# IL-1α AND TNF-α EXPRESSION DURING EARLY PHASES OF EXPERIMENTAL ORTHODONTIC TOOTH MOVEMENT

## An immunohistochemical study

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**ABSTRACT**

Remodeling of the periodontium after application of mechanical forces constitutes the basis of clinical orthodontics. Orthodontic tooth movement causes bone resorption in the pressure area and bone deposition in the tension side with various immunoregulatory molecules being involved in the above mentioned biological bone remodeling processes. The pro-inflammatory cytokines IL-1α and TNF-α are prominent examples of such molecules due to their involvement in bone resorption.

The aim of this study was to investigate the expression of IL-1α and TNF-α during early phases of experimentally induced orthodontic tooth movement. Eight-teen male Wistar rats were used. All maxillary right first molars were moved orthodontically with a force of 0.5 N for 3 hours, 1 day and 3 days (6 rats for each time period). The contra-lateral sides served as untreated controls. Parasagittal sections of the maxillary molars and the surrounding tissues were subjected to immunohistochemical staining for IL-1α and TNF-α. The mesial aspect of the distal root, the distal aspect of the mesial root, the gingiva and the periodontal ligament mesially and distally to the first molars, as well as the pulp tissue were evaluated using light microscopy.

IL-1α and TNF-α were expressed in both the mesial aspect of the distal root and the distal aspect of the mesial root (compression and tension zones respectively) of the experimental sides. The TNF-α expression began as early as 3 hours after force application, whereas IL-1α showed increased expression after 1 day. Increased intensity of staining for both cytokines was observed in the 1-day and 3-days groups. The compression zone exhibited heavier staining than the tension zone for both IL-1α and TNF-α. Moreover, these cytokines were expressed in the gingiva of both control and experimental sides. Nevertheless, the orthodontically moved teeth demonstrated heavier gingival staining than the controls. Minor changes in the expression of these cytokines were seen in the pulp tissue.

## 1. INTRODUCTION

Orthodontic tooth movement (OTM) is the result of organized remodeling of the periodontal tissues after application of mechanical forces. At the cellular level, remodeling of the periodontium consists of bone resorption adjacent to the periodontal ligament (PDL) in the compression zone, bone apposition in the tension zone, and degeneration and re-establishment of the PDL [1]. A number of factors have been recognized to participate in the rather complex orchestration of tissue remodeling during orthodontic tooth movement. It has been proposed that chemical mediators as cytokines play an important role [2]. Cytokines are small protein molecules that regulate cell communication and function and are actively secreted by different cell types in response to external stimuli. It has been proposed that during OTM, these signaling molecules are produced by inflammatory cells that migrated from dilated PDL capillaries after orthodontic force application [3].

Interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) are key mediators in acute-phase inflammatory reactions with overlapping activities. These two pro-inflammatory cytokines were members of the formerly known osteoclast-activating factor (OAF) and have been implicated in the bone remodeling process [4,5]. IL-1 exists in two isoforms with species differences in osteoclastic activity. In humans, IL-1β constitutes most of the OAF activity [4] whereas in rodents IL-1α appears to predominate [6]. In addition, both isoforms of IL-1 are more potent in stimulating bone resorption than the TNFs [7]. TNF-α induces the production of IL-1 [8] and IL-1 stimulates its own synthesis in a positive feedback loop [9,10]. At sites of inflammation IL-1 and TNF-α are expressed in large quantities by macrophages [11], as well as by many other cell types, including fibroblasts [12], osteoblasts [13] and osteoclasts [14]. IL-1 and TNF-α have been linked to various chronic inflammatory diseases where bone resorption is the main pathogenetic feature such as, rheumatoid arthritis, osteomyelitis, marginal and apical periodontitis. Furthermore, IL-1α and its receptor has been shown to be

of some importance in tissue homeostasis and remodeling during physiological tooth movement [15]. IL-1α and IL-1β have been demonstrated immunohistochemically in the PDL of cat canine in the early stages of orthodontic tooth movement, i.e., at 12 and 24 hr [3]. In rats, the induction of IL-1β was observed to reach a maximum 3 days after application of mechanical force [16]. Increased levels of IL-1 and TNF-α have been detected in gingival crevicular fluid of orthodontic patients[16-20] speculating that the elevated cytokines in GCF reflected the biological responses induced by mechanical stress. The IL-1β and TNF-α synthesis in GCF during tooth mobilization appeared to level off 24 hr after force application [19,20]suggesting a central role of these cytokines in the early phase of orthodontic tooth movement.

Although there is significant amount of data on cytokine production during orthodontic tooth movement, little information is available regarding the time frame where the cytokines are expressed. It was therefore, the purpose of this study to explore immunohistochemically the expression of IL-1α and TNF-α in the dental tissues 3 hours, 1 day and 3 days after orthodontic force application in order to provide more information on the involvement and interplay of these cytokines in the early events of orthodontic tooth movement. Furthermore, to observe if inflammatory changes are induced in the soft tissues at the onset of orthodontic tooth movement, the gingiva and the pulp of the orthodontically moved molars were also evaluated.

## 2. MATERIALS AND METHODS

### *2.1. Animal Preparation*

The material comprised of 18 male Wistar rats (Mol:WIST Han) weighing 255-247 g. All rats were housed in polycarbonate cages (2-3 rats per cage) and fed a standard pellet diet (801157W Expanded Pellets, Stepfield, Witham, Essex, UK) with tap water *ad libitum*. The

experimental protocol was approved by the Regional committee for Animal Research Ethics, University of Bergen, under the supervision of the Norwegian Experimental Animal Board. After one week of acclimatization period, the rats were anaesthetized with a subcutaneous injection of 0.15-0.2 ml/100 g body weight fentanyl (Dormicum; F.Hoffmann-La Roche & Co. AG, Basel, Switzerland)/fluanizon midazolam (Hypnorm; Janssen Pharmaceutica, Beerse, Belgium). The first right maxillary molar was moved mesially by a fixed orthodontic appliance consisting of a coil spring (Elgiloy spring, F-31 0.008 × 0.032, Rocky Mountain Orthodontics, Denver, Colorado, USA) ligated to the mesial aspect of the first maxillary molar [21]. The spring was activated to deliver a force of 50 g (500 mN) and ligated through the eyelet on an incisor band. The orthodontic force was applied for 3 hours (3hr), 1 day (1d) or 3 days (3d) (6 rats per time period). There was no reactivation of the spring throughout the experiment.

At the end of the experimental periods, the rats received an overdose of anesthesia (Dormicum/Hypnorm) and were transcardiacally perfused through the aorta with heparinized saline followed by 4% paraformaldehyde with 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. The orthodontic appliances were removed and the maxillae (both right and left halves) dissected out and postfixed overnight. The left maxillae were used as untreated controls. The specimens were rinsed in 0.1M phosphate buffer and decalcified in 10% EDTA for approximately 5 weeks. The decalcified jaws were then rinsed in 0.1M phosphate buffer, soaked in 30% sucrose overnight and stored frozen at -80$^{o}$C. Maxillae were embedded in Tissue-Tek OCT compound (Sakura, Zoeterwoude, Netherlands), and 20-25-$\mu$m-thick parasagittal sections including first, second and third molars were cut in a -20$^{o}$C cryostat.

*2.2. Immunohistochemistry*

The immunohistochemical procedure has been previously described [22]. The immunoreactions were performed on pre-coated glass slides (SuperFrost Plus, MenzelGlaser,

Braunschweig, Germany). Alternate serial sections of the maxillae were rinsed in Tris-buffered saline (TBS), treated with 5% polypeptide in TBS for 30 min, with 1% hydrogen peroxide for 30 min, followed by 2 hr incubation with 2.5% normal rabbit serum (Vector Laboratories Inc., Burlingam, CA, USA). The sections were thereafter incubated for 72 hr in rat IL-1α (dilution 1:400, Endogen, MA, USA) or TNF-α (dilution 1:300, Endogen) polyclonal antibodies raised in rabbit, at 4$^{o}$C. The specificity of the immune reactions was tested by omission of the primary antibody. Antigen-antibody complexes were detected by the avidin-biotin peroxidase (ABC) method, using a commercially available kit (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) and visualized by 3,3'-diaminobenzidine (DAB, Sigma) in the presence of 0.2% $(NH_4)_2Ni(SO_4)_26H_2O$ to enhance the immunostaining. Finally, the sections were counterstained with methylene blue/azure II in 1% sodium borate and distilled water. The sections were then dehydrated in graded alcohol series, cleared in xylene and cover slipped with Eukitt (O.Kindler, Freiburg, Germany).

*2.3. Section evaluation*

Serial sections of both right and left maxillae were evaluated in a Nikon photomicroscope (Nikon Eclipse E600, Nikon Instruments Inc., Japan). The sections containing at least 2/3rds of root length in the coronal-apical direction were chosen for evaluation. The areas of investigation were the mesial aspect of the distal root, the distal aspect of the mesial root, the gingiva and the PDL mesially and distally to the first molar and the pulp tissue along the mesial and/or the distal root, as well as the pulp adjacent to the furcation area (Fig. 1). If all the investigated areas were not properly seen in the same section, then the area in doubt was marked as missing.

At least six sections from each specimen, stained for each one of the two primary antibodies met the criteria and were chosen for further evaluation. Preliminary results from the contra-lateral control sides showed a similar staining pattern in the investigated areas regardless

experimental period and therefore, only 7 specimens (1-4 animals each time period) were sectioned and processed for immunohistochemistry.

The immunohistochemical staining reaction for either IL-1α or TNF-α was registered as no staining, light staining, moderate staining and heavy staining. The pulp tissue was only qualitatively evaluated and changes in the staining pattern were observed. When the individual rat served as unit, all the sections had to be in the "no staining" category in order to assign the rat as "no staining" unit. Otherwise, it was placed in the "staining" category.

## 3. RESULTS

All the negative controls, where primary antibody was omitted, showed a lack of specific immunohistochemical staining reaction.

### *3.1. Controls*

No staining for IL-1α was found in the investigated areas, along the mesial and the distal aspects of the molar roots, in all control specimens (Table 1, Figs. 2 and 3a,b). However, light TNF-α staining was observed in 5 of 7 animals (Table 2). In the gingiva, light to moderate staining was observed in all rats, for both IL-1α and TNF-α. The staining was mainly localized in the oral sulcular and gingival epithelium (Fig. 4a,c). In the pulp, the odontoblast layer stained for IL-1α whereas scarce cells in the pulp body were stained for TNF-α.

### *3.2. Experimental tooth movement*

#### *3.2.1. IL-1α*

*Compression area (mesial aspect of the distal root)*

Three hours after force application, light staining for IL-1α appeared in low number of sections (4 of 6 animals) (Table 1). As the time of force application progressed, positive staining was seen in all animals (Table 1) but it did not appear in all evaluated sections, indicating a localized reaction for this cytokine in specific area. Increased staining intensity

was observed toward a longer experimental period, as more sections were registered as moderate and heavy staining in the 3d than the 1d group (Fig. 2).

*Tension area (distal aspect of the mesial root)*

Only 2 of 40 sections (5%) were registered as lightly stained for IL-1α 3hr after force application (Fig. 2). The staining intensity increased with time as more sections were registered as light and moderate staining in the 3d than in the 1d group (Table 1 and Fig. 2). None of the evaluated sections exhibited heavy staining for this cytokine (Fig. 2).

*Gingival tissue and PDL mesially to 1$^{st}$ molar*

After 3hr of orthodontic force application, there were no changes in the staining pattern of the gingiva. Light to moderate staining was seen in the gingival epithelium. One and 3 days after OTM, the staining in the mesial gingiva was heavier than the controls and extended to the subjunctional area, the PDL and the alveolar bone (Table 1 and Fig. 2).

*Gingival tissue and PDL distally to 1$^{st}$ molar*

All sections from the three groups stained positively for IL-1α (Table 1). Three hours of force application did not seriously affect the staining pattern in the distal gingiva. Eight of 37 sections (21.6%) demonstrated heavy staining at 3hr (Fig.2). As the time of force application progressed, the staining intensity increased. After 3 days of tooth movement, the majority of the sections stained heavily for this cytokine (Figs. 2 and 4b).

*Pulp*

Generally, no differences in IL-1α staining were observed in the pulp tissue of orthodontically moved teeth compared to controls. The staining reaction for IL-1α at the odontoblast layer could be compared with the control sections. Occasionally, some sections had lighter staining of the odontoblasts in the root pulp adjacent to the compression zone compared with the same area on the control side.

*3.2.2. TNF-α*

*Compression area (mesial aspect of the distal root)*

More than half of the sections showed light staining for TNF-α 3hr after force application (Fig. 5). More sections with heavy staining reaction were observed as the time of OTM progressed (Fig. 5). Only moderate and heavy staining was registered after 3 days of tooth movement (Fig. 5), indicating continuous up-regulation of this cytokine (Fig. 6).

*Tension area (distal aspect of the mesial root)*

Early up-regulation of TNF-α was seen at the tension area, as half of the sections exhibited light staining after 3 hours (Fig. 5). Increased staining intensity was observed over time, nevertheless, only a small number of sections were registered as heavy staining for this cytokine in both 1d and 3d groups (Figs. 5, 6).

*Gingival tissue and PDL mesially to 1$^{st}$ molar*

There was an immediate increase in staining intensity after force application (Fig. 5). After 3 days, the majority of the sections were stained moderate (56.8%) to heavy (21.6%) (Fig. 5).

*Gingival tissue and PDL distally to 1$^{st}$ molar*

There was a continuous up-regulation of TNF-α in this area. Heavy staining reaction was registered in 10% of the sections after 3 hours of tooth movement (Fig. 5). All the sections stained positively in the 1d and 3d groups with increasing number of sections stained heavily as the time progressed (Table 2 and Fig. 5). Additionally, the staining was extended to the subjunctional area, the PDL and the alveolar bone (Fig. 4d).

*Pulp*

Frequently, more stained cells appeared in the pulp body, especially in the 3d-group. These stained cells were located at the pulp floor. There was no difference in TNF-α expression in the root pulp compared to the control side.

## 4. DISCUSSION

The pro-inflammatory cytokines IL-1 and TNF-α as typical mediators of acute phase inflammatory reaction have been shown to play an important role in the pathogenesis of inflammatory periodontal disease [23-25]. In the present study, using immunohistochemistry, we have explored the expression of the pro-inflammatory cytokines IL-1α and TNF-α in the periodontal tissues during the early stages of experimental orthodontic tooth movement. The induction of IL-1α and TNF-α was observed at 3hr, 1 and 3 days after mesial movement of the maxillary first molar. In general, only light staining reaction for both IL-1α and TNF-α was observed after 3 hr of orthodontic force application while the intensity of the staining increased as the time of force application progressed. Moreover, extensive staining reaction was observed in the proximal gingiva, mainly in the junctional epithelium that extended toward the subjunctional area and the PDL after insertion of the orthodontic appliances. After orthodontic force application, necrosis of the PDL in the compressed areas leading to the formation of a cell-free so called "hyalinized" tissue, followed by osteoclastic resorption of the adjacent alveolar bone, have been repeatedly demonstrated [21,26-28]. Chemical mediators from the necrotic tissue, neurotransmitters as well as inflammatory mediators such as cytokines, have been claimed to play an important role in the recruitment, invasion and stimulation of the resorptive cells [29]. Recruitment of osteoclasts to compression sites in orthodontic tooth movement requires 1 to 3 days after initial appliance activation [30,31]. Hence, it is rational that up-regulation of IL-1α and TNF-α takes place earlier on and the staining becomes heavier over time as a result of the osteoclasts' contribution since they also express these cytokines upon activation. In addition, the compression area exhibited heavier staining for the examined cytokines than the tension area. This observation can be attributed

to the fact that most of the tissue re-organization phenomena during orthodontic tooth movement occur at the compression zone.

The increased IL-1α staining over time in tension and compression areas was in accordance with previous animal experiments [3,16]. It has been shown that the induction of IL-1β after mechanical stress in rats reaches a maximum on day 3 and declines thereafter [16]. The present findings are related since the 3d group exhibited heavier staining than the 1d group and clearly demonstrate that up-regulation of this cytokine begins as early as 1 day after force application.

In the present study, up-regulation of TNF-α in response to orthodontic movement began earlier than IL-1α, already at 3 hours after force application. In contrast to our findings, Alhashimi et al., [2001] found no TNF-α mRNA expression detected in rats 3, 7 and 10 days after application of orthodontic force. The conflicting results in the former study were explained as a negative signal during the process of gene transcription. In other words, when TNF-α protein is produced and released it acts as a negative feedback mechanism for the gene transcription in order to shut down quickly the production of this cytokine. It is possible that TNF-α is produced and released in the very early stages of the inflammatory reaction that takes place during orthodontic tooth movement; TNF-α exerts its biological activities such as induction of IL-1 [8] and its increased protein levels down-regulate further production. As IL-1α is 25-fold more potent than TNF-α in stimulating bone resorption [7], it is rational to assume that an earlier appearance of TNF-α plays an indirect role in bone resorption by stimulating IL-1 production.

Additionally, light staining reaction of TNF-α was not uncommon in the control specimens whereas no IL-1α staining was found in the PDL of the control specimens. This finding is partly in accordance with previous data that showed the above cytokines focally transcribed in bone marrow cells and in single osteoblasts and osteoclasts in rat jaws under physiological

condition [15]. However, only IL-1α was detected at the protein level in that study. Interpreting the current TNF-α immunohistochemical observations of the control specimens, it is likely that TNF-α contributes in tissue homeostasis and remodeling during physiological tooth drift.

Besides cells of the monocytes/macrophage lineage, another source of cytokines appeared to be cells in the gingiva. It was clearly demonstrated in this study that the gingival epithelium was constitutively stained for both IL-1α and TNF-α. Moreover, the staining reaction was heavier than the one observed in the investigated areas along the roots. This observation was in agreement with previous data [15,22,32]. The increased gingival staining reaction as well as the extension of the staining at the subjunctional area and the PDL as the time of force application progressed is more likely attributed to the orthodontic appliance *per se* rather than the mechanical force. Mechanical trauma upon insertion of the orthodontic band and food impaction between the 1$^{st}$ and 2$^{nd}$ molars can explain the more severe inflammatory reaction in the distal compared to the mesial gingiva. However, we cannot completely exclude the contribution of orthodontic forces in the cytokine production at these sites. Elevated levels of IL-1 and TNF-α have been also demonstrated in GCF of human orthodontic patients when plaque induced inflammation was prevented [18-20] Taken together, we hypothesize that the gingival tissues may participate in bone remodeling via paracrine pathways during orthodontic tooth movement.

No major changes were seen in the molar pulp regarding cytokine expression during early phases of orthodontic tooth movement. As previously described [22], IL-1α and TNF-α immunoreacted cells are present in the pulp and they have different localization. The constitutive expression of IL-1α by odontoblasts resembles that of keratinocytes. Keratinocyte proliferation is stimulated by IL-1 via an autocrine manner [33,34]. As odontoblasts represent the first line of defense in the dental pulp, this cytokine expression

could be of significant importance in pulp homeostasis. Loss of the odontoblast staining capacity for IL-1α has been attributed to pulpal inflammation [22]. Although the majority of the examined sections showed no differences in the pulp of orthodontically moved molars and controls, there were occasional sections where the root pulp adjacent to the compression zone exhibited lighter IL-1α staining implying some degree of pulpal inflammation. Furthermore, increased number of TNF-α stained cells was seen in the pulp body at day 3 indicating that some of the pulps might have developed an aseptic pulpitis due to mechanical forces. Increased blood flow and recruitment of CD43+ cells in the pulp have been previously reported after orthodontic tooth movement in rats, suggestive of inflammatory changes [35,36].

Summarizing, the present study showed that up-regulation of TNF-α occurred as early as 3 hours after mechanical force application and it followed by IL-1α up-regulation (after 1 day) at both compression and tension areas. This finding indicates that probably TNF-α induced up-regulation of IL-1α in this model. Cytokine expression in the compression area was always more intense than the tension area. The gingiva around the orthodontically moved molars proved to be a significant source of cytokines and might be involved in bone remodeling. There were no spatial differences in the expression of the investigated cytokines with the exception of pulp. Mild inflammatory reactions may be initiated in the pulp in the very first stages of orthodontic tooth movement. Mechanical stress results in almost immediate inflammatory reactions in various dental tissues.

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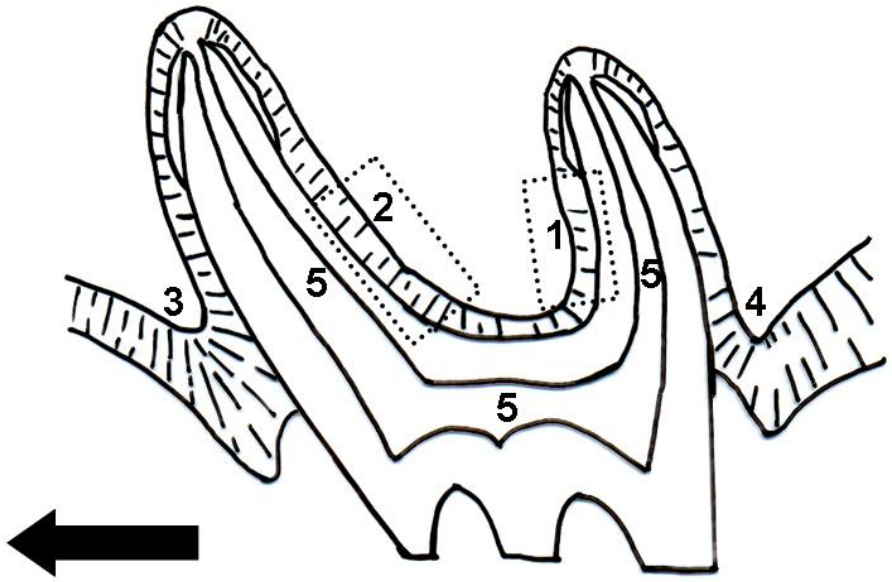


**Fig. 1. Schematic representation of the investigated areas at the first maxillary molar: 1, bone and periodontal ligament (PDL) at the mesial aspect of the distal root (boxed area); 2, bone and PDL at the distal aspect of the mesial root (boxed area); 3, gingiva and PDL mesially to 1st maxillary molar; 4, gingiva and PDL distally to 1st maxillary molar; 5, coronal and root pulp. Arrow indicates the direction of tooth movement.**

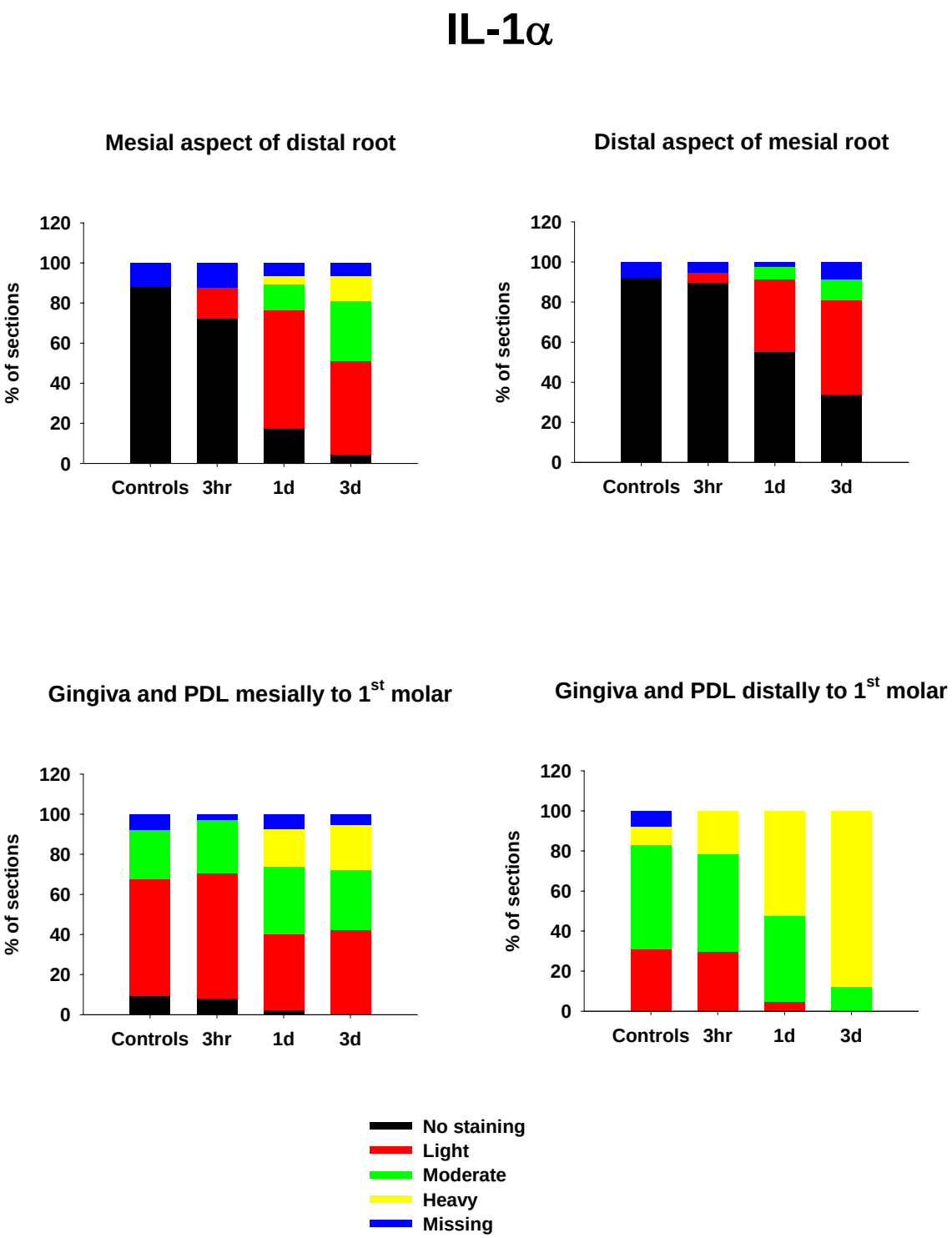


**Fig. 2. Distribution of the evaluated sections in the different staining categories. Immunohistochemical staining for IL-1α. Data are presented as % of evaluated sections for each group. Control sections from all experimental groups have been pooled together.**

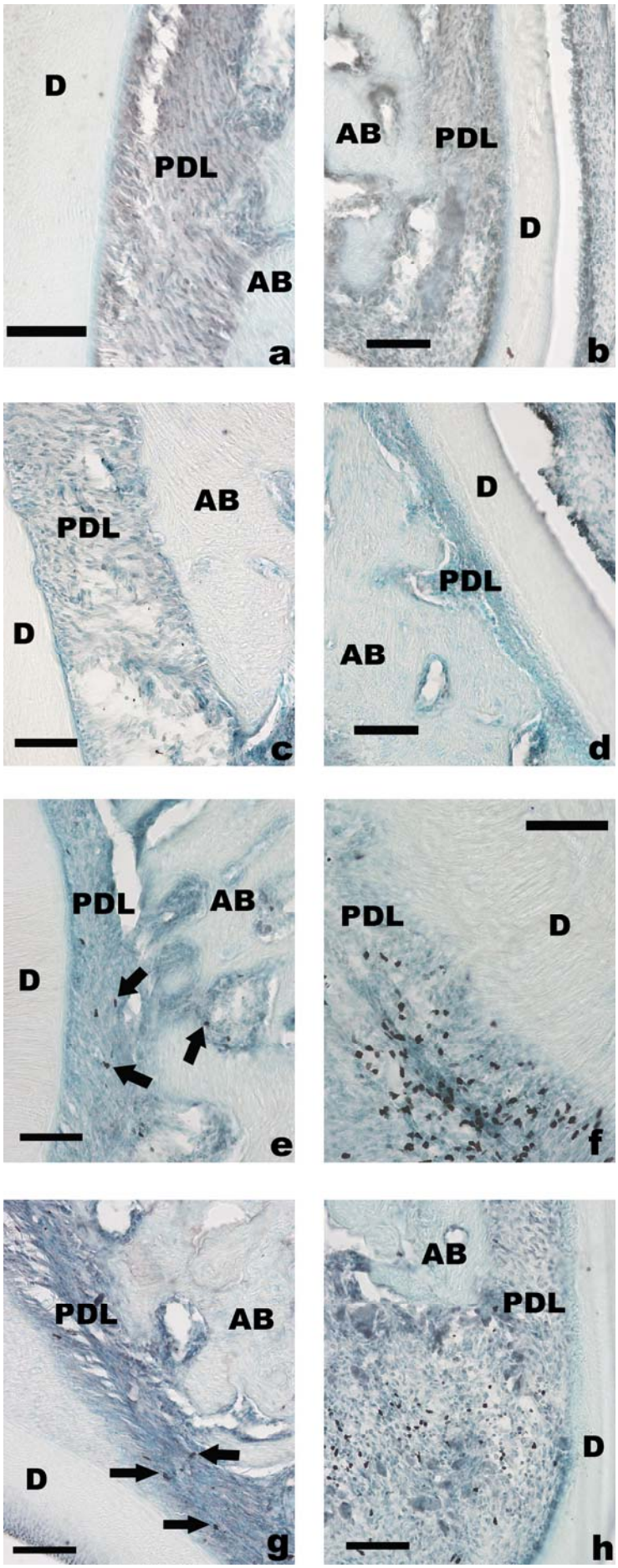


**Fig. 3. Immunohistochemical microphotographs (20x) from the distal aspect of the mesial root (a, c, e, g) and from the mesial aspect of the distal root (b, d, f, h) of the first maxillary molar stained for IL-1α. D: dentin; AB, alveolar bone; PDL, periodontal ligament; arrows point at stained cells. Bars, 100 μm. (a, b) No staining for IL-1α is observed in the untreated-control side. (c,d) Three hours after tooth movement, there is still no IL-1α staining. (e,f) One day after tooth movement there is up-regulation of IL-1α in both tension (e) and compression (f) areas. (g, h) The IL-1α up-regulation continues at 3 days after tooth movement in tension (g) and compression (h) areas. Note that the compression areas stained heavier than the tension areas.**

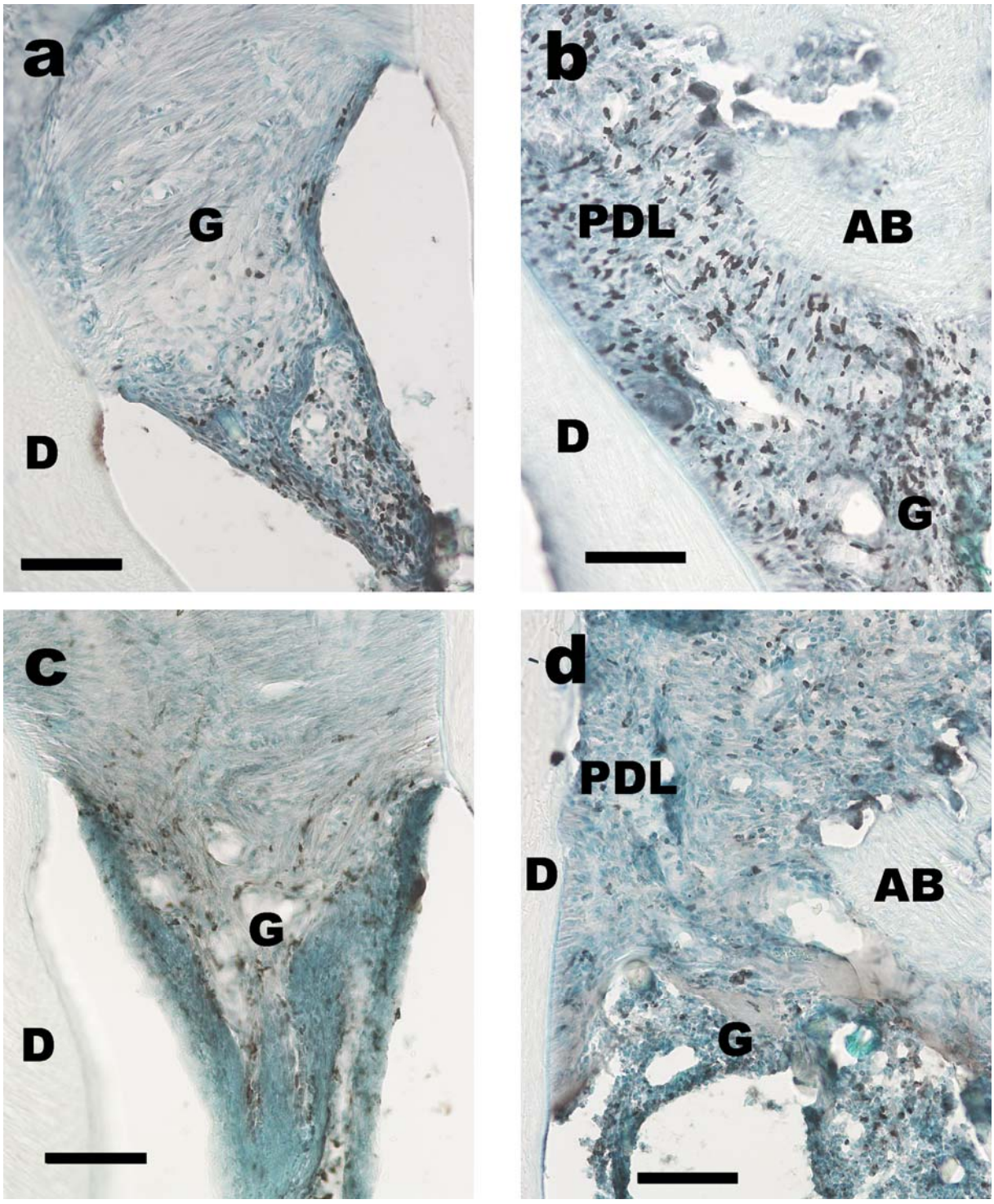


**Fig. 4. Immunohistochemical microphotographs (20x) of the gingiva and periodontal ligament distally to the 1st maxillary molar stained for either IL-1α (a, b) or TNF-α (c, d). D, dentin; G, gingiva; AB, alveolar bone; PDL, periodontal ligament. Bars, 100 μm. The interdental papilla in untreated-control side (a, c) exhibited strong staining reaction for the above cytokines at the gingival epithelium. Three days after the orthodontic appliance was placed and force was introduced (b, d) the interdental papilla was severely damaged and heavy staining for both IL-1α and TNF-α extended towards the PDL and the alveolar bone.**

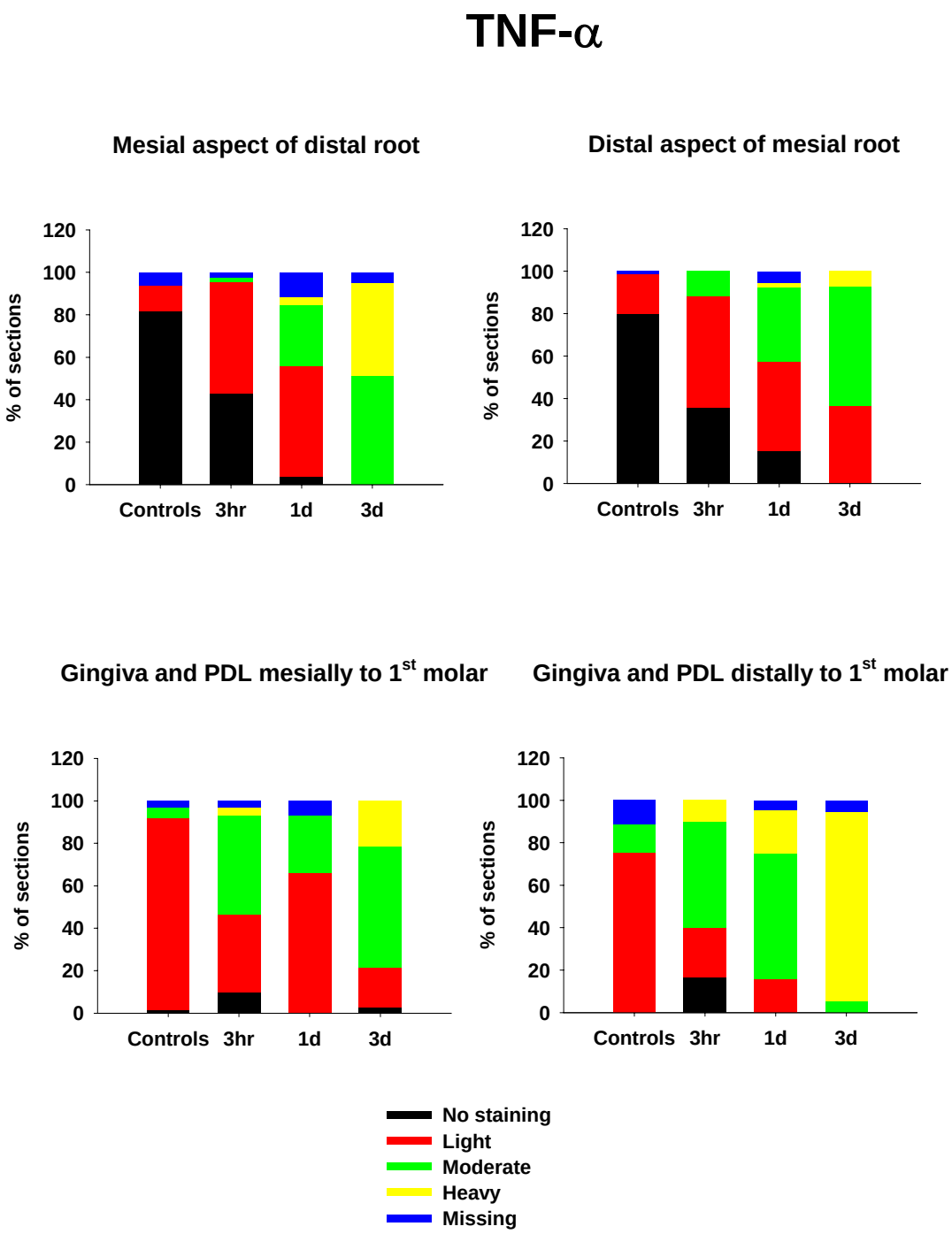


**Fig. 5. Distribution of the evaluated sections in the different staining categories. Immunohistochemical staining for TNF-α. Data are presented as % of evaluated sections for each group. Control sections from all experimental groups have been pooled together.**

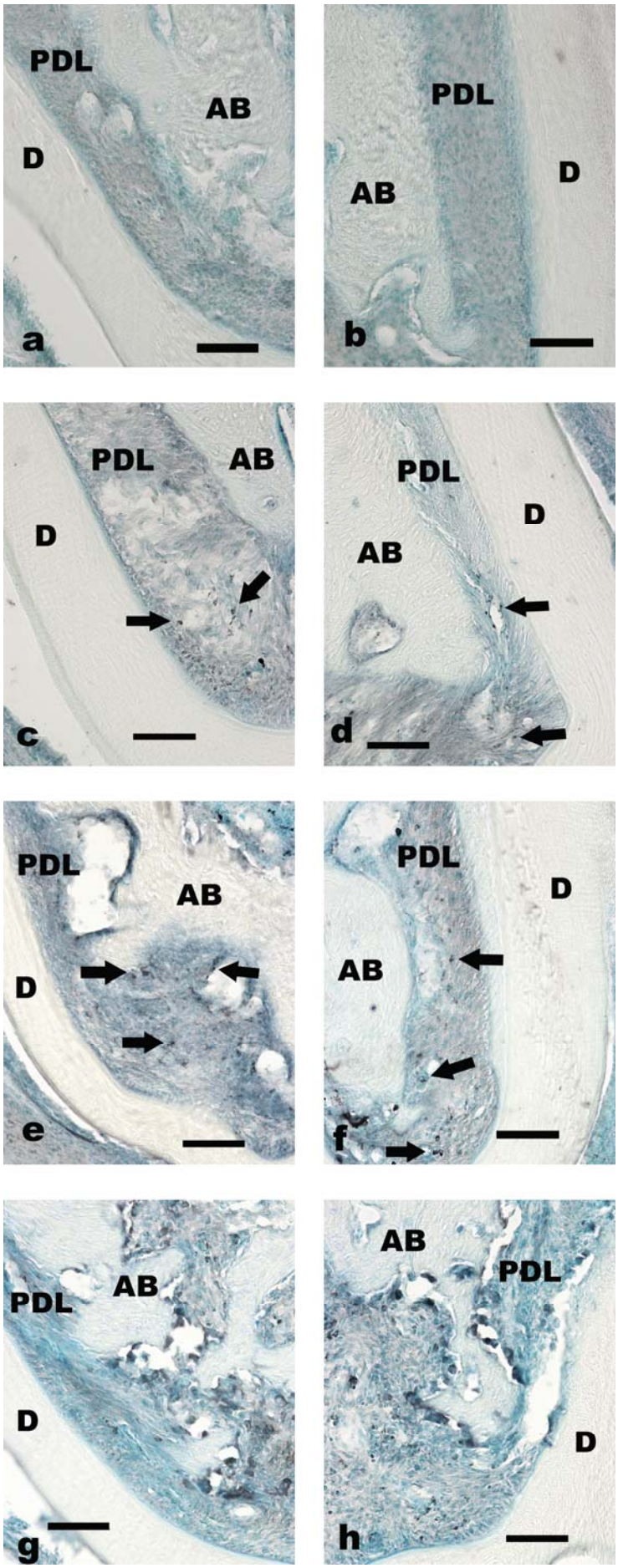


**Fig. 6. Immunohistochemical microphotographs (20x) from the distal aspect of the mesial root (a, c, e, g) and from the mesial aspect of the distal root (b, d, f, h) of the first maxillary molar stained for TNF-α. D, dentin; AB, alveolar bone; PDL, periodontal ligament; arrows point at stained cells. Bars, 100 μm. (a, b) No staining for TNF-α is visible in the untreated-control side. (c,d) Three hours after tooth movement TNF-α appears in both tension (c) and compression (d) areas. Up-regulation of TNF-α continues at 1 day (e,f) and 3 days (g, h) after tooth movement in both tension and compression areas with more staining in the compression areas (f, h) than in the tension areas (e, g).**

**Table 1**. IL-1α staining in dental tissues during early phases of experimental tooth movement. Data are number of rats.

| Groups | | Mesial aspect distal root | | | Distal aspect mesial root | | | Gingiva and PDL mesially to 1st molar | | | Gingiva and PDL distally to 1st molar | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No stain | Stain | Total | No stain | Stain | Total | No stain | Stain | Total | No stain | Stain | Total |
| **3hr** | **Experimental** | 2 | 4 | 6 | 2 | 4 | 6 | 1 | 5 | 6 | 0 | 6 | 6 |
| | **Control** | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 2 |
| **1d** | **Experimental** | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 |
| | **Control** | 4 | 0 | 4 | 4 | 0 | 4 | 0 | 4 | 4 | 0 | 4 | 4 |
| **3d** | **Experimental** | 0 | 6 | 6 | 1 | 5 | 6 | 0 | 6 | 6 | 0 | 6 | 6 |
| | **Control** | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |

**Table 2**. TNF-α staining in dental tissues during early phases of experimental tooth movement. Data are number of rats.

| Groups | | Mesial aspect distal root | | | Distal aspect mesial root | | | Gingiva and PDL mesially to 1st molar | | | Gingiva and PDL distally to 1st molar | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No stain | Stain | Total | No stain | Stain | Total | No stain | Stain | Total | No stain | Stain | Total |
| **3hr** | **Experimental** | 1 | 5 | 6 | 1 | 5 | 6 | 1 | 5 | 6 | 1 | 5 | 6 |
| | **Control** | 1 | 1 | 2 | 1 | 1 | 2 | 0 | 2 | 2 | 0 | 2 | 2 |
| **1d** | **Experimental** | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 |
| | **Control** | 1 | 3 | 4 | 1 | 3 | 4 | 0 | 4 | 4 | 0 | 4 | 4 |
| **3d** | **Experimental** | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 | 0 | 6 | 6 |
| | **Control** | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |