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# A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation

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***Abstract***

**Objective:** To establish and characterise a rat model of periodontitis that reiterates the features of human disease.

**Methods:** Periodontal inflammation was induced by a single injection of 10 $\mu$g liposaccharide (LPS) (*Salmonella typhimurium*) in 1 $\mu$l saline into rat mandibular gingiva at the buccomesial aspect of the second molar. Animals were killed after 3, 7 and 10 days, mandibles dissected and sectioned for histological and immunocytochemical analysis.

**Results:** LPS injection resulted in a significant gingival and periodontal inflammation with inflammatory infiltrate, apical migration of the junctional epithelium, interdental bone loss, and activation of osteoclasts at the site of injection 7 and 10 days after injection. At 10 days post injection, there was a significant trend for bone loss on both sides of the mandible. Periodontal inflammation was associated with alteration in the levels of calcitonin gene-related peptide-like immunoreactivity in nerve terminals innervating the inflamed gingival papilla.

**Conclusion:** Intragingival injection of LPS in the rat provides an easily induced reproducible experimental model of periodontal inflammation that reiterates features of human disease.

Key words: bone resorption; calcitonin gene-related peptide; inflammation; lipopolysaccharide; osteoclast activation; periodontitis

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Chronic adult periodontitis is a symmetrical disease characterised by gingival and periodontal inflammation, gingival recession, apical migration of the junctional epithelium with pocket formation, and alveolar bone loss (Listgarten 1986). Human longitudinal studies of periodontal diseases pose many problems in the study of the mechanisms of disease as there are many interpatient variables that are difficult to control, such as the level of disease activity, individual disease susceptibility and time of disease progression. Different animal models of periodontitis have therefore been used to evaluate both the pathogenesis of periodontal diseases and various periodontal treatment modalities (Klausen 1991). The aim of this study was to define a simple animal model of experimental periodontal disease that has features similar to those seen in human periodontal disease, which could be used to study mechanisms of disease progression. As features of periodontal disease in humans and animals may vary greatly depending upon the form of the disease and/or the stage of disease development, a further aim was to characterise the optimum time at which this model could be used to study these features.

Periodontal disease can be induced in rats by dietary manipulation (Robinson et al. 1991), introduction of pathogenic microorganisms (Jordan et al. 1972, Fiehn et al. 1992), placement of a

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ligature that acts as a site for bacterial colonisation (Klausen 1991), or by injection of bacterial toxins (Klausen 1991, Llavaneras et al. 1999). Many of these models of experimental periodontal disease have not been fully characterised in terms of their time course of inflammation, gross or histological features.

Lipopolysaccharide (LPS) is a cell wall constituent of virtually all subgingival Gram-negative organisms (Garrison & Nichols 1989). These molecules are known to induce polymorphonuclear leukocyte infiltration, oedema and vascular dilatation in inflamed periodontal tissues (Page 1991). Hard tissue may be affected by LPS through production of inflammatory mediators such as interleukins-1, 6 and 8, tumour necrosis factor (Lindemannet al. 1988, Agarwal et al. 1995) or prostaglandins (Garrison et al. 1988, Garrison & Nichols 1989), and osteoclast activation subsequent to osteoblast stimulation. It has also been postulated that LPS may activate osteoclasts through a direct effect of LPS on circulating leukocytes, with no involvement of osteoblasts (Jiang et al. 2002).

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide that is principally found in sensory neurons. CGRP is a potent vasodilator (Brain et al. 1985) and as such, when released from sensory neurons, can contribute to local inflammatory effects. Neuropeptide containing sensory nerve terminals have been located in the gingivae of many species including rat, cats and humans (Heyeraas et al. 1993, Jacobsen et al. 1998) and bone is innervated by CGRP-containing sensory neurons (Bjurholm et al. 1988). Study of the sensory innervation of inflamed gingivae from patients with gingival or periodontal inflammation using immunocytochemistry for neuropeptides has generated conflicting results. In patients with phenytoin-induced gingival hyperplasia or periodontitis, innervation density was reduced (Luthman, et al. 1988a, b, 1989), whereas in inflamed gingivae from patients with Downs syndrome, there was an increased peptidergic innervation density (Barr-Agholme, et al., 1991). These discrepancies may be due to the difficulties in comparing data from patients with different disease profiles and time courses. Innervation of inflamed gingivae by CGRP-containing nerve terminals has not been studied in a controlled situation such as a model of experimental periodontal disease.

In this study a previously described experimental model of periodontitis in the rat has been modified and fully characterised. A simple method of induction of periodontal disease is described, and the time course of inflammation and bone loss, potential symmetry of disease and gingival CGRP-like immunoreactivity (CGRP-LI) have been determined, in order to characterise this model for use in studies of gingival and periodontal inflammation.

## Material and Methods

### Animal preparation – induction of periodontal inflammation

All animal experiments were done in accordance with UK legislation (Animals (Scientific Procedures) Act 1986). Periodontitis was induced by the method of Llavaneras et al. (1999) with important modifications. Male Wistar rats (225–250 g) were anaesthetised with a mixture of fentanyl citrate (0.1 mg/kg) and fluanisone (3 mg/kg) (Hypnorm®; Janssen Beerse 0.3 mg/kg) (Jansen Pharmaceutica, Beerse, Belgium) administered intramuscularly, followed by 2.5 mg/kg diazepam administered intraperitoneally. Periodontitis was induced by intragingival injection of 1 $\mu$l of LPS (10 $\mu$g/$\mu$l) derived from *Salmonella typhimurium* (Sigma, Poole, UK) in sterile saline. A fine hypodermic needle was inserted at the mesiolateral aspect of the first right mandibular molar and the tip moved distally so that the injection was made at the interdental papilla between the first and second molars. The total volume of injection was reduced from 10 $\mu$l as originally described to 1 $\mu$l, as in initial experiments, the tightly bound tissue of the gingivae would not expand to accommodate an injection volume of 10 $\mu$l and much of the injected volume was lost along the needle track. The injection was made slowly and the needle held in place for several seconds post injection to ensure that the LPS was not lost through the needle track.

Fifteen animals were injected with LPS. Five animals were untreated to act as completely uninflamed control animals for comparison. Animals were weighed at regular intervals as an index of food intake/masticatory behaviour. Animals were killed at 3, 7 and 10 days post injection ($n = 5$ per group) by decapitation under halothane-induced anaesthesia. Control animals were killed in the same manner.

Mandibles were dissected from all animals, soft tissues were removed and the mandibles separated at the mandibular symphysis. Each half of the mandible was immersed directly in Zamboni's fixative for 3 days and then decalcified in a 10% EDTA/7.5% polyvinylpyrolidone solution for 2 weeks at 4°C. EDTA solution was changed 3 times per week. The mandibles were cryoprotected in 30% sucrose solution overnight, embedded in OCT and sagittal sections of 20–30 $\mu$m were cut on a cryostat. Sections were mounted on either gelatine coated, or Superfrost Plus microscope slides (BDH, Poole, UK) and stored at –20°C until used in histological, histochemical or immunocytochemical procedures.

For image analysis, images were captured on an image analysis system (colour still or video camera attached to a Nikon E600 Eclipse) (Nikon UK Ltd, Kingston upon Thames, UK). All analysis was performed on a computer using the public domain program Image J (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

### Histological staining and calculation of alveolar bone loss

Sections from control and inflamed animals were stained with Masson's trichrome (to identify changes in collagen) and Giemsa stain (for inflammatory infiltrate).

Alveolar bone loss in the interseptal area between the first and second mandibular molars was measured on stained sections in accordance with previously published methods (Guggenheim & Schroeder 1974, Amstad-Jossi & Schroeder 1978). On each side of the mandible, the distance between the level of alveolar bone crest and the amelocemental junction was measured on three random sections at the mesial side of the mandibular second molar (Fig. 1A), and the mean amelocemental junction to alveolar crest height (ACJ-AC) was calculated. Mean ACJ-AC height was then calculated for each group of animals. The degree of alveolar bone loss induced by LPS injection was assumed to be the difference of this measurement in the LPS-injected animals compared to controls.

### Histochemical staining for tartrate-resistant acid phosphatase (TRACP)

TRACP histochemistry was used to identify active osteoclasts in sections

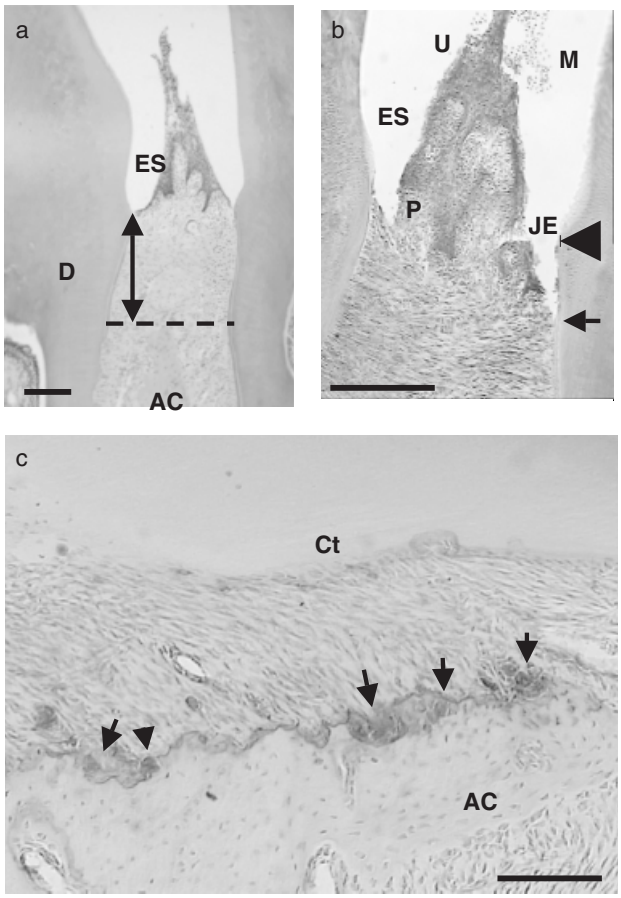


*Fig. 1.* (a) Schematic diagram showing measurement of ACJ-AC distance on a decalcified, haematoxylin and eosin stained section of normal periodontium. Dotted line shows the top of the alveolar crest and the ACJ-AC distance is the vertical distance from this to the amelocemental junction (double headed arrow). ES = enamel space; D = dentine; AC = alveolar crest. (b) Histological appearance of the interdental papilla 7 days after LPS injection showing apical migration of the junctional epithelium (JE) from the amelocemental junction (arrow head) to the root surface (arrow). Other features include pocket formation (P), ulceration of the epithelium (U) and monocytic extravasation into the gingival crevice (M). (c) Histological appearance of active osteoclasts (arrows) on the mesial surface of the interdental bone (AC) stained for tartarate-resistant acid phosphatase. The root of the first molar (Ct) is at the top of the photomicrograph and the oral cavity at the right. Scale bars = 100 $\mu$m (A, B), 50 $\mu$m (C).

of mandible to assess the degree of active bone resorption. TRACP staining was done according to the method described by Katayama et al. (1972). Active osteoclasts were defined as multinucleated TRACP-positive cells in contact with the bone surface. The number of active osteoclasts on the mesial and distal surfaces of the interdental alveolar bone between the first and second mandibular molar were counted. The mesial surface is that adjacent to the first molar and the distal that adjacent to the second molar. The mean number of active osteoclasts per mm bone surface on the mesial, distal and the total surfaces of the interdental septum was calculated for each group of animals.

#### CGRP immunocytochemistry

CGRP immunocytochemistry was performed according to the protocol of Lawson et al. (1996). Sections of mandibles were brought to room temperature and washed in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 several times. Endogenous peroxide was quenched in 2% hydrogen peroxide in PBS and sections blocked in 10% normal goat serum in PBS. The sections were then incubated overnight with rabbit polyclonal anti-CGRP antibody (1:2000; Peninsula Labs, St Helen's, UK) at 4°C. Sections were incubated with an anti-rabbit secondary antibody conjugated with horseradish peroxidase (1:200) for 30 min at room temperature. Detection of secondary antibody was performed using the tyramide amplification kit (NEN, Beaconsfield, UK), the ABC detection kit (Vector Labs, Peterborough, UK) and 3′,3-diaminobenzidine (Sigma) according to the manufacturer's instructions.

CGRP staining intensity was scored on a scale of 1–4. Scores were based on the intensity of staining and the number of nerve terminals innervating the papilla with 1 being low intensity and few terminals, 2 representing moderate intensity and number of fibres, 3 indicating high intensity and many fibres, while 4 signifying very high staining intensity with numerous terminals.

#### Statistical analysis

All results are expressed as mean $\pm$ SEM except for scoring of CGRP-LI intensity, which is expressed as median $\pm$ interquartile range. Data were analysed by one way ANOVA followed by post-hoc Bonferroni or tests for linear trend (animal weights, bone loss, osteoclast number), or by Kruskal–Wallis non-parametric analysis of variance followed by Dunn's post-hoc test (CGRP-LI intensity). The Null hypothesis was rejected at $P<0.05$.

## Results

Animals showed no obvious signs of systemic illness throughout the period of the study. All animals gained in weight over the 10 days of the study, although the weight gain in LPS injected animals was significantly less than controls at 3 days. By 7 days, however, weights of LPS-injected animals had regained control levels (not shown).

#### Histological appearance

Compared to control animals, all LPS-injected animals showed at least one of the following signs in the interdental area between the mandibular first and second rat molars: crater-like breakdown of the interdental papilla, disruption of transeptal collagen fibres or disruption and apical migration of the junctional epithelium (Fig. 1b). Giemsa staining revealed inflammatory cell infiltrate in epithelial and subepithelial connective tissues in all LPS-injected animals (Fig. 1b).

#### Alveolar bone level

There was no difference between ACJ-AC distance on the left and right sides of control rats, so these results were pooled as a control group for data analysis and presentation. On both sides of the mandible, there was a significant trend for ACJ-AC to increase with time in LPS-injected animals ($P<0.001$; Fig. 2a, b). When compared to uninjected controls, there was no change in ACJ-AC distance on either side of the mandible 3 days after LPS injection. At 7 and 10 days, the ACJ-AC distance was significantly greater in LPS-injected rats than controls ($P<0.01$; Fig. 2a).

#### Osteoclast activation

Active osteoclasts stained with TRACP were particularly noticeable on the mesial surface of the interseptal bone in LPS-injected animals (Fig. 1c). Active osteoclast numbers were calculated for the mesial (adjacent to first molar) and distal (adjacent to second molar) surfaces of the interdental septum. There was a greater number of active osteoclasts on the mesial surface of the interdental septum compared to the distal surface in all animals (Fig. 2e, f), and these differences were highly significant in all animals ($P<0.0002$). Following LPS injection, the numbers of active osteoclasts on the distal surface of the septum did not change significantly, whereas the numbers of active osteoclasts on the mesial surface of the septum increased at all time points and interestingly on both right (Fig. 2e, injected) and left (Fig. 2f, uninjected) sides of the mandible. On the injected side of the mandible, the numbers of mesial active osteoclasts (corrected for the length of bone surface) was significantly higher than controls at 10 days ($P=0.004$; Fig. 2e). At the site of LPS injection, the difference in numbers of mesial and distal osteoclasts was significantly increased at 10 days ($P=0.004$). On the left, uninjected side of the mandible, the mesial/distal difference in active osteoclast number also showed a tendency to increase at 7 days, but was at no time significantly different from control animals (Fig. 2f).

There was a highly significant positive relationship between the numbers of active osteoclasts either on the surface of bone ($r^2=0.27$; $P=0.0009$), or within the bone of the interdental septum ($r^2=0.33$, $P=0.0002$), and the ACJ-AC distance when data from control and experimental animals were pooled. Interestingly, there was also a significant positive correlation between the number of active osteoclasts and the length of resorbing bone surface at LPS-injected sites ($r^2=0.14$, $P=0.02$) and at contralateral non-LPS-injected sites from experimental animals ($r^2=0.26$, $P=0.0007$; Fig. 2c, d). Comparison of the linear regression lines for the controls and experimental animals showed a significant difference (shift of the

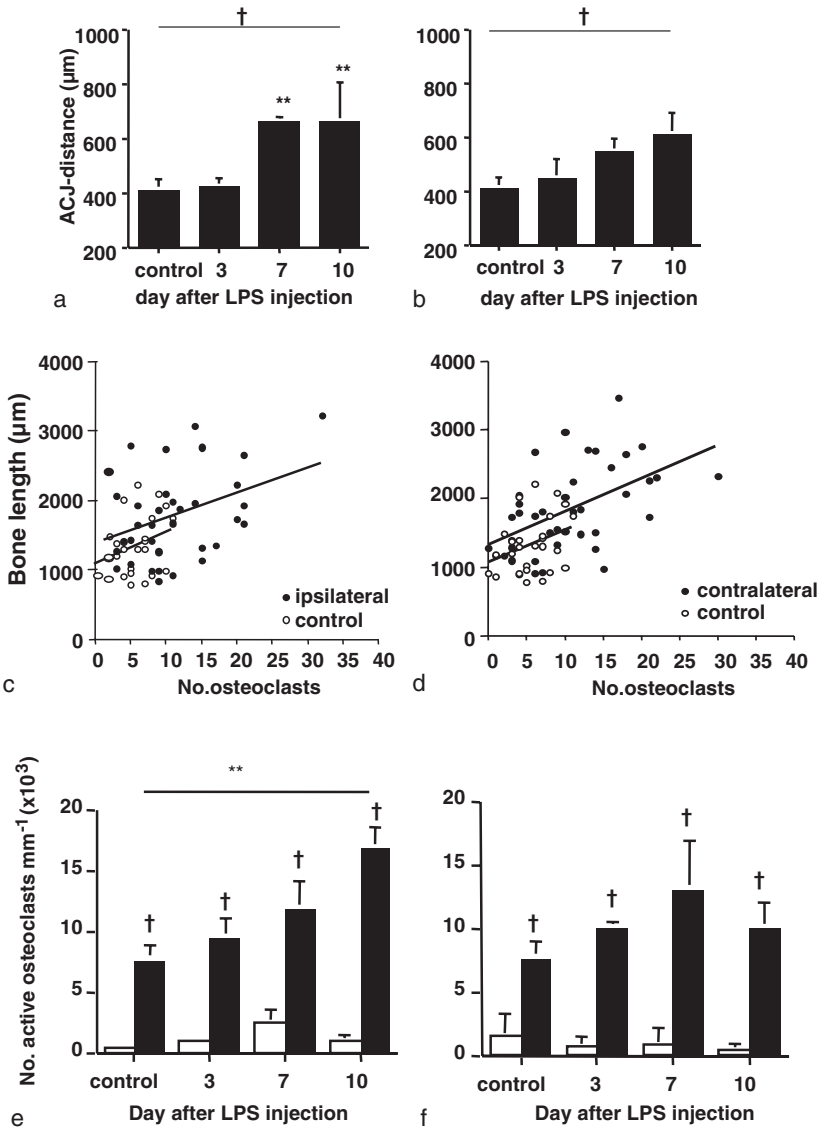


*Fig. 2.* Bone loss and osteoclast activation in control and LPS-injected rats (a & b). There was a significant increase in ACJ-AC distance at the interdental septum between the first and second molars in LPS-injected animals on the side of injection (a) at 7 and 10 days. The contralateral mirror image site (b) also showed a significant trend for increased ACJ-AC distance over the time course of the study. ($^{**}P<0.01$ compared to control; $^{\dagger}P<0.001$, linear test for trend) (c & d). There was a significant correlation between active osteoclast number and length of resorbing bone surface at both LPS injected (c) and contralateral sites (d). Comparison of the linear regression lines for LPS injected and control animals on both sides of the mandible showed a significant ($P<0.001$) upward shift following LPS injection, indicating increased osteoclastic activity following LPS injection (e & f). Active osteoclast numbers were significantly increased on the mesial surface of the alveolar bone both at the site of LPS injection (e) and contralaterally (f) 10 days after injection. Active osteoclasts were found in significantly higher numbers on the mesial surface (filled bars) of the interdental septum compared with the distal surface (open bars). ($^{**}P<0.01$, $^{\dagger}P<0.001$). This mesial:distal difference became more pronounced after LPS injection.

regression line upwards, $P<0.0001$) indicated by a significant increase in $y$ intercept. This shows that for a given number of osteoclasts the length of bone surface is significantly greater after LPS injection than in controls, either ipsilateral or contralateral to the LPS injection (Fig. 2c, d).

#### CGRP-like Immunoreactivity (CGRP-LI)

CGRP-LI was evident in control interdental papilla with an appearance similar to that reported by Kimberly and Byers (Kimberly & Byers 1988), suggesting that immunoreactivity was in nerve terminals innervating this area (Fig. 3c, d). There was also an increase in the background staining in sections from LPS-injected animals that was confined to the gingival papilla. This appearance has been previously attributed to increased local CGRP release (Kimberly & Byers 1988). Relative staining intensity was assessed on a ranked scale with sections from each animal being scored on a scale of 1–4. Three days after LPS injection there was a tendency for a reduction in the score based on the intensity of immunostaining and the number of nerve terminals innervating the interdental papilla (Fig. 3a). Staining intensity was highly variable between animals, as evidenced by the range of the data (Fig. 3). Seven days after LPS injection, CGRP-LI staining intensity was significantly increased on both the side of injection, and at the contralateral site ($P<0.05$; Fig. 3a, b), while 10 days post LPS injection the staining intensity was still raised but was not significantly different from controls. Thus CGRP-LI was initially decreased, then increased at 7 days and was returning to control levels by 10 days after LPS injection.

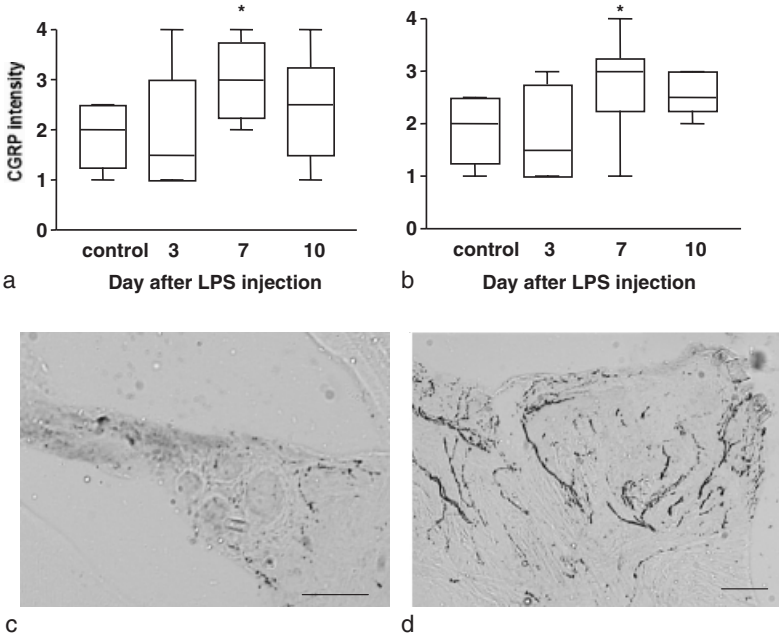


*Fig. 3.* Calcitonin gene-related peptide immunoreactivity staining intensity in LPS-injected rats Box and whisker plots (showing medians, interquartile range (box) and high/low values (whiskers)) of CGRP staining intensity in control and LPS-injected rats ipsilateral (a) and contralateral (b) to the LPS injection ($^*P<0.05$). Panels (c) and (d) show CGRP-LI in sections from control (c) and LPS-injected rats ((d); 7 days ipsilateral to the injection). Scale bar = 10 $\mu$m.

## Discussion

#### Establishment and characterisation of a model of periodontitis in the rat

In this study, we have described a modification of a model of experimental periodontal disease in which we have characterised the time course of inflammation and bone loss. We modified the model of LPS-induced periodontitis described by Llavaneras et al. (1999) for several reasons. Firstly, there was no indication in the latter study of the time at which bone loss and inflammation was maximal and therefore the best time at which to study possible therapies that might affect disease progression. Secondly, in our hands, injection of 10 $\mu$l of LPS solution directly into the interdental papilla was impossible as most was lost through the needle track. We have attempted to define a model in which the injection of LPS is easier and the amount injected, and therefore the inflammation induced, is more consistent between animals.

Several studies have used LPS to induce periodontal inflammation in rats. Topical application of LPS results in typical inflammatory changes such as junctional epithelial disruption, infiltration of leukocytes and oedema of the subepithelial connective tissue (Ijuhin, et al. 1992, Miyauchi et al. 1998). Injection of LPS into rat or rabbit gingiva has been described, but although both bone loss (Kawai et al. 2000), and connective tissue breakdown have been reported (Llavaneras et al. 1999, Ramamurthy et al. 2002), there has been, to our knowledge, no description to date of the time course of inflammation and bone loss following a single injection in the rat. Although *Salmonella typhimurium* is not a usual oral micro-organism, there is evidence that LPS derived from this bacterium is the most potent in stimulating inflammatory mediator release (Newton 1986, Garrison et al., 1988) and it was for this reason that we chose to use this source as our inflammatory stimulus.

Histologically, we have noted many of the features of human periodontitis in our experimental animals, particularly apical migration of the junctional epithelium, collagen breakdown and significant alveolar bone loss. The animals do not appear to be greatly affected by the presence of periodontal inflammation as weight gain is not significantly disrupted over the 10 days of study. We have also shown that the inflammation in this model in the rat shows the symmetry postulated to occur in human chronic periodontitis (Listgarten 1986, Mombelli & Meier 2001). These observations suggest that this LPS-induced model of periodontitis is a reasonable one for the study of periodontitis as it shows features that are similar to those of human disease.

#### Changes in histological, histochemical and immunochemical parameters at the site of injection

##### *The time course of inflammation and bone loss*

Evaluation of the histological features of inflammation in this model showed

that 3 days after LPS injection there was no significant bone loss or change in osteoclast number at the site of LPS injection. At seven and ten days post-injection bone loss (both time points) and osteoclast number (day 10 only) were significantly increased compared to controls, and inflammatory infiltrate was apparent. These observations suggest that between 1 week and 10 days after LPS injection the inflammation is well established at the site of LPS injection, and that studies of bone loss are best performed during this time window.

### *Osteoclastic activity*

Study of active osteoclasts at the interdental septum between the first and second molars showed that there was active bone resorption in control animals as indicated by the presence of these cells on the bone surface. In the rat, molars undergo distal drift (Roux et al. 1993), therefore physiologically there are always active osteoclasts present on the mesial surface of the interdental septum (at the distal aspect of the tooth). Analysis of the osteoclasts following LPS injection showed a significant increase in the number of active cells present (corrected for the length of bone surface) and an increase in their activity (upward shift in the regression line compared to control data) at the site of injection. This indicates that LPS may have a dual action in stimulating osteoclasts in bone resorption—stimulation of activity in addition to increased number/recruitment. The recruitment of osteoclasts appears to be specific to the mesial surface of the interdental septum, i.e. the site at which active resorption is already occurring, rather than a general increase in osteoclast number at the entire site of LPS injection.

The increase in osteoclast number and activity correlated well with the simple measure of vertical distance from ACJ-AC, suggesting that a change in this distance is a good measure of active bone remodelling in this model.

### Contralateral changes in histological, histochemical and immunocytochemical parameters - inflammatory effects distant from the site of LPS injection

Although LPS injection resulted in local bone loss (increased ACJ-AC distance) and increased osteoclastic activity at the site of injection, we also observed that there was increased bone loss, and osteoclastic activity in the contralateral mandible, at the mirror image site to that of injection. Human periodontal disease has been described as a symmetrical disorder (Listgarten 1986, Mombelli & Meier 2001). Analysis of the incidence of active disease and bacterial colonisation at symmetrical sites in humans has concluded that the development of disease in the light of existing contralateral lesions is not solely dependent on the presence of local factors (Mombelli & Meier 2001). There has been no previous report of symmetry of periodontitis in experimental animals.

It is well known that LPS can have extremely potent systemic effects in rodents, e.g. 1 ng of LPS injected intraperitoneally in a mouse can result in a pyrexia lasting several hours (Li et al. 1999). It is possible that the contralateral changes observed may be due to a systemic action of LPS; however, we do not believe that this is the case for the following reasons. Firstly, our significant findings on osteoclast activity and bone loss are evident one week or more after LPS injection, when we assume that the potent systemic effects of the initial LPS injection are over. Secondly, if a systemic action of LPS was important in the increased bone loss and osteoclast activity seen, we would expect to see effects on both sides of the mandible sooner, i.e. at 3 rather than 7 days, and simultaneously on both sides of the mandible. Our observations suggest that ipsilateral changes occur prior to contralateral ones. Finally, if LPS was stimulating general osteoclast activity and bone resorption through a systemic mechanism, we would not expect the recruitment of osteoclasts to be so specific as to be restricted to the mesial bone surface. However, LPS can have longer term effects due to the initiation of inflammation, and the production of inflammatory mediators can have widespread systemic effects on many parameters, including osteoclast function. A systemic effect of LPS on alveolar bone resorption cannot therefore be definitively ruled out.

It is possible that altered bone loss and osteoclast activity could be a result of an alteration of mechanical stimulation of the teeth due to altered patterns of mastication, for example increased chewing on the side contralateral to the site of injection due to ipsilateral discomfort. The rats injected with LPS gained weight at the same rate as control animals, although there was less weight gain at 3 days post injection, suggesting that in the acute stages of inflammation the animals may have been eating less. However, at 7 days, when inflammation, bone loss, osteoclast number and activity and CGRP staining are all at high levels, weights are not significantly altered. This implies that the animals did not generally eat less than controls at this point, and indeed between 3 and 7 days they possibly ate more than controls resulting in similar weights at day 7. Thus masticatory patterns may have been altered but not sufficiently to reduce food intake after a week.

An alternative explanation for these results is that contralateral inflammation with resulting bone loss is stimulated through a neurogenic mechanism. Despite the observation that periodontitis is a symmetrical disease in humans (Listgarten 1986, Mombelli & Meier 2001), it is as yet unknown whether there could be a neurogenic component in periodontal disease that might result in symmetrical disease. Neurogenic inflammation has been hypothesised to occur in unilateral experimental periodontitis as denervation reduces inflammation (Gyorfi, et al. 1994) and delays vascular responses (Vandevska-Radunovic et al., 1998), but the potential for a neurogenic mechanism underlying the symmetry of the disease has not been explored. Neurogenic mechanisms are thought to underlie contralateral inflammation in other symmetrical inflammatory diseases such as arthritis (Levine, et al. 1985, Donaldson 1999) with contralateral effects being qualitatively similar to those occurring at the ipsilateral side, but usually smaller in magnitude and with a briefer time course. The data reported here do fit this general pattern as the contralateral bone loss and osteoclast activation are both of smaller magnitude than ipsilateral to the injection, and peak at 7 days rather than 10 days.

In order to address the possibility that altered neuronal function could contribute to the contralateral inflammation in our model of periodontitis, the expression of CGRP-LI in nerve terminals innervating the site of LPS injection and the contralateral mirror image site was studied. Normal periodontium had a sparse CGRP-ergic innervation as has been described in other species (Luthman et al. 1988b, Kondo et al. 1995). Nerve

terminals were evident in the gingivae and close to the junctional epithelium (Kondo et al. 1995).

Analysis of the intensity of CGRP-LI in nerve terminals at the site of LPS injection showed an initial decrease at 3 days, an increase at 7 days and return to control levels at 10 days post-LPS injection. We propose that CGRP may be initially released from nerve terminals in the acute stages of inflammation, resulting in a loss of staining, and then increased in these terminals at later stages once synthesis is increased. Recent evidence shows that LPS directly stimulates release of CGRP from DRG neurons in culture (Xing et al., 2002) and our data suggest that neuronal CGRP synthesis is increased in the later stages of periodontitis in this model (Abd El-Aleem, Morales-Aza & Donaldson, in preparation), supporting this proposal. In addition, inflammatory mediators such as $PGE_2$, bradykinin, histamine, and serotonin can produce dose-related stimulation of immunoreactive CGRP release from pulpal terminals (Hargreaves et al. 1994), and basal gingival perfusion is maintained by local neuropeptides CGRP and Substance P (Berggreen & Heyeraas 2000). In patients with periodontitis, however, CGRP is not increased in gingival crevicular fluid (Lundy et al. 1999) and this has been attributed to an increased degradation of CGRP in GCF in active disease (Lundy et al. 2000). These observations suggest an important role for CGRP in both normal and inflamed gingivae.

A contralateral increase in CGRP in periodontal tissues may stimulate bone resorption, as sensory denervation inhibits osteoclast recruitment and activation (Adam et al. 2000); alternatively, CGRP may initiate contralateral inflammation, and the consequent release of inflammatory mediators then results in osteoclast activation. Interestingly, there is also a contralateral increase in CGRP mRNA expression in sensory neurons in the later stages of periodontitis in this model (Abd El-Aleem et al., unpublished observations) that may be associated with the contralateral inflammation seen in the mandible. The possibility that experimental periodontitis may be a symmetrical disease like the human counterpart has not been previously investigated; indeed many studies use the contralateral jaw as an internal control and therefore would not necessarily see any contralateral effects (Fischer & Klinge 1994). However, contralateral increases in CGRP-LI have also been reported following orthodontic tooth movement in the rat, a condition in which there is also periodontal inflammation (Norevall et al. 1995). Ipsilateral inflammation consequent to LPS injection may, therefore, result in a neurogenic activation of contralateral neurons that results in contralateral release of CGRP and other proinflammatory neuropeptides. This may initiate contralateral inflammation and contralateral bone loss, thereby leading to symmetrical disease.

In summary, a simple reproducible rat model of experimental periodontitis using a local injection of LPS into mandibular interdental papilla is described. This model shows marked osteoclast activity, alveolar bone loss and alterations in calcitonin gene-related peptide immunoreactivity at the site of injection after 1 week. These changes were mirrored in the contralateral mandible. It is possible that these symmetrical inflammatory changes result through a local neurogenic mechanism, although systemic or mechanical effects cannot be excluded. This model closely resembles human disease and can therefore be used for the study of mechanisms of inflammation and bone loss in periodontitis.

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