Neural modulation of inflammatory reactions in dental tissues incident to orthodontic tooth movement.
A review of the literature

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SUMMARY This article reviews the current knowledge of the biological aspects of dental tissue changes incident to orthodontic tooth movement. The inflammatory nature of these tissue changes was first recognized in the early 1970s, and since then a number of morphological and quantitative investigations have been published in support of this view. The studies dealing with vascular and cellular dental tissue changes, as well as those concerned with inflammatory mediators present at sites of orthodontic tooth movement are systematized and presented accordingly. Special emphasis is placed upon the role of the sensory nerve fibres and their neuropeptides in the control, and development of an inflammatory process, i.e. their role in tooth movement.

Introduction

Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodelling of the periodontal ligament (PDL) and alveolar bone. A pre-condition for the remodelling activities, and ultimately tooth displacement, is the occurrence of an inflammatory process. Vascular and cellular changes were the first events to be recognized and described. With the advancement of research techniques, a number of inflammatory mediators, growth factors, and neuropeptides have been demonstrated in the periodontal supporting tissue. Their increased levels during orthodontic tooth movement have led to the assumption that interactions between cells producing these substances, such as nervous, immune, and endocrine cells, regulate the biological responses after the application of an orthodontic force.

This article reviews the current knowledge of the biological aspects of inflammatory changes in dental tissues incident to orthodontic tooth movement. Special emphasis has been placed upon the role of the sensory nerve fibres and their neuropeptides, in the control and development of inflammation, i.e. their role in tooth movement.

Inflammation is a local response of the host to tissue injury, commonly as a reaction to invasion by microbial material, but also to chemical and/or physical stimuli. Regardless of the injurious agent, inflammation is characterized, in its acute form, by the classical signs of redness, heat, swelling, pain, and loss of function. These visual tissue changes are the result of a complex series of events that can be detected microscopically and functionally, and include: (a) vasodilation with increased vessel permeability and blood flow; (b) exudation of fluids; and (c) leukocyte migration into extravascular spaces. The spread of the inflammatory response to a wider tissue area is propagated, as well as amplified by a number of endogenous chemical mediators. Histamine, prostaglandins (PGs), leukotrienes, and cytokines are among the substances that mediate blood flow changes, augment adherence of circulating leukocytes to vascular endothelium, and promote migration of leukocytes into tissues (Gallin *et al.*, 1992; Stephenson, 1992; Guyton and Hall, 1996).

More recently, it has been recognized that, in addition to the classical constituents, peripheral nerve fibres participate in inflammation (Basbaum

and Levine, 1991; Heller *et al.*, 1995; Brain, 1997). This neural contribution is termed 'neurogenic inflammation' and involves release of neuropeptides after antidromic stimulation of afferent nerve endings and initiation of an inflammatory reaction, which can be significantly attenuated by depletion of neuropeptides or by denervation.

Inflammatory reactions in dental tissues incident to orthodontic tooth movement

Prolonged application of mechanical forces that exceed the bio-elastic limits of tooth-supporting structures induce tooth movement (Storey, 1973). These forces represent a physical agent capable of inducing an inflammatory reaction in the connective dental tissues leading to adaptive proliferation and remodelling, mainly in the PDL and alveolar bone (Storey, 1973; Rygh, 1983; Davidovitch *et al.*, 1988). The PDL is confined in a low compliance environment and even light, but continuous orthodontic forces can exert pressure that compromises the integrity of the vascular compartment. Over-compression results in ischaemia, interruption of nutrition and cell death (Rygh, 1972a,b; Storey, 1973), with almost unavoidable formation of a necrotic or hyalinized zone (Reitan, 1951; Reitan and Kvam, 1971; Kvam, 1972; Rygh, 1972b, 1983). These tissue changes, although aseptic, are beyond the limits of a physiological response. The necrotic periodontal structures are potent inflammatory stimuli (Lilja *et al.*, 1983; Stephenson, 1992) leading to blood vessel and cell proliferation in the surrounding areas, with removal of the hyalinized structures and subsequent tissue repair (Rygh, 1974, 1983; Vandevska-Radunovic *et al.*, 1994, 1997a,b; Hellsing and Hammarström, 1996). The remodelling processes in the PDL and alveolar bone represent only one aspect of the alterations of tooth supporting structures. Orthodontic forces also affect the dental pulp and gingiva, inducing vascular changes that are inflammatory in nature (Stenvik and Mjør, 1970; Guevara and McCluggage, 1980; Grieve *et al.*, 1994; Vandevska-Radunovic *et al.*, 1994; Brodin *et al.*, 1996). The anatomy and physiology of the PDL, alveolar bone, gingiva

and dental pulp are closely associated, and the pattern of reaction in one of them is related to the responses in the other tissue compartments. Therefore, the inflammatory changes preceding and coinciding with the remodelling processes necessitated by tooth movement, have to be seen as a part of a wider biological system, involving continuous adjustment of all dental tissues to changed functional demands.

Clinical evidence of inflammation in dental tissues

Gingiva is the only dental tissue visually accessible for direct clinical evaluation of the classic signs of inflammation. Redness and swelling are common clinical observations during fixed orthodontic therapy. In addition, clinical tests such as the Gingival Index and pocket depth probing confirm the development of an inflammatory reaction (Zachrisson and Zachrisson, 1972; Pender *et al.*, 1994). Although gingival inflammation can be due to plaque retention, bracket, or adhesive irritation (Zachrisson and Brobakken, 1978; Davidson *et al.*, 1982), investigation of the gingival crevicular fluid (GCF), its flow rate and glycosaminoglycan (GAG) content indicate that orthodontic tooth movement *per se* induces inflammatory changes in the underlying dental tissues, particularly in the alveolar bone and PDL (Last *et al.*, 1988; Samuels *et al.*, 1993; Pender *et al.*, 1994).

Pain, being one of the cardinal signs of inflammation, is almost inevitable and, for the patient, the most unpleasant reaction to orthodontic therapy. It begins a few hours after application of an orthodontic force and lasts for approximately 5 days (Furstman and Bernik, 1972; Jones, 1984; Kvam *et al.*, 1987; Ngan *et al.*, 1989; Scheurer *et al.*, 1996). Pain results, partly, from stretching and distortion of tissues (Stephenson, 1992) due to mechanical forces, and from interaction of multiple inflammatory mediators with the local pain receptors (Davies and MacIntyre, 1992; Hargraves *et al.*, 1995). Many of the inflammatory mediators shown to elicit hyperalgesic responses, including histamine, PGs, serotonin, bradykinin, and substance P (SP), have increased levels in dental tissues following orthodontic

tooth movement (Yamasaki, 1983; Davidovitch *et al.*, 1989; Nicolay *et al.*, 1990; Saito *et al.*, 1992; Grieve *et al.*, 1994). As pain leads to impaired function, biting and chewing are reported to be the source of the most intense discomfort during fixed orthodontic therapy (Scheurer *et al.*, 1996).

However, administration of anti-inflammatory drugs which suppress PG synthesis, reduces the discomfort after insertion of an orthodontic appliance (Ngan *et al.*, 1994). Taken together, these findings signify initiation of an inflammatory reaction in the peripheral target tissues, with activation and involvement of central mechanisms in response to orthodontic tooth movement.

Experimental evidence of inflammation

Direct clinical evaluation of any inflammatory alterations in the PDL, pulp, and alveolar bone is impossible because of their anatomy. Therefore, and in order to obtain a thorough insight into the changes elicited by orthodontic forces, it is necessary to evaluate these tissues experimentally. Thus, the use of human material is restricted, and most of the findings of vascular, cellular, and neural changes in dental tissues incident to orthodontic tooth movement are derived from animal experimental models.

Vascular changes following orthodontic tooth movement

Numerous investigations have been performed to elucidate the effects of orthodontic forces on the vascular response in dental supporting tissues. Application of both tipping (Gianelly, 1969; Rygh, 1972a,b; Gaengler and Merte, 1983; Nakamura *et al.*, 1986; Rygh *et al.*, 1986; Hosoyama, 1989; Kvinnsland *et al.*, 1989; Vandevska-Radunovic *et al.*, 1994), and extrusive (Cooper and Sims, 1989; Lew *et al.*, 1989; Brodin *et al.*, 1996) and intrusive forces (Stenvik and Mjør, 1970; Guevara and McCluggage, 1980; Ng *et al.*, 1981; Brodin *et al.*, 1996) seem to induce rapid vascular responses, although the severity of the tissue reaction is dependent on the force magnitude and duration (Gianelly, 1969; Storey, 1973; Ng *et al.*, 1981). The immediate changes in blood vessel morphology
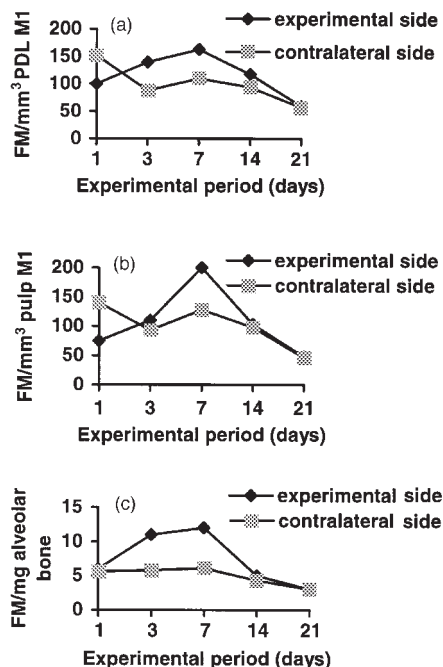
Figure 1 Blood flow changes in the (a) periodontal ligament (PDL) and (b) pulp of a first maxillary rat molar (M1) and (c) alveolar bone, 1, 3, 7, 14 and 21 days after experimental tooth movement. The number of fluorescent microspheres (FM) per mm^3 and mg tissue is taken as a measure of blood flow changes between the experimental and contralateral molars.

on the pressure side of the PDL and alveolar bone are characterized by compression, and reduction in the number of patent capillaries (Macacanpan *et al.*, 1954; Nakamura *et al.*, 1986), occlusion and partial disintegration of blood vessels (Gianelly, 1969; Rygh, 1972a), and ischaemia (Gaengler and Merte, 1983). Pulpal tissues demonstrate reduced linear velocity (Guevara and McCluggage, 1980), hyperaemia, and margination of white blood cells (Stenvik and Mjør, 1970). Correspondingly, periodontal and pulpal blood flow is reduced (Vandevska-Radunovic *et al.*, 1994; Brodin *et al.*, 1996), while blood circulation in the alveolar bone seems to be unaffected (Vandevska-Radunovic *et al.*, 1994; Figure 1). The degenerative vascular changes, as well as the impaired blood supply, lead to formation of a necrotic, hyalinized zone (Reitan,
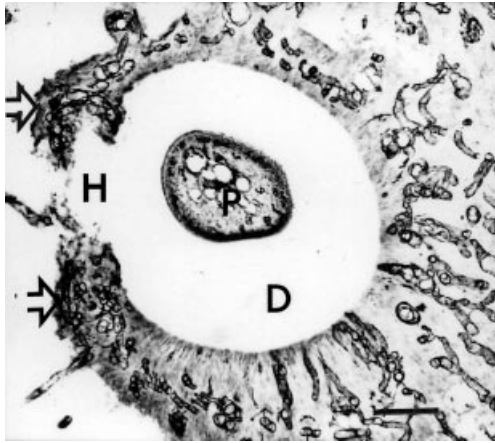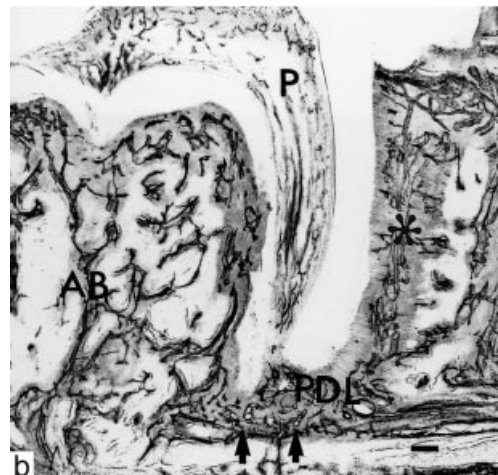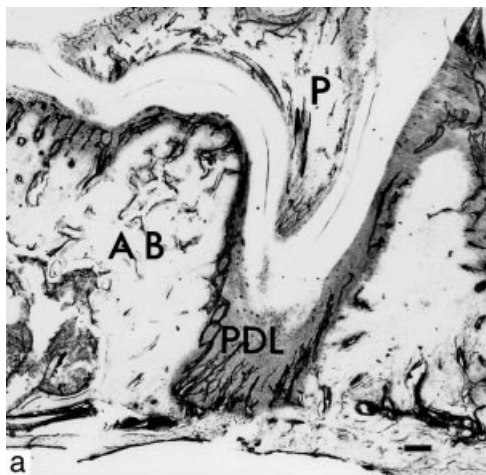
Figure 2 Horizontal section of a distal root of a first maxillary rat molar, 7 days after experimental tooth movement. Laminin immunopositive blood vessels (open arrows) proliferate and invade the hyalinized tissue (H). D = dentine; P = pulp. Bar = 0.1 mm.

1967; Rygh, 1972b, 1973, 1974; Lilja *et al.*, 1983; Nakamura *et al.*, 1996), which temporarily arrests tooth movement (Reitan, 1967; Rygh, 1976). On the tension side, periodontal vascular changes are not that dramatic (Rygh, 1976). However, early signs of acute inflammation can be observed with distension of blood vessels in

the direction of strain, blood vessel dilation and red blood cell diapedesis (Rygh, 1976; Rygh *et al.*, 1986; Cooper and Sims, 1989). Simultaneously, an increase in the mean vascular volume and number of fenestrae in capillaries and post-capillary venules is observed, indicating that the microvascular bed maintains local blood flow and fluid exchange in accord with tissue needs (Lew *et al.*, 1989).

The sequence of events following the initial vascular responses to orthodontic tooth movement denotes increased vascular activity and propagation of an inflammatory reaction. Morphologically, in the periphery of the hyalinized zone, dilation of blood vessels, proliferation, and ingrowth of vascular structures into the necrotic tissue can be observed (Rygh, 1972a, 1983; Rygh *et al.*, 1986; Hosoyama, 1989; Vandevska-Radunovic *et al.*, 1997b; Figure 2). The vascular changes are not confined only to the PDL, but encompass also the alveolar bone (Rygh *et al.*, 1986; Vandevska-Radunovic *et al.*, 1997b), whereby proliferation of blood vessels and blood-borne cells resorb the bone tissue behind the hyalinized area (Gianelly, 1969; Hosoyama, 1989; Figures 2 and 3). Vasodilation and increased vascular permeability (Warita, 1990), with fluid exudation in the connective tissue

Figure 3 Density and distribution of laminin immunopositive blood vessels in the periodontal ligament (PDL), pulp (P), and alveolar bone (AB) of a (a) control and (b) experimental first maxillary rat molar, 7 days after experimental tooth movement. Proliferation of blood vessels can be seen in the peri-apical (arrows) and distal tension areas (*) of the PDL, and in the alveolar bone. Bar = 0.1 mm.

compartment (Lew *et al.*, 1989; Tang and Sims, 1992), do not necessarily lend evidence for increased blood flow, as dilation of blood vessels can also occur during stasis and in connection with reduced blood circulation (Heyeraas-Tønder, 1983). However, quantitative analysis of the blood flow rates in the PDL, pulp, and alveolar bone support data obtained from other histological studies, and demonstrate enhanced blood flow in these tissues (Kvinnsland *et al.*, 1989; McDonald and Pitt Ford, 1994; Vandevska-Radunovic *et al.*, 1994). Knowing that remodelling of bone and periodontal structures is a cell-mediated process crucial for facilitation of tooth movement, it is conceivable that these tissues show increased blood flow and prepare for the increased cellular demand and activity.

Orthodontic tooth movement is not purely a local phenomenon, but affects the properties of neighbouring teeth and tissues. Evaluation of blood flow changes in the PDL and pulp of rat molars adjacent to the actively moved tooth, also shows significantly increased blood flow values (Kvinnsland *et al.*, 1989; Vandevska-Radunovic *et al.*, 1994). It has also been demonstrated that local application of orthodontic force induces decreased mechanical properties of the PDL of all teeth in the same dental arch during extraction (Ki, 1990). Administration of anti-inflammatory drugs during orthodontic tooth movement improves the mechanical properties of the PDL in rat molars (Ohkawa, 1982), thus confirming the development of an inflammatory reaction and the importance of the vascular elements in the re-organization of the dental supporting tissues.

Increased blood vessel volume, blood vessel dilatation and proliferation (Rygh *et al.*, 1986; Murrel *et al.*, 1996; Vandevska-Radunovic *et al.*, 1997b), and increased blood circulation (Vandevska-Radunovic *et al.*, 1994) can still be observed 2 weeks after the application of an orthodontic force in rat PDL, but not in the pulp (Vandevska-Radunovic *et al.*, 1994). The inflammatory vascular reactions subside within 3 weeks in all tissues in the rat model, provided that there has been no reactivation of the orthodontic force (Rygh, 1972a, 1973; Vandevska-Radunovic *et al.*, 1994, 1997b).

Cellular changes in dental tissues following orthodontic tooth movement

Periodontal and alveolar bone remodelling are cell-mediated processes, and require increased cell attendance, activity, and renewal. They can be provided both by proliferation and differentiation of resident periodontal cells, and by cell migration from the microvasculature to the remodelling sites. Orthodontic forces induce increased mitotic activity in fibroblasts, osteoclasts, and osteoblasts (Kvam, 1972; Roberts and Jee, 1974; Roberts *et al.*, 1974; Smith and Roberts, 1980), and proliferating cells from throughout the PDL are shown to migrate towards the alveolar bone (Roberts and Chase, 1981; McCulloch and Melcher, 1983).
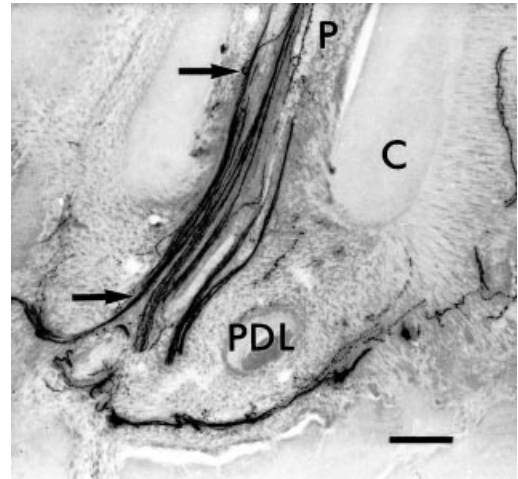
Electron microscopic investigation of the bone resorbing areas demonstrates an increased number of three different cell types: osteoclasts, mononuclear cells such as macrophages, and undifferentiated cells (Kurihara, 1977). Increased number and activity of macrophage-like cells and osteoclasts is also noticed close to the hyaline zone (Kvam, 1972; Brudvik and Rygh, 1993, 1994; Vandevska-Radunovic *et al.*, 1997a), indicating the importance of these cells not only in bone turnover, but also in the removal of the necrotic periodontal tissue (Figure 4). It has been suggested that the undifferentiated, progenitor cells can transform into osteoclasts, osteoblasts, fibroblasts, or phagocytic cells (Kurihara, 1977), depending on the local tissue need. As the progenitor cells in the PDL are primarily located in paravascular sites and/or vascular channels of the alveolar bone (McCulloch *et al.*, 1987; Lekic and McCulloch, 1996), it is clear that an adequate blood supply is necessary for periodontal cell renewal and also in cases of increased cellular demand.

The development of an inflammatory reaction in the dental tissues following experimental tooth movement is further supported by data from experiments using intra-vital microscopy. Continuous compression, imitating initial application of orthodontic force, induces increased vascular permeability (Warita, 1990; Iida *et al.*, 1992) and leukocyte migration from the micro-vasculature into the extra-vascular spaces (Iida
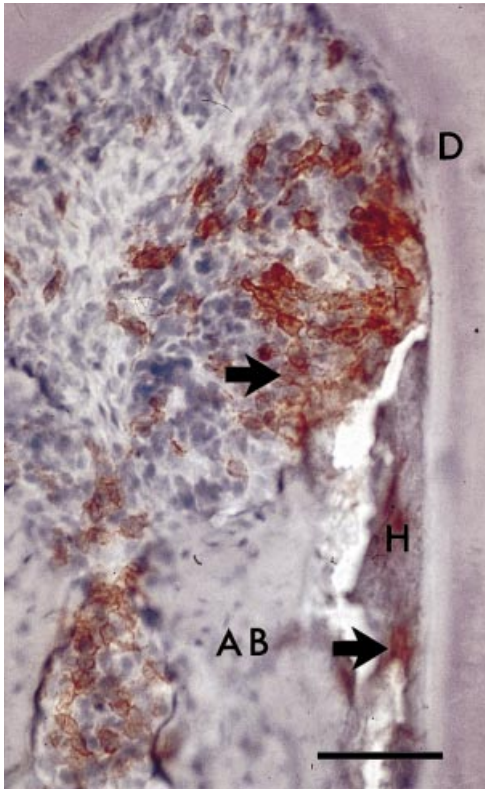
Figure 4 Inter-radicular area of a first maxillary rat molar, 7 days after experimental tooth movement. Numerous macrophage-like (CD11b+) cells surround and invade the hyalinized tissue (H). AB = alveolar bone; D = dentine. Bar = 0.1 mm. (*European Journal of Oral Science* 1997; 105: 36–44, published with kind permission.)


Figure 5 Peri-apical area of a maxillary rat molar distal root, 7 days after experimental tooth movement. CGRP immunoreactive nerve fibres (arrows) demonstrate increased density both in the peri-apical area of the periodontal ligament (PDL) and root pulp (P). C = cementum. Bar = 0.1 mm.
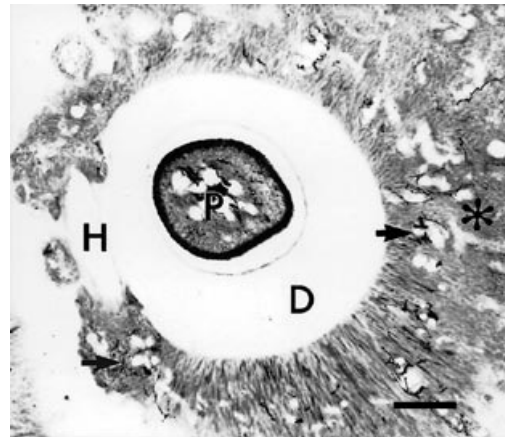
Figure 6 Serial section to Figure 2. Numerous nerve sprouts immunoreactive to protein gene product (PGP) 9.5 are seen close to blood vessels (arrows) both in the distal tension area (*) and near hyalinized tissue (H). D = dentine; P = pulp. Bar = 0.1 mm.

et al., 1996). Electron microscopic observations identify these cells as monocytes and polymorphonuclear leukocytes (Iida *et al.*, 1992), suggesting that cells from the monocyte granulocyte lineage differentiate into macrophages and osteoclast precursor cells. There is extensive evidence to support the concept that osteoclasts arise by fusion of mononuclear phagocytes derived from blood monocytes (Kahn *et al.*, 1978; Teitelbaum and Kahn, 1980; Sminia and Dijkstra, 1986). While increased number and distribution of mononuclear phagocytic cells have been clearly demonstrated, especially in the vicinity of hyalinized tissues (Jäger *et al.*, 1993; Vandevska-Radunovic *et al.*, 1997a), no changes in the number and distribution of lymphocytes
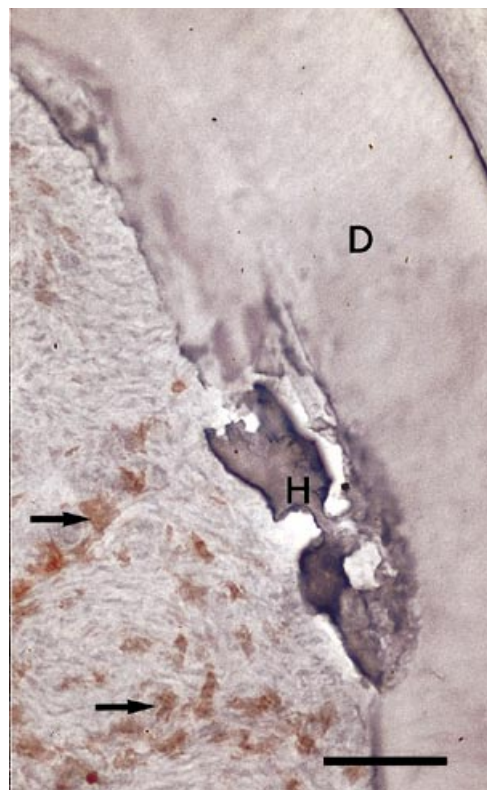
and granulocytes have been reported (Kurihara, 1977; Rygh *et al.*, 1986; Vandevska-Radunovic *et al.*, 1997a). Absence of these cell types provides evidence for an aseptic inflammatory reaction to experimental tooth movement, where macrophage-like cells are the dominant cell
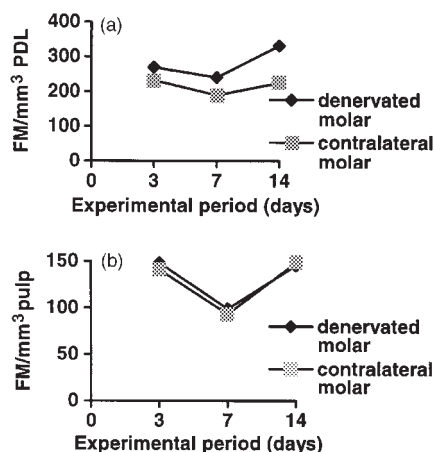
Figure 7 Blood flow changes in the (a) periodontal ligament (PDL) and (b) pulp of a denervated and contralateral, innervated first mandibular rat molar, 3, 7 and 14 days after axotomy of the inferior alveolar nerve (IAN) and experimental tooth movement. The number of fluorescent microspheres (FM) per mm^3 tissue is taken as a measure of blood flow. Re-innervation of the periodontal and pulpal tissues initiates at approximately 14 days, and coincides with the statistically significant difference in periodontal blood flow between the denervated and contralateral molar.

Figure 8 Inter-radicular area of a rat molar, 7 days after axotomy of the inferior alveolar nerve (IAN) and experimental tooth movement. Macrophage-like (CD11b$^+$) cells (arrows) approach, but do not invade the hyalinized tissue (H). D = dentine. Bar = 0.1 mm. (*Journal of Dental Research* 1999; in press, published with kind permission.)

type. They serve as scavengers, which remove damaged and necrotic material, and secrete a number of biologically active substances that control growth and replication of other cells, such as fibroblasts (Nathan, 1987; Adams and Hamilton, 1992).

Another cell type identified in dental tissues known to participate in the development of inflammatory reactions is the mast cell (Zachrisson, 1967; Yamasaki *et al.*, 1982; Church *et al.*, 1989; Silberstein *et al.*, 1991; Matsson *et al.*, 1995). The early change in the number of toluidine positive mast cells after initiation of orthodontic tooth movement has been interpreted as an early stage of inflammation due to the release of chemical mediators (Yamasaki *et al.*, 1982). Despite the evidence that mast cells and their mediators participate in bone remodelling and provide signals for the recruitment of osteoclast progenitors (Silberstein *et al.*, 1991), they represent only one of the redundant mechanisms providing for skeletal homeostasis (Silberstein *et al.*, 1991; Zernik and Minken, 1992).

Inflammatory mediators and orthodontic tooth movement

Vascular and cellular inflammatory changes are mediated and maintained by a number of biochemical substances. These mediators are detected in increased levels in dental tissues incident to orthodontic tooth movement and are secreted by the existing inflammatory cells (Henson *et al.*, 1992; Postlethwaite and Kang, 1992). They can act in autocrine or paracrine fashion, either stimulating or inhibiting cellular activity (Tsunawaki *et al.*, 1988; Pretus *et al.*, 1989; Ozaki *et al.*, 1996).

Interleukin-1 (IL-1) and tumour necrosis factor (TNF) are pro-inflammatory cytokines known to induce synthesis of various proteins that, in turn,

elicit acute or chronic inflammation (Dinarello, 1992; Ozaki *et al.*, 1996). Furthermore, *in vitro* studies show that these molecules are stimulators of bone resorption (Gowen and Mundy, 1986; Pfeilschifter *et al.*, 1989). A marked increase in the staining intensity for IL-1 and TNF is noticed in cells of the PDL and alveolar bone of ortho-dontically moved cat canines, implying their activity in the bone remodelling processes (Davidovitch *et al.*, 1988; Davidovitch, 1991; Saito *et al.*, 1991b).

Orthodontic mechanical forces are also shown to induce increased levels of PGs (Yamasaki, 1983; Davidovitch *et al.*, 1989) and leukotrienes (Mohamed *et al.*, 1989) in both periodontal and bone cells. Together, PGs and leukotrienes are potent inflammatory mediators that lead to increased blood flow and blood vessel permeability, and induce chemotaxis (Lewis and Austen, 1981; Davies and MacIntyre, 1992; Lam and Austen, 1992). They are believed to acceler-ate bone remodelling processes (Leiker *et al.*, 1995), as substances that inhibit PG and leuko-triene synthesis lead to significant reduction of tooth movement (Sandy and Harris, 1984; Mohamed *et al.*, 1989; Giunta *et al.*, 1995). It has been suggested that one of the possible mech-anisms through which PGs and leukotrienes affect tooth movement is via inhibition of the inflammatory response (Mohamed *et al.*, 1989).

Innervation and inflammation incident to orthodontic tooth movement

Innervation of dental tissues

Dental tissues receive sensory, sympathetic and parasympathetic innervation, but the majority of the nerve fibres belong to the trigeminal sensory neurones (Byers and Holland, 1977; Silverman and Kruger, 1987; Wakisaka *et al.*, 1987, 1996; Linden, 1990; Wakisaka, 1990; Heyeraas *et al.*, 1993; Hildebrandt *et al.*, 1995; Byers and Maeda, 1997). In addition to nerves having their perikarya in the trigeminal ganglion, the PDL receives axons from the mesencephalic trigeminal nucleus (Byers *et al.*, 1985, 1986; Millar *et al.*, 1989; Linden *et al.*, 1994). The latter have their nerve endings mainly in the apical periodontal

area, while nerve endings from the trigeminal ganglion are concentrated in the cervical and mid-root area. Periodontal nerve endings consist of mechanoreceptors, mostly Ruffini-like (Byers *et al.*, 1985; Maeda *et al.*, 1989; Loescher and Holland, 1991) and nociceptors (Jyväsjärvi *et al.*, 1988). The mechanoreceptors are low-threshold, respond to stretch, and are concentrated between the fulcrum and the apex of the tooth (Linden, 1990). Nociceptors are high-threshold, activated by heavy forces, tissue injury, and inflammatory mediators (Jyväsjärvi *et al.*, 1988; Dong *et al.*, 1993), and belong either to the unmyelinated C fibres or small myelinated Aδ fibres (Sato *et al.*, 1992; Byers and Maeda, 1997). They occur less frequently than the mechano-receptors in the PDL (Byers and Maeda, 1997), but predominate in the pulp (Hildebrand *et al.*, 1995). Under physiological conditions periodontal and pulpal nociceptors are silent in terms of electrophysiology, but not inactive (Byers and Maeda, 1997). They contain various neuro-peptides (Olgart *et al.*, 1977; Gazelius *et al.*, 1981; Holzer, 1988; Heyeraas *et al.*, 1994; Maggi, 1995), including calcitonin gene-related peptide (CGRP) and SP, which are synthesized in the perikaryon and transported to both central and peripheral processes (Hökfelt *et al.*, 1975). Peripherally, the neuropeptides are released in the tissues where they take part in tissue homeostasis (Olgart *et al.*, 1991; Olgart, 1996; Jacobsen and Heyeraas, 1997). Centrally, evidence exists that CGRP and SP, in addition to other neuropeptides, act as neurotransmitters (Hökfelt *et al.*, 1980; Salt and Hill, 1983).

Nociceptor endings in the PDL and pulp demonstrate plasticity in response to different stimuli. Pulpitis (Byers *et al.*, 1990), traumatic occlusion (Kvinnsland and Heyeraas, 1992) and orthodontic tooth movement (Kvinnsland and Kvinnsland, 1990; Vandevska-Radunovic *et al.*, 1997b) induce sprouting of CGRP- and SP-containing nerve fibres, and increase in neuro-peptide intensity.

Neuropeptides and inflammation

The contribution of distinct neuropeptides to particular aspects of tissue-specific inflammatory

responses is firmly established and proves that afferent nerve fibres not only serve a sensory role, but take part in local effector mechanisms (Basbaum and Levine, 1991; Payan, 1992; Agro and Stanisz, 1995; Heller et al., 1995). CGRP and SP are potent vasodilators (Brain et al., 1985; Gazelius et al., 1987), induce increased vascular permeability (Mayer et al., 1988; Ohkubo et al., 1993), stimulate plasma extravasation, and proliferation of endothelial cells and fibroblasts (Hægerstrand et al., 1990; Figini et al., 1997), and upregulate the expression of endothelial cell adhesion molecules (Smith et al., 1993). These neuropetides are considered to be mediators of neurogenic inflammation, as depletion of CGRP- and SP-immunoreactive nerve fibres by capsaicin pre-treatment or surgical denervation attenuates the vascular inflammatory responses (Jansco et al., 1967; Kowalski and Kaliner, 1988; Mayer et al., 1988; Fazekas et al., 1990; Pedersen-Bjergaard et al., 1991; Ohkubo et al., 1993; Olgart et al., 1993; Györfi et al., 1994; McDonald et al., 1996; Vandevska-Radunovic et al., 1998).

Apart from these vasoregulatory effects, CGRP and SP are shown to have direct and indirect influence on cells known to participate or be affected by the inflammatory responses. Monocytes, lymphocytes, and mast cells express specific neuropeptide receptors (Umeda and Arizawa, 1989; Abello et al., 1991; Crivellato et al., 1991), which transduce intracellular signals giving rise to cellular responses (Roch-Arveiller et al., 1986; Foreman, 1987; Payan et al., 1987; Peck, 1987; Lotz et al., 1988; Laurenzi et al., 1990; Foster et al., 1992; Payan, 1992; Chancellor-Freeland et al., 1995). These responses include altered cytokine synthesis and release, production of other peptides that may amplify response or cause release of additional neuropeptides, changed expression of adhesion receptors, or direct release of mediators (Roch-Arveiller et al., 1986; Foreman, 1987; Peck, 1987; Lotz et al., 1988; Laurenzi et al., 1990; Foster et al., 1992; Payan, 1992; Chancellor-Freeland et al., 1995). Moreover, neuropeptides can directly influence connective tissue cells, either by inducing their proliferation, or by changing the expression of surface molecules (Nilsson et al., 1985; Payan, 1992).

The ability of neuropeptides to act on endothelial, connective and especially immune cells is not unidirectional. Expression of classic cytokines and their corresponding receptors by endocrine and nervous tissue cells has been clearly demonstrated (Arzt et al., 1993; Cunningham and De Souza, 1993; Blalock, 1994). Cytokines of the interleukin family are involved in regulation of neuronal differentiation (Mehler et al., 1993) and pituitary cell proliferation (Arzt et al., 1993), providing evidence that the immune and neuroendocrine systems share common ligands and receptors, representing an integrated information circuit (Blalock, 1994; Savino and Dardenne, 1995). These neuro-immune interactions can occur, and most probably take place in dental tissues during orthodontic tooth movement.

Sensory innervation and orthodontic tooth movement

Orthodontic tooth movement affects the number, functional properties and distribution of both mechanoreceptive and nociceptive periodontal nerve fibres. Mechanoreceptors deriving mainly from myelinated fibres, show higher force thresholds, reduced conduction velocities, and decreased number, even after the removal of the orthodontic appliance (Loescher et al., 1993; Long et al., 1996). These changes are attributed to nerve fibre degeneration, being in favour of the notion that orthodontic forces induce nerve injury which might be permanent (Long et al., 1996).

Nociceptive nerve fibres demonstrate different reactions to experimental tooth movement. In stressed teeth, both the PDL and pulp show intensified staining, and pronounced sprouting of nerve fibres immunoreactive to CGRP and SP (Davidovitch et al., 1988, 1989; Kvinnsland and Kvinnsland, 1990; Nicolay et al., 1990; Saito et al., 1991a; Vandevska-Radunovic et al., 1997b; Figure 5). In addition, these fibres show changed morphology and distribution (Kvinnsland and Kvinnsland, 1990; Vandevska-Radunovic et al., 1997b), which all together coincide and co-localize with the vascular and cellular dental tissue changes (Vandevska-Radunovic et al.,

1994, 1997a,b; Figure 6). It has also been shown that the increased density of CGRP- and SP-immunopositive nerves persists in the rat PDL, pulp and marginal gingiva after the removal of the orthodontic appliance (Norevall *et al.*, 1995) and concurs with the reparative tissue processes.

Davidovitch *et al*. (1988) were the first to propose the hypothesis that neurotransmitters, released in the PDL following the application of an orthodontic force, interact with endothelial cells and lead to rapid vasodilatation, followed by extrusion of plasma and cells. They suggested that the peripheral nervous system acts as a link between the physical stimulus and the bio-chemical responses, as paradental tissues demon-strate not only increased SP immunoreactivity, but also elevation in cyclic nucleotides (Davidovitch *et al*., 1988, 1989). Although the increased density of CGRP and SP containing fibres, which coincides with the vascular and cellular changes during tooth movement (Rygh *et al*., 1986; Davidovitch *et al*., 1988; Vandevska-Radunovic *et al*., 1994, 1997a,b), is strongly in favour of this hypothesis, there has so far been no direct evidence that peptidergic sensory nerves control the development of the early inflammatory and later reparative dental tissue reactions.

Evidence exists that SP and CGRP play a pro-inflammatory role in disease, and a beneficial role in wound healing in various tissues (for review see Maggi, 1995; Brain, 1997). During inflammation, and especially after nerve axotomy, changes in peptide expression in primary sensory neurones occur, which can have functional implications in the course of an inflammatory condition (see Hökfelt *et al*., 1994). Therefore, it was logical to assume that deprivation of the sensory nerve supply in dental tissues could lead to a changed inflammatory response once ortho-dontic forces are applied (Vandevska-Radunovic *et al*., 1998, 1999). Recently, axotomy of the inferior alveolar nerve (IAN) has been shown to impede and postpone periodontal and pulpal blood flow, until a sensory tissue re-innervation is established (Vandevska-Radunovic *et al*., 1998; Figure 7). Re-innervation processes and the con-current blood flow increase during orthodontic

tooth movement are found to precede the recruitment of immunocompetent cells of the mononuclear phagocytic lineage (Vandevska-Radunovic *et al*., 1999; Figure 8). This observation suggests that not only sensory re-innervation, but also increased blood supply is a prerequisite for the deployment of mononuclear phagocytes into inflamed dental tissues. Furthermore, it has been found that denervation reduces bone formation (Duan *et al*., 1993). Collectively, these data clearly show that neurogenic mechanisms take part and modulate the development of the early inflammatory, as well as later reparative processes incident to orthodontic tooth movement.

Concluding remarks and future research

Denervation of dental tissues by sectioning the IAN has proven to be a suitable model system for analysing efferent actions of sensory nerves. Such studies clearly demonstrate the regulatory role of the sensory nerve fibres in the control and development of inflammatory reactions during orthodontic tooth movement. Thus, the dental tissue reactions are a result of both direct and indirect activity of the neuropeptides released from periodontal nerve endings after application of orthodontic force as schematically presented in Figure 9.

Development of an inflammatory reaction is a prerequisite for tooth movement. As sensory denervation of dental tissues attenuates the inflammatory response, several questions arise: can teeth be moved if their sensory innervation is compromised? If they can, will it be slower and to what extent will it have clinical relevance? The issue seems to be of importance, especially in cases where combined orthodontic and extensive surgical treatment is chosen, and where sensory innervation is severed. Other aspects that require investigation are the effects of sensory nerves and neuropeptides on hard tissue and cement formation, and, in this respect, root resorption. The mechanisms of dental root resorption related to orthodontic tooth movement are still obscure and it is possible that by using the denervation-model-system this phenomenon can partly be elucidated.
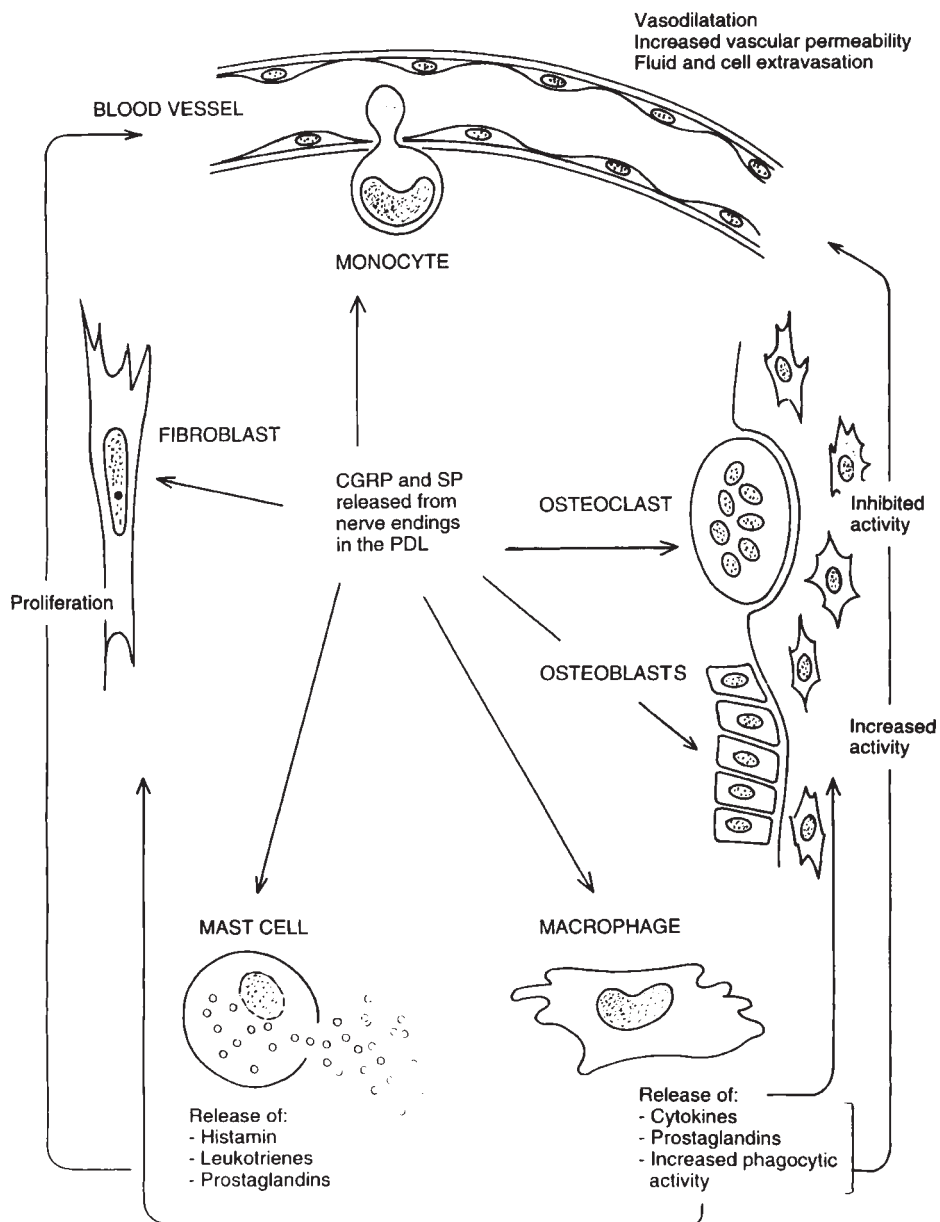
Figure 9 Schematic presentation of the tissue responses to calcitonin gene-related peptide (CGRP) and substance P (SP) released from periodontal nerve endings following application of an orthodontic force.

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