Peripheral sympathetic nerves innervate tissues locally at sites of inflammation and regionally in immune organs such as the thymus, spleen and lymph nodes. Noradrenaline is released from sympathetic nerve terminals into these tissues in response to danger signals or stressful situations (15, 16). The binding of noradrenaline to specific adrenergic receptors on immune cells importantly modulates a number of immune and inflammatory responses (7, 8). Interaction of noradrenaline with immune and tissue cell receptors may affect immune cell trafficking, circulation and proliferation, as well as modulate cytokine production (7, 8). Through these mechanisms, SNS activation may cause a selective suppression of T helper (Th)1 responses, and a shift towards Th2 dominance, much in the same way as HPA axis activation (17).

One widely used method to study how SNS influences immune responses, disease development and progression, is peripheral chemical sympathectomy by means of the noradrenaline-selective neurotoxic drug 6-hydroxydopamine (6-OHDA) (7, 18). 6-OHDA does not cross the blood–brain barrier when given to adults. Systemically administrated, it selectively enters and destroys peripheral terminals of sympathetic noradrenergic nerve fibres (19). Thus, systemic SNS denervation by 6-OHDA is an effective technique and an ideal tool to examine the potential role of the peripheral SNS and noradrenaline in modulating immune responses, as well as disease development and progression. Since the SNS and the HPA axis interact, it is also important to evaluate 6-OHDA-induced changes on HPA axis responsiveness (20).

The present study was designed to investigate the effects of 6-OHDA-induced sympathectomy on the progression of ligature-induced periodontal disease, as well as cytokine and HPA axis responses to a Gram-negative bacterial LPS challenge in Fischer 344 rats. These rats have been found to be genetically HPA axis high responders, and they are highly susceptible to periodontal disease (11, 13). The rationale for this study is clinical observations and epidemiological evidence in humans suggesting that emotional stress, such as the loss of a spouse by death, and poorly developed coping strategies to manage such negative life events, may be important determinants of susceptibility to severe periodontal disease (9, 21). Psychological stressors are known to stimulate the HPA axis and the SNS, resulting in increased release of glucocorticoids and catecholamines (including noradrenaline and adrenaline), and both systems are highly involved in immune regulation by means of these neuromediators (8, 10).

Material and methods

Animals

Twenty male Fischer 344 rats, weighing 270–280 g, were obtained from Möllegaard Breeding Center (Ejby, Denmark), and used after 2 weeks of acclimatization. Standard rat chow pellets and tap water were available *ad libitum*. The animals were housed in groups of five under a 12/24 h light/dark cycle (light on from 7.00 AM to 7.00 PM) with temperature and humidity at 22°C and 40–60%, respectively. The experiments were registered and approved by the Norwegian Experimental Animal Board (NEAB).

Chemical sympatectomy

Denervation of peripheral sympathetic nerves was produced in 10 rats by intraperitoneal (i.p.) injections of the neurotoxin 6-hydroxydopamine (2,4,5-trihydroxyphenethylamine; 6-OHDA, Sigma, St. Louis, MO, USA) dissolved in 0.1% ascorbate and 0.9% saline solution (7). On day 1, 10 days before experimental ligature induction, 40 mg/kg body weight of 6-OHDA was injected i.p., followed by 60 mg/kg on days 3, and 5. Fourteen days after the first OHDA-injection, and 4 days after periodontal disease induction, 80 mg/kg of 6-OHDA was injected i.p., and thereafter the same dose was injected once a week for a further 7 weeks to prevent sympathetic nerve reinnervation (7). Ten control rats received equal volumes of the vehicle.

Experimental periodontal disease

Ten days after the first injection of 6-OHDA, all animals were anaesthetized by a subcutaneous injection in the neck with Hypnorm-Dormicum (fentanyl/fluanizone, midazolam), 0.2 ml/100 g body weight. A sterile silk ligature (Ethicon Perma-hand® size 3/0, Norderstedt, Germany) was tied around the neck of the maxillary right second molar tooth in the gingival sulcus. The ligatures served as a retention device for oral microorganisms. Forty-nine days after application of the ligatures, all animals were killed by decapitation. The maxillae were excised and fixed in 4% formaldehyde.

Lipopolysaccharide challenge

The peripheral blood monocytes of humans with periodontal disease release an altered profile of mediators when stimulated by LPS (22). LPS is a potent activator of the immune system and the HPA axis, which in turn regulates or modulates various immune cell activities (10). Therefore the animals were injected with LPS (*Escherichia coli* serotype 0111:B4, Sigma) i.p. (150 μg/kg; 100 μg/ml) 24 and 2 h before ending the experiment to assess whether the treatment regimen influenced on cytokine and corticosterone responses. After decapitation of the rats, the blood samples were collected (6–10 ml from each animal) in vacutainer tubes (10 ml without additives) and allowed to clot on ice for 1 h. Thereafter, the samples were

centrifuged for 20 min at 2000 *g*, and the serum samples were removed, aliquoted and stored at −20°C prior to analysis of cytokines and corticosterone.

Assay of serum tumour necrosis factor-α, interleukin-6, interleukin-10, and transforming growth factor-1β

The levels of the cytokines tumour necrosis factor-α (TNF-α), transforming growth factor-1β (TGF-1β), interleukin-6 (IL-6), and IL-10 in the serum samples were measured by means of enzyme-linked immunosorbent assays (ELISA) kits from R & D systems, Inc. (Minneapolis, MN, USA), with catalogue numbers RAT00 for TNF-α, MB100 for TGF-1β, R6000 for IL-6, and R1000 for IL-10. The minimum detectable concentration for TNF-α is less than 12.5 pg/ml, less than 31.2 pg/ml for IL-10 and TGF-1β, and less than 62,5 pg/ml for IL-6.

Assay of corticosterone

Corticosterone was measured with ^{125}I radioimmunoassay (RIA) coat-A-count kit from Diagnostic Products Corporation (Los Angeles, CA, USA), catalogue number TKRC1. The detection limit was 5.7 ng/ml.

Radiographic examination

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 mesial surfaces of the second molars were displayed digitally. The examiner was unaware whether the specimens came from experimental or control animals. Each X-ray was read three times, and the mean of the three readings calculated. The reliability of the method has been tested earlier (23). The average percentage difference between individual readings and the mean of the respective triplicate was 3.48 ± 5.12%.

Statistics

Because some of the data were not normally distributed, the non-parametric Mann–Whitney test was used for between-group comparisons, and the non-parametric Spearman rank order test to seek correlations between parameters. In the text, data are expressed as means ± SD throughout.

Results

The treatment impeded the weight gain of the animals. The 6-OHDA-treated and control animals weighed 282.6 ± 8.1 g and 285.7 ± 6.3 g ($p = 0.25$), respectively, at 6-OHDA induction, and 286.9 ± 10.5 g and 374.2 ± 10.6 g ($p < 0.01$), respectively, at the termination of the experiment, 59 days later, and 49 days after ligature induction (Table 1).

Radiographically, the mean bone loss measured as the distance between the cemento-enamel junction and the most coronal bone was 0.93 ± 0.18 mm in the 6-OHDA-treated rats. In the control rats, the same distance was 1.18 ± 0.30 mm (Table 1). The bone loss in the treatment group was significantly reduced compared to that seen in the vehicle-treated controls ($p < 0.01$). As the 6-OHDA-treated rats showed reduced weight, the length of their teeth could be shorter. We therefore compared the root-length of the right second molar teeth in the two groups by measuring the distance between the cemento-enamel junction and the apex on mesial root surfaces. There was no difference between the root-length in the 6-OHDA-treated and saline-treated control rats (1.97 ± 0.09 mm in 6-OHDA-treated vs. 1.96 ± 0.11 mm in the controls; $p = 0.93$).

The 6-OHDA-treated rats had significantly higher TGF-1β (31.8 ± 6.7 pg/ml) serum levels compared to controls (26.2 ± 5.7 pg/ml; $p = 0.05$). Also, the serum levels of IL-6 were significant higher in the 6-OHDA-treated rats (2329.6 ± 1916.1 pg/ml) compared to controls (819.7 ± 1168.9 pg/ml; $p = 0.05$). In addition, the 6-OHDA-treated rats showed a tendency to higher IL-10 serum levels (275.6 ± 256.0 pg/ml in 6-OHDA-treated rats vs. 152.6 ± 140.7 pg/ml in controls; $p = 0.11$), as well as higher TNF-α serum levels (542.8 ± 601.5 pg/ml) compared to controls (274.1 ± 408.5 pg/ml; $p = 0.18$).

Treatment with 6-OHDA significantly enhanced the HPA axis response as measured by plasma corticosterone to the LPS challenge (6-OHDA-treated rats 1758.4 ± 343.0 nm/l; controls 1384.2 ± 245.5 nm/l; $p = 0.01$; Table 1).

Table 1.

	Treatment		
	6-OHDA ($n = 10$)	Saline ($n = 10$)	Mann–Whitney *p*-value
Weight at 6-OHDA induction (g)	282.6 ± 8.1	285.7 ± 6.3	0.25
Weight at ligature placement (g)	262.6 ± 9.9	315.1 ± 9.4	< 0.01
Weight at death (g)	286.9 ± 10.5	374.2 ± 10.6	< 0.01
Bone loss, X-ray (mm)	0.93 ± 0.18	1.18 ± 0.30	< 0.01
Root-length, X-ray (mm)	1.97 ± 0.09	1.96 ± 0.11	0.93
TGF-1β (pg/ml after i.p. LPS at death)	31.8 ± 6.7	26.2 ± 5.7	0.05
IL-6 (pg/ml after i.p. LPS at death)	2329.6 ± 1916.1	819.7 ± 1168.9	0.05
IL-10 (pg/ml after i.p. LPS at death)	275.6 ± 256.0	152.6 ± 140.7	0.11
TNF-α (pg/ml after i.p. LPS at death)	542.8 ± 601.5	274.1 ± 408.5	0.18
Corticosterone (nm/l after i.p. LPS at death)	1758.4 ± 343.0	1384.2 ± 245.6	0.01

All data are shown as means ± SD.
IL-6, interleukin-6; IL-10, interleukin-10; i.p., intraperitoneally; LPS, lipopolysaccharide; 6-OHDA, 6-hydroxydopamine; TGF-1β, transforming growth factor-1β; TNF-α, tumour necrosis factor-α.

Discussion

The present study shows that selective destruction of peripheral sympathetic nerve terminals with 6-OHDA strengthened the resistance to ligature-induced periodontal disease in periodontal disease susceptible Fischer 344 rats. The treatment also boosted immune and HPA axis responses to a robust LPS challenge. The results support other studies showing that 6-OHDA-induced chemical sympathectomy alters immune and HPA axis responses to antigenic challenges, and influences the clinical course of infections and inflammatory disorders (15, 20, 24). These data, suggesting that denervation of the SNS inhibits periodontal disease, are, to our knowledge, the first report demonstrating that the SNS is involved in the pathogenesis of periodontal disease.

It is well established that noradrenaline is released from nerve terminals within the microenvironment of immune cells after SNS activation (15, 16). Studies have also verified synaptic-like contacts between sympathetic nerve fibres and T-lymphocytes and macrophages in lymphoid organs. In addition, immune cells possess specific noradrenaline receptor mechanisms to assist in modulating or fine tuning immune responses. For example, Th1 cells (but not Th2 cells) and macrophages express noradrenaline binding β2-adrenergic receptors (25). Systemic noradrenaline (and adrenaline) inhibit the production of the cytokines IL-12, TNF-α and interferon-γ. Collectively, germane studies show that SNS activation and noradrenaline inhibit Th1 responses, while stimulating Th2 responses (8, 15, 17). Noradrenaline also inhibits LPS-induced TNF-α and IL-6 production in human whole blood (26). Furthermore, it alters chemokine and nitric oxide production by macrophages (17, 24, 27, 28). In this way SNS activation by immune cells or emotional and physical stress shifts the Th1/Th2 balance towards Th2 dominating responses, much in the same manner as does HPA axis activation (17). Noradrenaline can also be a powerful chemoattractant for monocytes (29).

Experimental studies have also demonstrated that chemical sympathectomy with 6-OHDA can alter immune responses to antigenic challenges. For example, treatment with 6-OHDA augmented mitogen-induced T-cell proliferation, macrophage phagocytosis (30), and the production of IL-2 in splenocytes stimulated with concavalin A (31). Furthermore, chemical sympathectomy with 6-OHDA has been found to enhance the TNF-α contents in the lungs of rats exposed to haemorrhagic shock (32). In immune cells of mice infected with the intracellular pathogen *Listeria monocytogenes* the production of TNF-α, interferon-γ and IL-12 was up-regulated (24). Chronic treatment with 6-OHDA may also alter the susceptibility to infections and inflammatory disorders. For example, acute cold/restraint stress, which stimulates both the SNS and the HPA axis, has been found to increase the susceptibility to infection with *Listeria monocytogenes*. Administration of 6-OHDA inhibited this effect in a mouse model (20). On the other hand, chemical sympathectomy with 6-OHDA has been shown to aggravate experimental allergic encephatomyelitis in rats (33). This Th1-mediated autoimmune disease mimics multiple sclerosis in humans.

Experiments with the noradrenaline reuptake inhibitor and antidepressant, desipramine, have demonstrated that 6-OHDA owes its effects on immune responses and inflammatory disorders to reduced noradrenaline stimulation (15, 20). By blocking the uptake of 6-OHDA into nerve terminals, desipramine prevents sympathetic nerve destruction. The cytokine enhancing and periodontal disease inhibitory effect of the 6-OHDA treatment, as found in the present study, may therefore be a result of reduced noradrenaline stimulation.

However, although loss of SNS and reduced release of noradrenaline may be responsible for many of the changes relating to immune function, recent studies have shown that 6-OHDA treatment may also increase the reactivity of the HPA axis (34). This property is supported by the present study, as illustrated by significantly elevated blood corticosterone levels after the LPS challenge. Thus, 6-OHDA-induced sympathectomy may modulate immunity not only due to the absence of sympathetic neuromediators, but even by activating the HPA axis and increasing the release of glucocorticoid hormones.

HPA axis activation and the attending stimulation of glucocorticoid secretion, are known to down-regulate Th1-mediated immunity and bias immune responses towards Th2 and T-regulatory responses (35, 36), partly evoked by down-regulating IL-12 and increasing IL-10 release from antigen-presenting cells (36, 37). The increased activation of the HPA axis in response to LPS in the 6-OHDA-treated rats may thus reduce the propensity to shift the T-helper lymphocyte activity towards the Th1 direction. However, the impact of the HPA axis activation apparently does not exceed the effects of 6-OHDA on the SNS. In line with this, we have previously shown that enhanced HPA axis responsiveness is associated with increased susceptibility to periodontal disease, whereas decreased responsiveness strengthens the resistance (9). It is therefore possible that the increased HPA axis responsiveness in the 6-OHDA-treated rats may be a compensatory response that reduces the shift from Th2 to Th1, while leaving the effects of SNS denervation nonetheless dominating. The effects of 6-OHDA on the SNS may thus solely be responsible for the heightened resistance to periodontal disease as found in this study. The increased activation of the HPA axis may reduce this effect, but only to such a degree that the SNS denervation remains dominating. This suggestion is supported by a recent study in mice showing that 6-OHDA-induced sympathectomy influences immune function primarily by activating β2-adrenergic receptors, and that activation of glucocorticoid receptors conceivably plays a minor role (20).

In conclusion, we have described a potent inhibiting effect of 6-OHDA-induced peripheral sympathectomy on periodontal breakdown. We have thus identified a novel mechanism that may

be involved in immune and inflammatory processes controlling the growth of pathogens (periodontopathogenes) in subgingival plaques, as well as the subsequent tissue destructive immune and inflammatory responses to the over-growth of these pathogens. This is supported by a recent study showing that the growth of pathogenic bacteria is significantly reduced in 6-OHDA-treated mice (24). These findings are in agreement with several other studies showing that the brain and immune systems are functionally linked by SNS and HPA axis regulation, and that these two systems are highly involved in immune regulation and control of pathogens. Thus, these mechanisms may be major contributors to the immunodeficiency associated with the increased growth of periodontopathogens in patients exposed to conditions associated with SNS and HPA axis over-responsiveness. Interestingly, all the known periodontal disease risk factors, including smoking, poorly controlled diabetes, and traumatic life experiences, such as the loss of a loved one by death, and poorly developed psychological coping strategies to manage such traumatic life events, are associated with over-responsiveness of these stress response systems (9, 21). A better understanding of the mechanisms by which the brain regulates the neuroendocrine system, including HPA axis and SNS activation by Gram-negative bacteria and other threatening stimuli or situations, may likely improve the treatment of periodontal disease. The results may also suggest that, apart from dental plaque control, stress-protection may represent a novel approach, which may be capable of alter immune-to-brain-to-immune regulatory pathways, and thereby modulate the predisposition to the disease.

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References

1. Blalock JE. The syntax of immune-neuroendocrine communication. *Immunol Today* 1994;**15:**504–511.
2. Ader R, Cohen N, Felten D. Psychoneuroimmunology: Interactions between the nervous system and the immune system. *Lancet* 1995;**345:**99–103.
3. Maier SF, Watkins L. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev* 1998;**1:**83–107.
4. Straub RH, Westerman J, Schoelmerich J, Falk W. Dialog between the CNS and the immune system in lymphoid organs. *Immunol Today* 1998;**166:**232–413.
5. Danzer R, Konsman JP, Bluthe RM, Kelly KW. Neural and hormonal pathways of communication from the immune system to the brain: parallel or convergent? *Auton Neurosci* 2000;**85:**60–65.
6. Hosoi T, Okuma Y, Nomura Y. The mechanisms of immune-to-brain communication in inflammation as a drug target. *Curr Drug Targets Inflamm Allergy* 2002;**1:**257–262.
7. Lorton D, Lubahn C, Klein N, Schaller J, Bellinger DL. Dual role for noradrenergic innervation of lymphoid tissue and arthritic joints in adjuvant-induced arthritis. *Brain Behav Immun* 1999;**85:**60–65.
8. Eskandari F, Sternberg EM. Neural immune interaction in health and disease. *Ann New York Acad Sci* 2002;**966:**20–27.
9. Breivik T, Thrane PS. Psychoneuroimmune interactions in periodontal disease. In: Ader R, Felten DL, Cohen N, eds. *Psychoneuroimmunology*, 3rd edn. San Diego, CA: Academic Press, 2001: 627–644.
10. Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;**332:**1351–1361.
11. 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.
12. 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.
13. 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.
14. Sanders VM, Kasprowicz DJ, Kohm AP *et al*. Neurotransmitter receptors in lymphocytes and other lympoid cells. In: Ader R, Felten D, Choen N, eds. *Psychoneuroimmunology*, 3rd edn. San Diego, CA: Academic Press, 2001, 161–196.
15. Madden KS. Catecholamines, sympathetic nerves, and immunity. In: Ader, R, Felten, D, Choen, N, eds. *Psychoneuroimmunology*, 3rd edn. San Diego, CA: Academic Press, 2001, 161–196.
16. Felten DL. Neural influence on immune responses: underlying suppositions and basic principles of neural-immune signalling. *Prog Brain Res* 2002;**122:**381–389.
17. Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol Metab* 1999;**10:**359–368.
18. Piclo MJ. Methods of sympathetic denervation and alteration. *J Auton Nerv Syst* 1997;**62:**11–125.
19. Kostrzewa RM, Jacobowictz DM. Pharmacological actions of 6-hydroxydopamine. *Pharmacol Rev* 1974;**26:**199–288.
20. Cao L, Filipov NM, Lawrence DA. Sympathetic nervous system plays a major role in acute cold/restraint stress inhibition of host resistance to *Listeria monocytogenes*. *J Neuroimmunol* 2002;**125:**94–102.
21. Hugoson A, Ljungquist B, Breivik T. The relationship of some negative events and psychological factors to periodontal disease in an adult Swedish population 50–80 years of age. *J Clin Periodont* 2002;**29:**247–253.
22. Fokkema SJ, Loos BG, Slegte C, van der Velden U. A type 2 response in lipopolysaccharide (LPS)-stimulated whole blood cell cultures from periodontitis patients. *Clin Exp Immunol* 2002;**127:**374–378.
23. Breivik T, Rook GA. Prevaccination with SRL172 (heat-killed Mycobacterium vaccae) inhibits experimental periodontal disease in Wistar rats. *Clin Exp Immunol* 2000;**120:**463–467.
24. Miura T, Kudo T, Matisuki A *et al*. Effects of 6-dydroxydopamine on host resistance against Listeria monocytogenes infection. *Infect Immunol* 2001;**67:**7234–7241.
25. Sanders VM, Baker RA, Ramer-Quinn DS, Kasprowicz DJ, Fuchs BA, Street NE. Differential expression of the beta-2-drenergic receptor by Th1 and Th2 clones: implication for cytokine production and B cell help. *J Immunol* 1997;**158:**4200–4210.
26. Van der Poll TJ, Jansen E, Endert HP, Sauerwein HP, van Deventer SJH. Noradrenaline inhibits lipopolysaccharide-induced tumor necrosis factor and interleukin 6 production in human whole blood. *Infect Immun* 1994;**62:**2946–2050.
27. Bloomershine CS, Lafuce WP, Zwilling BC. Beta 2-adrenergic receptor stimulation inhibits nitric oxide generation by mycobacterium ovium infected macrophages. *J Neuroimmunol* 1999;**101:**68–75.

28. Szelenyi J, Kiss JP, Vizi ES. Differential involvement of sympathetic nervous system and immune system in the modulation of TNF-alpha production by alpha (2) - and beta adrenoreceptors in mice. *J Neuroimmunol* 2000;**103:**34–40.
29. Straub RH, Mayer M, Kreutz M, Leeb S, Schoelmerich J, Falk W. Neurotransmitters of the sympathetic nerve terminal are powerful chemoattractants for monocytes. *J Leukocyte Biol* 2000;**67:** 553–558.
30. Lyte M, Ernst S, Driemeyer J, Aissa B. Strain-specific enhancement of splenic T cell mitogenesis and macrophage phagocytosis following peripheral axotomy. *J Neuroimmunol* 1991;**31:**1–8.
31. Madden KS, Stevens SY, Felten DL, Bellinger DL. Alteration in T lymphocyte activity following chemical sympathectomy in young and old Fischer 344 rats. *J Neuroimmunol* 2000;**103:**131–145.
32. Molina PE. Noradrenergic inhibition of TNF upregulation in hemorrhagic shock. *Neuroimmunomodulation* 2001;**9:**125–133.
33. Chelmicka-Schorr E, Kwasniewski MN, Wollmann RL. Sympathectomy augments adoptively transferred experimental allergic encephalomyelitis. *J Neuroimmunol* 1992;**37:**99–103.
34. Leo NA, Bonneau RH. Mechanisms underlying chemical symphathectomy-induced suppression of herpes simplex virus-specific cytotoxic T lymphocyte activation and function. *J Neuroimmunol* 2000;**110:**45–56.
35. Rook GA. Glucocorticoids and immune function. *Baillieres Best Pract Res Clin Endocrinol Metab* 1999;**13:**567–581.
36. Vieira PL, Kalinski P, Wierenga EA, Kapsenberg ML, de Jong EC. Glucocorticoids inhibit bioactive IL-12p70 production by in vitro-generated human dendritic cells without affecting their T cell stimulatory potential. *J Immunol* 1998;**161:**5245–5251.
37. Visser J, van Boxel-Dezaire A, Methorst D, Brunt T, de Kloet ER, Nagelkerken L. Differential regulation of interleukin-10 (IL-10) and IL-12 by glucocorticoids in vitro. *Blood* 1998;**91:**4255–4264.