

Histomorphometric study of the periodontal ligament in the initial period of orthodontic movement in Wistar rats with induced allergic asthma

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Introduction: Asthma is a common systemic disease occurring in infancy and adolescence, time periods that could encompass orthodontic treatment. Asthma is an inflammatory disease; therefore, it might interfere with orthodontic tooth movement. The purpose of the study was to analyze the histomorphologic aspects of the periodontal ligament of asthmatic Wistar rats in the initial period of orthodontic movement. **Methods:** Thirty-two Wistar rats were divided into 4 groups: 2 control groups consisting of rats without induced allergic asthma, and 2 experimental groups consisting of rats with induced allergic asthma. The animals of the first control and experimental groups did not receive orthodontic forces, whereas those in the second control and experimental groups were subjected to mesial movement of the maxillary left first molar for 3 days. The samples were prepared for histomorphometric analysis of the periodontal ligament. The area of the periodontal ligament was calculated as a function of root length in the cervical and apical regions of the distal face of the maxillary first molar mesial root. The Student *t* test and the Welch correlation test were applied to the data obtained. **Results:** There was a statistically significant difference ($P < 0.05$) between the control and experimental groups. An enhanced response to orthodontic force was observed in the asthmatic animals: the periodontal ligament was more compressed at the pressure area and more stretched in the traction area. **Conclusions:** Our findings indicate that experimental allergic asthma seems to exacerbate orthodontic movement in rats. (Am J Orthod Dentofacial Orthop 2012;142:333-8)

Asthma is a disease of the airways characterized by hypersensitivity of the tracheobronchial tree to several stimuli. This condition is evidenced by generalized narrowing of airways and can be alleviated spontaneously or with medication. Clinically, asthma manifests itself through dyspnea, coughing,

and wheezing. Asthma is an episodic disease in which acute exacerbations are intercalated with asymptomatic periods.¹ It can be unchained as a consequence of inhalation of domestic dust, hair, smoke, chalk powder, strong odors, or chemical sprays; ambient temperature changes; emotional disturbance; hyperventilation; physical exercise; and viral infection. Exposure to allergens, particularly during the first years of life, can determine the chronic inflammation of the airways of those who are genetically susceptible to asthma development. Asthma affects about 5% to 10% of the population,² and its incidence has increased significantly during the last 20 years in industrialized countries.³

Asthma's etiology is complex and multi-factorial. There is an interaction between genetic factors and environmental stimuli contributing to the development of a mediated immune response, predominantly by activation of TH2 lymphocytes.^{4,5} Laboratory analysis of the bronchoalveolar lavage fluid from asthmatic patients shows inflammatory reactions characterized by increased amounts of mast cells, epithelial cells,

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eosinophils, lymphocytes, and inflammatory mediators. Once released, such mediators produce immediate and intense inflammatory reactions, involving bronchoconstriction, vascular congestion, and tissue edema. Generalized increases in cellularity and the amount of capillaries are the most frequent anomalies observed in asthma. Eosinophilia is the most common finding in the blood count.^{1,6,7}

Similarly to the asthma process, the inflammatory process is also found in the periodontum during the initial period of orthodontic movement. In the phase of acute inflammation, one can observe an increase in the caliber of blood vessels in the periodontal ligament and structural changes in this tissue. Therefore, soon after force application, a rapid and small dental displacement occurs for 4 to 7 days as a result of inflammatory changes and gradual compression of the periodontal ligament.⁸

Such considerations support the purpose of our study: ie, to analyze the histomorphologic aspects of the periodontal ligament of asthmatic Wistar rats in the initial period of orthodontic movement. Because asthma is an inflammatory disease, it can interfere with orthodontic movement.

MATERIAL AND METHODS

Male Wistar rats (180–200g) were obtained from the Oswaldo Cruz Foundation breeding center (Rio de Janeiro, Brazil). All procedures involving the care and use of laboratory animals in this study were approved by the Animal Ethics Committee of the Federal University of Rio de Janeiro (reference number, ODONTO 004).

The animals were divided into 2 groups: the control group consisting of 16 animals was actively sensitized and stimulated with saline solution, and the experimental group consisting of 16 animals was also actively sensitized and stimulated with antigen. In both groups, dental movement was conducted for 3 days in half of the animals, and the rest had no orthodontic intervention. This determined the establishment of the subgroups: control group 1, control group 2, experimental group 1, and experimental group 2 with 8 animals in each.

All the animals were sensitized through subcutaneous injection containing 50 µg of ovalbumin and 5 mg of aluminum hydroxide, for a total volume of 0.2 mL. After 14 days, the reinforcement procedure was carried out with an intraperitoneal injection of antigen solution. The antigenic challenge was performed only in the experimental groups through nasal instillation with the antigen (ovalbumin, 700 µg per animal) diluted in sterile saline solution of 0.1 mL in each animal. The animals of the control group were challenged with 0.1 mL of saline solution. Such challenges were performed for 3 consecutive days from day 7 after reinforcement. These

procedures ensured the maintenance of basal metabolism in these animals. The orthodontic devices were installed in control group 2 and experimental group 2 on the day after the last challenge.^{5,9,10}

Blood samples were obtained from all animals. Total leukocytes were counted in a Neubauer chamber, and a differential analysis was performed under oil-immersion objective in blood samples and cytocentrifuged smears stained with May-Grunwald-Giemsa stain. Orthodontic devices were installed after verifying the increase in total leukocyte numbers in the experimental group compared with the control group. Differential blood cell counts were obtained from laboratory prepared slides containing blood smear stained by the May-Grunwald-Giemsa method. The slides were analyzed under a light microscope with oil-immersion objective lenses. The results obtained from the total leukocyte count and the differential blood cell count were submitted to the Student *t* test for nonpaired samples.

An intraperitoneal single dose of 40 mg per kilogram of sodium thiopental was administered for fitting the orthodontic device in all animals of control group 2 and experimental group 2. Mesial movement of the maxillary left first molar was obtained by attaching a nickel-titanium coil spring 7 mm in length and calibrated at 40 gf. The mandibular incisors had their height reduced with a high-speed dental hand piece to prevent damage to the orthodontic appliance. Only the animals in control group 2 and experimental group 2 were anesthetized and had the device installed and the mandibular incisors worn.

All animals were killed with a lethal intraperitoneal injection of sodium thiopental (60 mg/kg) 3 days after dental movement in experimental group 2 and control group 2. Soon after checking the loss of vital signs, bronchoalveolar lavage was performed by inserting a polyethylene cannula into the trachea for administration and aspiration of 15 mL of heparinized phosphate buffered saline (10 IU/mL) in 3 successive lavage procedures of 5 mL each. Bronchoalveolar lavage fluid was collected, and total leukocytes were counted as previously described for blood analysis. Differential counts were performed in cytocentrifuged smears stained with May-Grunwald-Giemsa stain.

Anatomic pieces containing the maxillary left first molars and lungs were fixed in 4% paraformaldehyde solution buffered with 0.1 mol per liter of sodium phosphate for 48 hours. The dental specimens were prepared with a decalcification method by using 40% formic acid and 20% sodium citrate solution for subsequent hematoxylin and eosin staining of the histologic sections. The dissected lungs were submitted to the classic histotechnical procedures with Giemsa staining after the fixation period.

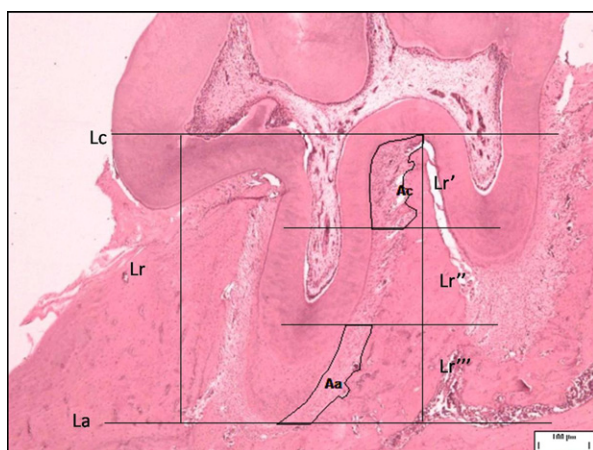


Fig. Micrograph illustrating the histomorphometric analysis of the periodontal ligament on the distal face of the maxillary first molar mesial root. *Lc*, The cervical line tangent to the furca; *La*, the apical line tangent to the root apex; *Lr*, the total root length; *Lr'*, the cervical third length; *Lr''*, the middle third length; *Lr'''*, the apical third length; *Ac*, the cervical area; *Aa*, the apical area. Preparation for decalcification, hematoxylin and eosin.

Lung tissue sections were studied under a light microscope, with peribronchial eosinophils counted in 4 bronchi per animal. Section thickness was 5 μm . A morphometric analysis was conducted in 3 regions around each bronchus. The values for the control and experimental groups were submitted to the Student *t* test for nonpaired samples.

Histologic sections containing dental samples were evaluated under a light microscope (Eclipse; Nikon, Tokyo, Japan) for histomorphometric analysis of the periodontal ligaments. Section thickness was 5 μm . Four sections were measured per animal; only those that included the dental pulp were used to ensure the evaluation of deep histologic sections. Software (Image-Pro Plus, version 4.5; MediaCybernetics, Bethesda, Md) was used on the digital images obtained for measurement of the apical and cervical regions of the periodontal ligament on the distal face of the maxillary left first molar mesial root.

A standardized analytic method was adopted for reading and comparing the data between the subgroups. The methodology consisted of tracing 2 straight lines parallel to each other: 1 tangential to the furca, limiting the cervical point of the mesial root, and the other tangential to its most apical point, both parallel to the lower border of the photograph. Next, another straight line was traced perpendicular to both parallel lines, which was measured to determine root length. By dividing the root length by 3, the length of each root third was

Table I. Examinations for asthma confirmation in the experimental group

Before installation of orthodontic device

	Control group	Experimental group	P value
<i>Blood</i>	<i>Cells $\times 10^{-3}/\text{mm}^3$</i>	<i>Cells $\times 10^{-3}/\text{mm}^3$</i>	
Total leukocytes	88.1 ± 6.2	132.4 ± 8.7	<0.05
Eosinophils	5.0 ± 1.2	16.8 ± 1.4	<0.0001

Before death

<i>Blood</i>	<i>Cells $\times 10^{-3}/\text{mm}^3$</i>	<i>Cells $\times 10^{-3}/\text{mm}^3$</i>	
Eosinophils	1.0 ± 0.4	11.1 ± 1.0	<0.0001

After death

<i>Bal</i>	<i>Cells $\times 10^5/\text{bal}$</i>	<i>Cells $\times 10^5/\text{bal}$</i>	
Total leukocytes	7.4 ± 0.8	10.4 ± 0.7	<0.05
Eosinophils	0.1 ± 0.1	1.1 ± 0.2	<0.05
<i>Lung tissue</i>	<i>Cells $\times 10^4/\mu\text{m}^2$</i>	<i>Cells $\times 10^4/\mu\text{m}^2$</i>	
Eosinophils	0.1 ± 0.2	19.9 ± 5.9	<0.05

Values are expressed as means $\pm$ average SE ($n = 16$). The Student *t* test was used for nonpaired samples ($P < 0.05$).

Bal, Bronchoalveolar lavage.

obtained. Once the cervical and apical thirds were delimited, the area of the periodontal ligament was calculated for the respective regions. To eliminate comparison errors resulting from individual characteristics of each animal, the ratio was calculated between the periodontal ligament area and its corresponding third length. The results were submitted to statistical analysis with the Student *t* test and the Welch correlation (Fig).

RESULTS

Before fitting the orthodontic device, we first noted that the antigen challenge led to increases in the numbers of total leukocytes and eosinophils in the blood of experimental rats compared with the control animals. The experimental animals also showed significant increases in total leukocyte and eosinophil counts in the bronchoalveolar lavage (Table I). A marked leukocyte infiltration was observed in the lung parenchyma consisting mainly of eosinophils.

Histomorphometric analysis of the distal face of the maxillary first molar mesial root showed traction areas of the periodontal ligament in the cervical region and pressure areas in the apical region in control group 2 and experimental group 2, thus characterizing a movement of mesial inclination. The results regarding the cervical and apical regions of the periodontal ligament were statistically significant after being submitted to the Student *t* test and the Welch correction (Table II).

Table II. Evaluation of periodontal ligament areas regarding root length in the cervical and apical regions on the distal face of the maxillary first molar mesial root

Comparison between subgroups	CG1	CG2	EG1	EG2	P value
<i>Ratio of periodontal ligament cervical area to cervical root third ($\mu\text{m}^2/\mu\text{m}$)</i>					
CG1 $\times$ EG1	65.00 $\pm$ 2.220	—	36.00 $\pm$ 2.405	—	<0.001
CG1 $\times$ CG2	65.00 $\pm$ 2.220	82.13 $\pm$ 4.006	—	—	<0.01
EG1 $\times$ EG2	—	—	36.00 $\pm$ 2.405	148.80 $\pm$ 20.800	<0.01
CG2 $\times$ EG2	—	82.13 $\pm$ 4.006	—	148.80 $\pm$ 20.800	<0.05
<i>Ratio of periodontal ligament apical area to apical root third ($\mu\text{m}^2/\mu\text{m}$)</i>					
CG1 $\times$ EG1	66.75 $\pm$ 2.343	—	44.38 $\pm$ 4.456	—	<0.01
CG1 $\times$ CG2	66.75 $\pm$ 2.343	60.00 $\pm$ 1.402	—	—	<0.05
EG1 $\times$ EG2	—	—	44.38 $\pm$ 4.456	31.13 $\pm$ 1.432	<0.05
CG2 $\times$ EG2	—	60.00 $\pm$ 1.402	—	31.13 $\pm$ 1.432	<0.01

Values are expressed as means $\pm$ average SE (n = 8). The Student *t* test and the Welch correction were used for comparisons between the subgroups (*P* < 0.05).

CG, Control group; EG, experimental group.

Table III. Difference in mean ratios ($\mu\text{m}^2/\mu\text{m}$) (periodontal ligament area to root length) in the cervical and apical regions on the distal face of the maxillary first molar mesial root

Intragroup difference	Cervical region		Apical region	
CG: CG2 – CG1	+17.13 $\pm$ 4.580	+26.35%	–6.75 $\pm$ 2.730	–10.11%
EG: EG2 – EG1	+112.80 $\pm$ 20.940	413%	–13.25 $\pm$ 4.680	–29.85%

Values are expressed as mean differences $\pm$ average SE (n = 8) and percentages. The results show the periodontal ligament's response to orthodontic movement in the groups.

CG, Control group; EG, experimental group.

Orthodontic movement in the cervical and apical regions was more intense in the experimental group compared with the control group. In the cervical region, there was a 413% increase in periodontal ligament area per micrometer of root in experimental group 2 compared with experimental group 1, whereas control group 2 had a 26.35% increase compared with control group 1. With regard to the apical region, a 29.85% decrease was observed in the periodontal ligament area per micrometer of root in experimental group 2 compared with experimental group 1, and the periodontal ligament area decreased by 10.11% in control group 2 compared with control group 1 (Table III).

DISCUSSION

To confirm the establishment of an allergic asthma model in rats, an analysis of leukocyte populations was performed before installation of the orthodontic devices. The results showed that the ovalbumin-challenged animals had significant increases in blood and bronchoalveolar total leukocyte and eosinophil counts compared with the saline-challenged animals at 3 days after orthodontic movement. The count of peribronchial eosinophils in the pulmonary parenchyma was also noted.

Together, these data confirmed allergic asthma reactions in the experimental animals, thus supporting our progression to the histomorphometric analysis of the periodontal ligament (Table I). The model of inflammation that we observed simulates that in bronchial asthma in humans.^{5,9,10} The results of this experimental research corroborate previous clinical studies in the literature, all showing increased amounts of eosinophils, neutrophils, mastocytes, lymphocytes, epithelial cells, and inflammatory mediators in the bronchoalveolar lavage fluid of asthmatic patients. Endobronchial biopsy samples showed an inflammatory process involving infiltration of eosinophils, neutrophils, and lymphocytes. Eosinophilia was the most common finding in blood count results.^{1,6,7}

Histomorphometric evaluation of the periodontal ligament was performed with software (Image-Pro Plus, version 4.5). Because there was no previous similar study, it was necessary to determine a methodology for comparing the subgroups. Significant errors would be incorporated if the values of the periodontal ligament area had been compared; the comparison of a tooth with an extensive root length with another with a short root would result in an expressive difference that did not

correspond to reality. In a study regarding mediated re-implantation of teeth treated with sodium hypochlorite, however, Kanno et al¹¹ observed repair of the periodontum after histomorphometric analysis of transversal histologic sections. They used the ratio between the periodontal ligament and the root surface circumference, thus reducing the influence of individual variations. To make our study more precise, a ratio between periodontal ligament area and length of the corresponding root third was adopted. This eliminated possible comparison errors between longer and shorter roots.

Analysis of the possible influence of asthma on a periodontum not submitted to orthodontic force showed that rats from experimental group 1 (cervical region, $36.00 \pm 2.405 \mu\text{m}^2/\mu\text{m}$; apical region, $44.38 \pm 4.456 \mu\text{m}^2/\mu\text{m}$) had the smallest periodontal ligament area per root third length (μm) compared with the animals from control group 1 (cervical region, $65.00 \pm 2.220 \mu\text{m}^2/\mu\text{m}$; apical region, $66.75 \pm 2.343 \mu\text{m}^2/\mu\text{m}$). There is still no scientific basis to clarify this fact, and further studies can better define this relationship. Response to orthodontic movement was more intense in the animals in experimental group 2 (cervical region, $148.8 \pm 20.80 \mu\text{m}^2/\mu\text{m}$; apical region, $31.13 \pm 1.432 \mu\text{m}^2/\mu\text{m}$) compared with those in control group 2 (cervical region, $82.13 \pm 4.006 \mu\text{m}^2/\mu\text{m}$; apical region, $60.00 \pm 1.402 \mu\text{m}^2/\mu\text{m}$), in both traction and pressure areas in the cervical and apical regions, respectively (Table II). This histomorphometric study on the periodontal ligament yielded statistically significant results, which corroborate the histologic descriptive analyses of periodontal tissues in the animals of control group 2 and experimental group 2. Collapses in blood vessels and periodontal ligament fibers were observed in the pressure area, whereas bone resorption lacunae containing osteoclasts were present along the inner alveolar cortex, thus characterizing Howship's lacunae. Nevertheless, only the ovalbumin-challenged and sensitized animals exhibited indirect resorption areas in the alveolar bone adjacent to the pressure area of the periodontum, indicating a more intense tissue response in this subgroup.

The stress caused on periodontal ligaments from force application induces a stimulus that, in turn, triggers local inflammation. Chemotactic factors attract eosinophils, platelets, and polymorphonuclear leukocytes toward the inflamed region. Increased vascular permeability and formation of inflammatory infiltrates and exudates also occur, turning the pH acidic and favoring the arrival and permanence of clastic cells.¹²⁻¹⁴ It is possible that inflammation of the periodontal ligament was aggravated by the systemic condition in experimental group 2, because eosinophilia was present in the blood of the ovalbumin-challenged and sensitized animals.

Eosinophils are important tissue infiltrative components because they trigger the production of chemotactic cytokines, which increase the local inflammation.¹

The response in the cervical region was more exacerbated than in the apical region of the periodontal ligament. Animals from control group 2 had a 26.35% increase in the periodontal ligament area per root (μm) in the cervical region compared with control group 1. However, a 10.11% decrease in the periodontal ligament area was observed in the apical region compared with the control subgroup. With regard to animals from experimental group 2, a 413% increase in the periodontal ligament area per root (μm) was noticed in the cervical region, whereas a 29.85% decrease was observed in the apical region compared with experimental group 1 (Table III). Such evidence can be explained by the fact that force was directly applied to the dental crown, away from the resistance center, thus leading to inclination movement. Consequently, the periodontal ligament response was greater in the cervical region than in the apical region.

CONCLUSIONS

The results show that a more intense response to orthodontic forces was noted in the allergic asthmatic animals, as confirmed by greater compression in the pressure area of the periodontal ligament and greater stretching in the traction area compared with the control animals. Our findings suggest that allergic asthma generated an increase in the response caused by orthodontic movement in rats. Further experiments are required to elucidate the mechanisms involved in this phenomenon.

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