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Soluble cytokine receptor treatment in experimental orthodontic tooth movement in the rat

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SUMMARY Pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α), are believed to play a role in the biological processes involved in the course of orthodontic tooth movement and especially in root resorption. The inhibition of cytokine activity, e.g. by soluble receptors, could be beneficial in reducing this unwanted side-effect.

The aim of this study was to investigate the role of cytokines IL-1 and TNF-α in the course of experimentally induced tooth movement. The upper left first molar was moved orthodontically in 80 male Wistar rats using a coil spring with a force of 0.5 N. Starting at day –1, three groups of 20 animals each received daily intraperitoneal injections (ip) of 2 ml of 1 μg/ml soluble receptors (a) to IL-1(sIL-RII), (b) to TNF-α (sTNF-α-RI) and (c) a combination of (a) and (b). Twenty animals served as the control. After 3, 6, 9 and 12 days, the animals were killed in groups of five. The amount of tooth movement was registered and the maxillae were prepared for histological and histomorphometric analysis. Osteoclasts and odontoclasts were identified using tartrate-resistant acid phosphatase (TRAP) histochemistry.

The amount of tooth movement was reduced in all receptor-treated groups by approximately 50 per cent. At the same time, the number of TRAP-positive cells on the desmodontal bone surface and on the surface of the roots was reduced. Thus, systemic application of soluble receptors to IL-1 and TNF-α following experimental induction of tooth movement in the rat reduced the number of osteoclasts as well as odontoclasts.

Introduction

Orthodontic tooth movement is based on force-induced periodontal ligament (PDL) and alveolar bone remodelling. Necrosis of the PDL on the pressure side with formation of a cell-free hyaline zone followed by osteoclastic resorption of the neighbouring alveolar bone and bone apposition by osteoblasts on the tension side are the well-described typical histological characteristics of these processes (Thilander *et al.*, 2000). In the course of tooth movement, root resorption represents a negative side-effect in which dental hard tissues are attacked by odontoclasts, cells with an osteoclastic phenotype, whose relationship to osteoclasts and development is still a matter of debate (Ne *et al.*, 1999; Brezniak and Wasserstein, 2002).

There is evidence that pro-inflammatory cytokines play an important role in the regulation of osteoclastic alveolar bone resorption as well as odontoclastic root resorption. This evidence is provided by the fact that cytokines promote osteoclastic bone resorption. They are upregulated in response to inflammatory triggers and secreted by different cell populations, e.g. cells of the mononuclear phagocyte system. Cytokines contribute to the differentiation, chemotaxis and activation of osteoclasts and their precursors (Martin *et al.*, 1998; Lorenzo and Raisz, 1999; Horowitz *et al.*, 2001). In addition, other pro-inflammatory cytokines, especially interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α), as typical mediators of inflammatory responses, have been shown to be involved in the process of bone resorption (Tatakis, 1993; Lorenzo and Raisz, 1999). IL-1 mostly exerts local paracrine and autocrine functions and exists in two isoforms, IL-1α and IL-1β. These bind to two types of cellular receptors, IL-1-RI and IL-1-RII, where the latter has no signalling property and acts as a 'decoy' target for IL-1. Soluble and membrane-bound IL-1 is produced by macrophages, fibroblasts, lymphocytes, osteoclasts and other cells, and is able to strongly stimulate bone resorption (Dinarello, 1998; O'Neill and Dower, 2000). TNF-α is primarily produced by activated monocytes and macrophages, but also by osteoblasts, and stimulates osteoclastic bone resorption *in vitro* and *in vivo*. Its effects are mediated by binding to two distinct, ubiquitously expressed cell surface receptors, TNF-RI (p55) and TNF-RII (p75). Whereas most activities of TNF-α have been shown to be signalled by TNF-RI, the role of TNF-RII is less well defined (Aggarwal *et al.*, 2000). TNF-α is also an important factor for osteoclast differentiation (Azuma *et al.*, 2000).

IL-1 and TNF-α have been shown to play a role in orthodontically induced tooth movement and in the pathogenesis of several inflammatory periodontal

diseases in man and other species. During orthodontic tooth movement in man and rats, cytokines have been detected or expressed in periodontal tissues, as well as in crevicular fluid (Saito *et al.*, 1991; Sandy *et al.*, 1993; Grieve *et al.*, 1994; Lowney *et al.*, 1995; Uematsu *et al.*, 1996; Tzannetou *et al.*, 1999; Alhashimi *et al.*, 2001). In the rat, the IL-1α subtype is considered to be more important in the PDL than IL-1β (Wang and Stashenko, 1997). Under physiological conditions, TNF-α and IL-1α are already expressed in the rat periodontium and may contribute to remodelling processes in physiological tooth drift in these animals (Lossdörfer *et al.*, 2002).

Advances in the understanding of cytokine-mediated development and progression of rheumatoid arthritis have led to efforts to neutralize these cytokines by using antibody, antisense or soluble receptor techniques (Carteron, 2000). Soluble receptors are able to bind their ligands with high specificity and affinity and effectively neutralize the bioactivity of cytokines (Dinarello, 1998). Recently, it has been shown in an animal model of mechanically induced root resorption that systemic application of soluble receptors to IL-1 (sIL-RII) or TNF-α (sTNF-α-RI) leads to a reduction in, and even prevention of, root resorption (Zhang *et al.*, 2003). In the present study, blocking these cytokines was investigated in a rat model of orthodontically induced tooth movement.

Materials and methods

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the local district government and the Animal Care Commissioner of the University of Bonn (Germany).

Eighty 3-month-old male Wistar rats equally divided into four groups, with an average weight of 215 g, obtained from Charles River Laboratories (Sulzfeld, Germany) were used. The animals were kept in plastic cages with a standard 12-hour light–dark cycle, and fed a soft diet and water *ad libitum*. During the experiments the weight of the animals was recorded daily. Feeding behaviour was also controlled.

Experimental protocol

An orthodontic appliance, based on a modified technique Ong *et al.* (2000), was inserted under anaesthesia with Rompun® (Bayer, Leverkusen, Germany; 0.01 ml) and Ketavet® (Pharmacia and Upjohn, Erlangen, Germany; 0.24 ml). The appliance consisted of a stretched closed coil spring (0.012 inch nickel-titanium wire; GAC International, New York, USA) ligated between the maxillary right first molar and the incisors, moving the molar mesially with a force of 0.5 N. The molars and incisors were notched on the mesial and distal surfaces to ensure maximum retention of the spring. The appliance was activated immediately upon insertion and the fit was checked daily. No reactivation was performed during the experimental period.

Starting 1 day before the installation of the appliance, the animals in the control group received a sham intraperitoneal injection (ip) of 2 ml of sterile phosphate-buffered saline (PBS) daily. Following an identical time schedule, the animals in the sIL-1-R group were administered 2 ml of 1 μg/ml recombinant human sIL-1-RII (R&D Systems, Wiesbaden, Germany) ip diluted in sterile PBS. In the sTNF-α-R group, 2 ml of 1 μg/ml recombinant human sTNF-α-RI (R&D Systems) diluted in PBS was applied in the same manner. In group 4, sTNF-α-RI and sIL-1-RII diluted as described were applied simultaneously (combination group).

Five animals from each group were killed after 3, 6, 9 and 12 days. Following sacrifice, the maxilla of each animal was dissected, divided into two halves and prepared for light microscopic examination. The magnitude of tooth movement was determined by measuring the separation between the first and second maxillary molars using calibrated gauges with an accuracy of 0.05 mm. On day 12, loosening of the coil spring had occurred in five of the animals (three in the IL-1α receptor group and two in the group treated with both cytokine receptors). In these cases, no values for the amount of tooth movement were recorded.

Histology

The right maxilla of each animal was fixed in 4 per cent paraformaldehyde in 0.1 M PBS for 24 hours and decalcified in neutral 10 per cent ethylene diamine tetra-acetic acid (EDTA) at room temperature for at least 20 days. The EDTA solution was changed daily. To determine whether calcification was complete, a methyl red-ammonia-ammonium oxalate solution test was carried out. After dehydration and paraffin embedding, 5 μm serial sections in a mesio-distal direction parallel to the long axis of the mesial root of the first molar were cut on a microtome (HM 355s; Microm International, Walldorf, Germany) and mounted on glass slides (K. Roth, Karslruhe, Germany). Selected sections were stained with haematoxylin–eosin (HE).

Tartrate-resistant acid phosphatase (TRAP) histochemistry

In order to identify osteoclasts, odontoclasts and their precursors, selected tissue sections were stained to demonstrate TRAP, according to the method of Barka and Anderson (1962). In short, after TRAP activation in Tris hydrochloride acid buffer, the incubation

was carried out in a solution containing a mixture of pararosaniline hydrochloric acid with sodium nitrite, veronal acetate buffer and tartaric acid and of naphthol-AS-biphosphonate with dimethyl formamide for 1 hour at room temperature. Thereafter, the slides were rinsed, counterstained with Mayer's hemalaun and mounted. All chemicals were purchased from Sigma (Deisenhofen, Germany).

Histomorphometry

For quantitative evaluation of resorption activity, five representative TRAP-stained sections per animal were taken for histomorphometric analysis. The selection was based on morphological criteria such that central sections of the distal roots were chosen where these roots appeared to be as long as possible. The measurements were performed in the bifurcation area of the first molar between the middle and distal roots (Figure 1) using a light microscope (Axiophot 2; Zeiss, Göttingen, Germany) in combination with a colour scan camera (Praktika, Dresden, Germany) and imaging software (SilverFast V5, Lasersoft, Kiel, Germany) on a personal computer. Counts for nuclei of TRAP-positive cells were performed at a magnification ×200 and the measurement field on the computer screen was 1145 × 815. The counts were performed separately on (1) the root surface, (2) the desmodontal bone surface and (3) the neighbouring endosteal bone surfaces within the alveolar bone. TRAP-positive cells within the PDL were not considered. The measurements were taken twice for each section. The mean was determined and intra-operator error was assessed following repeated blind measurements.

For histomorphometric analysis, the animals killed after 3 and 6 days were placed in one group and those killed after 9 and 12 days in a second group. Possible overall differences between the groups were evaluated for the two time periods using the Kruskal–Wallis test. Significance levels were set at $P \leq 0.05$. In the case of significance, individual group differences were analysed with multiple pairwise comparisons using a *post-hoc* Mann–Whitney test. The level of significance was adjusted for multiple testing according to Bonferroni to ≤ 0.017.

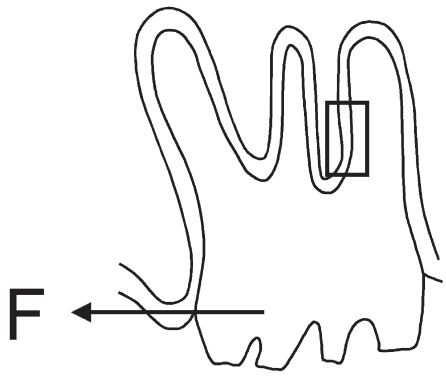

Figure 1 A schematic representation of the upper right molar and the area of bifurcation between the middle and distal roots where the histomorphometric measurements were performed. The arrow points in the direction of the applied force (F) vector.

Results

Animal health

All animals remained healthy during the study period, and food and water ingestion appeared unaffected by the orthodontic appliance. Except for a short period following surgery, there was an overall gain in weight throughout the lifetime of the animals.

Tooth movement

In the control animals there was a continuously increasing movement of the molars from days 3 to 12, ending with a mean of 400 μm (range 300–600 μm, Figure 2). Following the application of cytokine receptors, the amount of tooth movement after 12 days was reduced in all groups. This held true especially for the groups treated with receptors to TNF-α and the combination of receptors to TNF-α and IL-1α, where tooth movement was reduced to approximately 60 per cent (sTNF-α-R group: mean 240 μm, range 200–300 μm; combination group: mean 266 μm, range 200–300 μm; Figure 2). In the IL-1-R group after 12 days, tooth movement could only be registered in three animals (mean 300 μm, range 100–500 μm). In addition, in the groups receiving cytokine receptors, tooth movement seemed to be more intermittent over the experimental period.

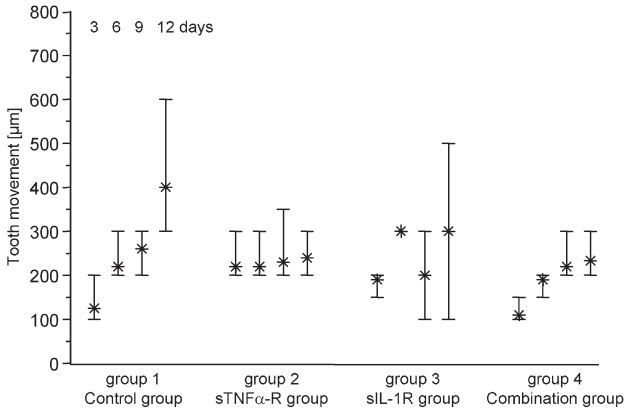

Figure 2 The extent of orthodontic tooth movement measured for the four groups as a function of time following application of the orthodontic force. Each asterisk represents the mean of five animals (in group 3, after 12 days, tooth movement could only be registered for three animals). The vertical bars represent the range of the measurements.

Histology

Control group (Figure 3a, b). The animals in the control group showed the histological characteristics that are well known from other experimental studies. Briefly, hyalinization of the PDL on the pressure sides of all roots was already visible on day 3 in nearly all animals and increased until day 12. Starting from the central parts of the PDL, they usually showed a coronal extension. Around the large mesial root, hyaline areas appeared mainly in the disto-apical and mesio-coronal regions, indicating tipping of the tooth. The initiation of hyalinization was characterized by structural disturbances of PDL fibres and fibroblast pyknosis with vessel damage and extravasation followed by formation of an eosinophilic, cell-free hyalinosis. In the later stages, hyaline zones transformed into more basophilic areas of disintegrating compact areas, which in some cases were artificially lost from the histological sections. Until day 12, complete removal of hyaline tissues was not observed. In the areas under tension, the typical morphology with stretched PDL fibres was visible. Apposition of bone on the mesial surfaces of the alveloar septa and of cementum on the distal root surfaces was seen. These surfaces were mostly covered by osteoblasts or cementoblasts.

On days 3 and 6, multinucleated clastic cells were scattered around the hyaline zones, but morphological signs of root resorption were not visible before day 9, except for some small pits in the cementum. At that time, resorption lacunae appeared at the surface apical and coronal to the hyalinized areas in most of the animals. On day 12, nearly all roots showed signs of resorption. Along some roots, extended undermining resorption showing accumulation of large odontoclasts was visible. No signs of resorption repair were observed.

On day 3, osteoclasts were seen along mesial alveolar bone surfaces, on endosteal surfaces and on the basal maxillary bone. From day 6 on, the number of osteoclasts along the mesial alveolar walls seemed to decrease. Generally, the number of bone osteoclasts showed individual variability among the animals in this group.

Animals treated with sTNF-α-RI (Figure 3c, d). In general, the morphology and time course of the orthodontically induced histological alterations were basically similar to the animals in the control group. While the structure and extension of hyaline areas appeared the same, the number of clastic cells around these areas seemed to be reduced. This was especially true for odontoclastic cells on the tooth roots. On days 9 and 12, the number and extension of root resorption lacunae also seemed to be reduced. In some animals killed on day 12, signs of early resorption repair showing cementum deposition and PDL fibre reinsertion were visible. However, individual variability with respect to the number of clastic cells and root resorption extension among the animals in this group was noted. Additionally, the number and distribution of bone osteoclasts varied among individual animals over time, but generally their number had decreased by day 9. Compared with the control group, fewer osteoclasts were observed on days 3 and 6.

Animals treated with sIL-1-RII (Figure 3e, f). The general histological findings in this group mostly resembled those in the sTNF-α-R group. In contrast, the variability of the number of resorbing cells seemed to be even more obvious. A variable morphology comprising all stages of early and late hyalinization was also seen over all days investigated.

Animals treated with sTNF-α-RI plus sIL-1-RII (Figure 3g, h). Individual morphological variability as in the other groups was again noted. The general histological appearance after 3 and 6 days was very similar to the control group. As in the other treated groups, only a few or no odontoclasts were observed on the root surfaces. For days 9 and 12, the number of root resorbing cells seemed to be increased, although root resorption did not appear to be as significant as in the control group. Qualitative findings for osteoclasts were comparable with the other groups.

Histomorphometry

The intra-operator error of the cell counts was established to be within the 5 per cent range.

Multinucleated and a few mononuclear osteoclasts and odontoclasts located on bone and root surfaces all stained definitely positive for TRAP. Mononuclear cells located in the PDL, in bone marrow spaces or around vessels probably resembling osteoclast precursors were also TRAP reactive. Overall, the morphometric results corroborated the qualitative histological observations, especially with respect to the number of clastic cells on the roots.

After 3 and 6 days, in the control group, the number of osteoclastic nuclei on the desmodontal bone surface had a median value of 8 (Figure 4a). In the three groups treated with cytokine receptors, the median values overall showed a significant tendency towards lower values (Figure 4a, Tables 1 and 2). After 9 and 12 days, this median value increased significantly to 21 for the animals in the control group (Figure 4a, Table 3). In the cytokine receptor groups, the number of osteoclastic nuclei seemed to be reduced to about one-half in the animals treated with sTNF-α-RI (median = 11) and in those treated with the combination of the two receptors (median = 9) (Figure 4a). These differences were highly significant (Tables 1 and 2).

There was a highly significant difference in the number of osteoclast nuclei on the endosteal bone surface for animals treated for 3 and 6 days compared with those

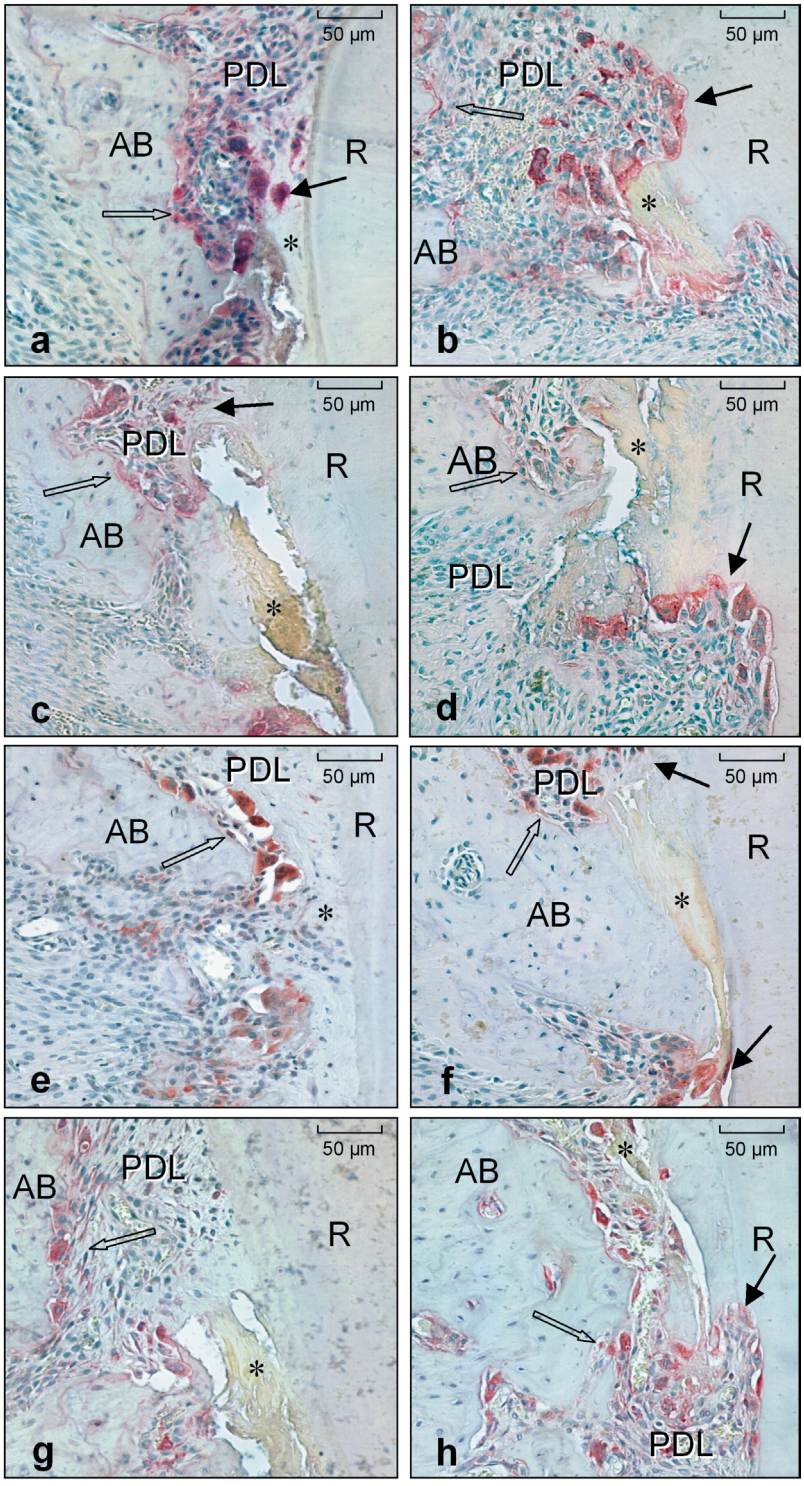

Figure 3 Histological sections of the periodontal tissues of the upper right molar showing the area of bifurcation between the middle and distal roots. AB, alveolar bone; PDL, periodontal ligament; R, root; asterisk, hyalinized periodontal tissue; open arrows, resorption lacunae on the root surface containing odontoclasts; arrows, resorption lacunae on the desmodontal bone surface containing osteoclasts; original magnification ×200. (a, b) Control group. (a) After 6 days, hyalinization of the PDL is visible. Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells are scattered around the hyaline zones. A few osteoclasts are housed on the desmodontal surface, but morphological signs of root resorption cannot be seen. (b) After 12 days, accumulation of multinucleated odontoclasts in resorption lacunae are obvious. Remnants of hyalinized PDL tissue can still be seen. No signs of resorption repair of the root can be observed. (c, d) Tumour necrosis factor-alpha receptor 1 (TNF-α-RI) group. (c) After 6 days, significant hyalinization of the PDL is obvious. Only a very few TRAP-positive cells are visible. (d) After 9 days, resorption of the necrotic PDL tissues has started. TRAP-positive cells can be seen over the desmodontal bone surface as well as over the root, although their number seems to be reduced with respect to the control group. (e, f) Interleukin-1α receptor II (IL-1α-RII) group. (e) After 6 days, few osteoclasts can be seen over the desmodontal bone surface. No root resorption is obvious. (f) After 12 days, the PDL is still necrotic over wide areas. TRAP-positive cells are starting resorption activity of hard tissues at the coronal and apical margins. (g, h) TNF-α-RI plus IL-1α-RII group. (g) After 3 days, besides hyalinized PDL tissue, a few TRAP-positive cells can be seen on the desmodontal bone surface. (h) After 9 days, hard tissue resorption is obvious at the desmodontal bone surface as well as on the root. The number of TRAP-positive cells seems to be lower than in the control group.

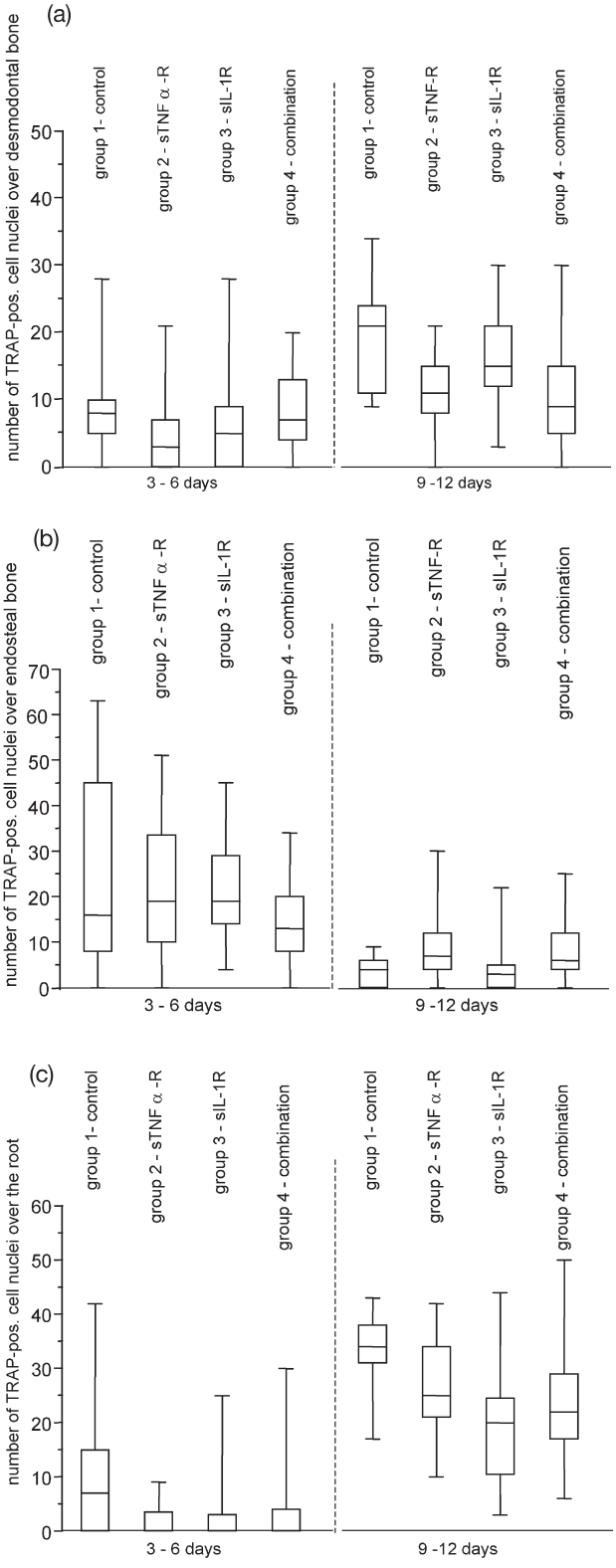

Figure 4 Box plots of the number of tartrate-resistant acid phosphatase (TRAP)-positive cell nuclei counted at (a) the desmodontal bone surface, (b) the endosteal bone surface and (c) the root surface. Animals treated for 3 and 6 days were pooled, as well as those treated for 9 and 12 days. Each box represents the measurements of five representative TRAP-stained sections of 10 animals each. In each box, the median as well as the 5, 25, 75 and 95 per cent quartiles are given.

Table 1 Results of Kruskal–Wallis tests to evaluate possible overall differences between the four groups for the two time periods. Significance levels were set at $P \leq 0.05$.

Days after treatment	Probability	Test criterion
Root surface		
3–6	0.0004	8.4180
9–12	0.0004	18.2244
Desmodontal bone surface		
3–6	0.0137	10.6547
9–12	0.0005	17.7416
Endosteal bone surface		
3–6	0.0552	7.5934
9–12	0.0302	8.9300

treated for 9 and 12 days for all experimental groups, the latter ones presenting a decrease of at least 50 per cent (Figure 4b, Table 3). On the other hand, although there was generally a tendency towards lower median values for the receptor groups, statistical testing revealed no significant differences for both time periods (Tables 1 and 2). The sum of the total numbers of osteoclast nuclei (those on the desmodontal surface plus those on endosteal bone surfaces) revealed that there was a significant decrease in the median values for the animals treated for 3 and 6 days compared with those treated for 9 and 12 days, but there was no significant difference between the control group and those treated with cytokine receptors (data not shown).

With respect to the number of odontoclastic nuclei on the roots of the teeth, the median in the control group after 3 and 6 days was 7. For all the cytokine receptor groups, this value was reduced to zero at this time (Figure 4c). Although there were large standard deviations, these differences from the control group were highly significant for all the receptor groups (Tables 1 and 2). After 9 and 12 days, the number of odontoclastic nuclei increased significantly in all groups (Figure 4c, Table 3). Nevertheless, there was still a significant difference between the animals in the control group and those treated with cytokine receptors, the latter demonstrating lower medians (Tables 1 and 2).

Discussion

The ultimate aim of orthodontic tooth movement would be to move teeth in the most effective way and at the same time to reduce side-effects, such as root resorption, as much as possible. In this study, the role played by the cytokines IL-1 and TNF-α in the course of experimentally induced tooth movement was investigated in the rat. Instead of using unspecific anti-inflammatory therapy (Kameyama *et al.*, 1994; Zhou *et al.*, 1997), soluble receptors to these cytokines were applied to specifically inhibit their biological activity.

Table 2 Results of multiple pairwise comparisons between the three receptor-treated groups and the control group using a *post-hoc* Mann–Whitney test. The level of significance was adjusted for multiple testing according to Bonferroni to ≤ 0.017.

Days after treatment	Group 2: sTNF-α-R (α)	Group 3: sIL-1-R (α)	Group 4: combination (α)
Root surface			
3–6	0.0001	0.0004	0.0010
9–12	0.0161	0.000	0.0013
Desmodontal bone surface			
3–6	0.0027	0.0003	0.4101
9–12	0.0006	0.0557	0.0010
Endosteal bone surface			
3–6	0.3862	0.4189	0.0402
9–12	0.0122	0.4569	0.0222

sTNF-α-R, soluble receptor to tumour necrosis factor-alpha; sIL-1-R, soluble receptor to interleukin-1.

Table 3 Results of Mann–Whitney tests comparing the measurements for each of the four groups with respect to the duration of treatment (3 and 6 days versus 9 and 12 days). The level of significance was adjusted for multiple testing according to Bonferroni to ≤ 0.017.

	Group 1: control (α)	Group 2: sTNF-α-R (α)	Group 3: sIL-1-R (α)	Group 4: combination (α)
Root surface (3–6 and 9–12 days)	0.0000	0.0000	0.0000	0.0000
Desmodontal bone surface (3–6 and 9–12 days)	0.0001	0.0001	0.0000	0.1149
Endosteal bone surface (3–6 and 9–12 days)	0.0000	0.0000	0.0000	0.0008

sTNF-α-R, soluble receptor to tumour necrosis factor-alpha; sIL-1-R, soluble receptor to interleukin-1.

The results demonstrate that under the applied experimental conditions, the amount of tooth movement reached following a constant tipping force of 0.5 N for 12 days was remarkably reduced in all receptor-treated groups. This confirms that the concentration of the soluble receptors in the local microenvirement of the periodontium reached following systemic application was sufficient to interfere with the remodelling processes induced in the periodontal tissues.

In contrast to these findings, King *et al.* (1991a, b), in their studies on alveolar bone remodelling following orthodontic tooth movement in the rat, found a significant decrease in the amount of tooth movement between days 7 and 10 after force application. At the same time, the number of osteoclasts at the compression site showed a significant decline. However, those authors used a coil spring fabricated from conventional steel, exerting an initial force of 0.4 N, and they expected a distinct deactivation of the force following initial movement of the teeth. The superelastic coil spring used in the present study was indicated to produce an initial force of 0.5 N. Because of the material properties of the spring, there should have been a constant force level for the whole treatment period of 12 days. Nevertheless, the amount of force applied in both studies would have to be ranked in a range called the 'high force level' (Melsen, 1999).

After application of this type of 'high' force, tooth movement follows a typical pattern of phases, namely displacement, delay and movement. At the same time, resorption of alveolar bone is initiated by the appearance of osteoclasts at the sites of compression of the periodontal membrane. In the rat, the appearance of osteoclasts lasts approximately 3–5 days (King *et al.*, 1991a; Brudvig and Rygh, 1994). This delay is caused by a series of intermediate steps, which are division of stem cells, chemotaxis of pre-osteoclasts, recognition of sites to be remodelled, attachment to bone surfaces and fusion, and differentiation into functional multinucleated osteoclasts.

For histomorphometric measurement, the number of TRAP-positive cells was counted. The technique of histochemical identification of cells presenting TRAP for the analysis of periodontal remodelling following orthodontic tooth movement has been used previously (King *et al.*, 1991a, b, 1998; Brudvig and Rygh, 1994; Zhou *et al.*, 1997; Hughes and King, 1998; Gu *et al.*, 1999; Noxon *et al.*, 2001; Rody *et al.*, 2001). Precisely, the number of TRAP-positive clastic cell nuclei was counted instead of the number of clastic cells. This procedure has been reported to be a more realistic

indicator of resorbing activity and less prone to error (Vignery and Baron, 1980). For statistical analysis, the animals being treated for 3 and 6 days and those being treated for 9 and 12 days were pooled into two larger groups. The reason for this procedure was that there was no statistical difference between the specimens originating from the respective groups. Morphologically, the PDL up to day 6 was dominated by significant amounts of coagulated necrotic tissues and there was no distinct difference with respect to morphology between the animals killed on days 3 and 6. The same held true for the animals killed after 9 and 12 days. The morphological appearance was rather similar and there seemed to be high variability in the resorbing and reparative processes occurring in the periodontal tissues. One possible explanation would again be the rather constant force that was applied.

As a result, it was found that after 3 and 6 days the number of TRAP-positive cell nuclei was highest at the endosteal bone surfaces. This seems to be in accordance with the fact that this is the site where new pre-osteoclasts develop and that at this time the desmodontal bone surface is mostly occupied by necrotic tissue. The histological picture presented is traditionally called 'undermining resorption' of alveolar bone. If the groups treated with cytokine receptors are compared with the control group, only the animals treated with both receptors presented a significantly reduced number of cell nuclei. The number of TRAP-positive cell nuclei lying on the desmodontal bone surface after 3 and 6 days was significantly reduced in all the receptor groups. After 9 and 12 days the number of TRAP-positive cell nuclei over endosteal bone surfaces significantly declined for all groups. At the same time, this number increased at the desmodontal bone surface. Again, at this site, the number was significantly lower in the animals administered cytokine receptors.

With respect to the mechanisms controlling the appearance of osteoclasts at the compression sites, the role of cytokines, growth factors and matrix components has been discussed (Sandy *et al.*, 1993; Thilander *et al.*, 2000; Brezniak and Wasserstein, 2002). However, the complex interrelationship between these factors is still not completely understood. Among the cytokines, IL-1 and TNF-α are thought to play a prominent role. Various studies have shown that IL-1 stimulates bone resorption and inhibits bone formation *in vivo* and that it is secreted in response to a variety of stimuli, be they mechanical, hormonal or inflammatory (Lorenzo and Raisz, 1999; Horowitz *et al.*, 2001). Exogenous IL-1 has been demonstrated to induce experimental bone loss, while IL-1 receptor antagonists were able to prevent this. In the field of dentistry, the role of various cytokines has been proven in the course of inflammatory periodontal disease (Seymour and Gemmell, 2001) and after the application of orthodontic force (Saito *et al.*, 1991; Uematsu *et al.*, 1996; Alhashimi *et al.*, 2001; Iwasaki *et al.*, 2001). IL-1 influences the process of bone resorption in different ways, but seems to have a primary effect on the division and differentiation of pre-osteoclasts and, thus, on the later stages of osteoclastogenesis (Lorenzo and Raisz, 1999; Horowitz *et al.*, 2001). In this context, Rody *et al.* (2001) pointed out that for orthodontic tooth movement, the recruitment of new pre-osteoclasts may be more important than the stimulation of resident cells.

TNF-α is produced primarily by activated monocytes and macrophages, but also by osteoblasts, and has been proven to be an activator of osteoclastic bone resorption *in vivo* and *in vitro* (Aggarwal *et al.*, 2000; Azuma *et al.*, 2000). Vargas *et al.* (1996) investigated the role of IL-1 and TNF-α in physiological bone remodelling in IL-1-RI and TNF-α-RI knockout mice. Those authors did not find any obvious structural bone abnormalities or resorption disturbances and concluded that these cytokines might only become involved in bone remodelling under non-physiological conditions. Under the condition of inflammation, one important effect of cytokines is the induction of prostaglandin E_2, which has been shown to be a strong osteoclast-activating factor (Pilbeam *et al.*, 2002). Accordingly, the expression of the isoenzyme cyclo-oxygenase-2 has been shown to be correlated with the induction of root resorption in root transplantation experiments (Shiraishi *et al.*, 2001).

For the morphometric results, it has to be emphasized that the remodelling processes at the compression site were considered to be at least partly influenced by inflammatory reactions to the coagulation necrosis of the periodontal tissues. Thus, while no differences could be found with respect to the cells lying over the endosteal bone, the reduced number of osteoclasts present on the desmodontal surface in all animals treated with cytokine receptors can be interpreted as an indicator of the role played by these cytokines in the bone resorption process that is triggered by the inflammation process. In addition, this offers a possible explanation for the reduced distance that the teeth were moved in the animals in the receptor groups. On the other hand, there are no obvious significant differences between the groups treated with either the IL-1 receptor, the TNF-α receptor or with both. In this context, it has to be considered that the cytokines are tightly regulated at the transcription and translation level. Each individual cytokine and different cytokines together are regulated in the manner of complex feedback loops. In addition, after secretion, the activity is controlled by the receptor level on the target cells (Mantovani, 1997; Oppenheim and Feldmann, 2000). Lastly, bone resorption is a complex process that involves more than just a definite number of individual osteoclasts to be present at a given site. Other factors that may be of significance include bone resorbing

activity and the capacity of individual osteoclasts, osteoclast adhesion to the bone surface, H^+-ion transport, enzyme secretion and matrix digestion.

With regard to the number of TRAP-positive cell nuclei lying over the root surface in the sites of compression, a remarkable number were already present at days 3 and 6. These were primarily situated at the margins of the most compressed PDL affected by coagulated necrosis. Comparing the control group with the animals treated with cytokine receptors, the latter showed significantly fewer cells. The same was true for the animals killed after 9 and 12 days. At this time there was a three-fold increase in the number of cell nuclei in the control group, whereas the number in the three receptor-treated groups was significantly lower.

Whereas numerous studies on the biological processes of bone remodelling have been carried out, the knowledge of mechanisms underlying root resorption is still incomplete. The process is probably related to a local injury to the periodontal tissues and, in particular, with the aseptic coagulation necrosis process occurring in the periodontal tissues. The process starts at the periphery of necrotic areas within the compression sites of the PDL and continues some days later beneath the central area (Brudvig and Rygh, 1994). Chemical signals from the necrotic tissue, together with neurotransmitters and inflammatory factors such as cytokines, chemokines and prostaglandins, are thought to be responsible for the recruitment, invasion and activity of resorbing cells (Brezniak and Wasserstein, 2002).

It is known that the amount of coagulated necrotic periodontal tissues is correlated with the extent of root resorption (Brudvik and Rygh, 1994). The cells involved in the resorption process are fibroblasts, macrophages and special multinucleated cells. Among the latter, a differentiation can be made between multinucleated giant cells removing necrotic soft tissues and hard tissue resorbing odontoclasts, which have both been shown to carry the TRAP enzyme (Brudvik and Rygh, 1994). Although there have been some studies reporting opposite osteoclastic and odontoclastic responses to either stimulation (Goldie and King, 1984) or inhibition (Lasfargues and Saffar, 1993) of bone resorption, the odontoclasts are believed to share a common origin with the osteoclasts. Namely, there is striking morphological and functional similarity between these two cells (Furseth, 1968; Tanaka *et al.*, 1990; Götz *et al.*, 2000) and cells resorbing bone and root simultaneously have been shown (Tanaka *et al.*, 1990). In addition, it has been demonstrated that following mechanical induction of root resorption, the number of odontoclasts decreases following the administration of aspirin (Kameyama *et al.*, 1994) or soluble receptors to IL-1 or TNF-α (Zhang *et al.*, 2003).

On the other hand, the quantitative relationship between the activity of bone resorbing osteoclasts and root resorbing odontoclasts does not seem to be a simple one. Following reactivation of an orthodontic appliance after 1 or 10 days, King *et al.* (1998) found a significant increase in the number of osteoclasts only 1 day later, but no increase in root resorption. The opposite effect could be seen when the appliance was reactivated 4 days after initial activation (Hughes and King, 1998).

Conclusions

The resorption process of dental hard tissues seems to be triggered by the activity of cytokines as well as that of bone. On the other hand, inhibition of cytokine activity using the application of their soluble receptors does not seem to be a suitable treatment regime for preventing root resorption in the course of orthodontic tooth movement. The results of the present study suggest that the number of odontoclasts, as well as osteoclasts, over the desmodontal bone surface is reduced and, thus, the amount of tooth movement. Future research will be focused on possible influences of soluble cytokine receptors on the individual cell activity of the involved cells.

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