Histochemical and Immunohistochemical Demonstration of Macrophages and Dendritic Cells in the Lingual Periodontal Ligament of Rat Incisors

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Summary. The distribution of macrophages in the lingual periodontal ligament of rat incisors was surveyed by histochemical and immunohistochemical methods. Numerous macrophages showing intense ACPase reactions were located primarily in the shear zone of the periodontal ligament. Immunostaining with an ED1-monoconal antibody that recognizes various subpopulations of macrophages revealed plentiful positive cells showing flamelike profiles throughout the periodontal ligament, in addition to regular macrophages associated with sinusoidal blood vessels. A similar distribution of flamelike cells expressing Ia antigens was demonstrable with immunostaining using an OX6-monoconal antibody. A consecutive staining of sections for ACPase histochemistry followed by immunoreactions for Ia antigens revealed the presence of two types of the flamelike cells in the periodontal ligament: one with and the other without distinct ACPase activity, corresponding to the macrophage and the dendritic cell, respectively. Either type of flamelike cells was located in the bone-related and shear zones, whereas only dendritic cells without ACPase activity were restricted to the tooth-related zone. OX6-immunonegative cells showing ACPase reactions were also found in the periphery of the sinusoidal blood vessels.

Our data are the first to demonstrate the abundance of macrophages and dendritic cells expressing Ia antigens throughout the lingual periodontal ligament of rat incisors. In addition to regular macrophages, an exclusive localization of macrophages with flamelike extensions has been demonstrated in the bone-related and shear zones of the ligament. The region-specific arrangement of macrophages and dendritic cells with various histochemical and immunological features suggests that the periodontal ligament of rat incisor is a useful model for analyzing the process of differentiation of antigen-presenting cells.

Dendritic cells have been identified in lymphoid tissues as cells with extensive cytoplasmic projections showing flamelike profiles; they have little or no endocytic activity (STEINMAN et al., 1986). They are known to have I-region associated (Ia) antigens and play an important role in the immune system as tissue antigen-presenting cells (STEINMAN et al., 1986). Recent immunohistochemical study on the dental tissues has revealed the presence of a large population of dendritic cells in the dental pulp, serving towards tissue protection under physiological as well as pathological conditions (KAWASHIMA, 1990).

Macrophages, a kind of antigen-presenting cells in the immune system, have been suggested in recent studies to cooperate with dendritic cells for antigen-presentation (KAPSENBERG et al., 1986). In gingival connective tissues constantly exposed to antigenic stimuli through sulcular epithelium, the role of the macrophage has been investigated in relation to the pathology of periodontal diseases (SEYMOUR et al., 1988; MATSUKI et al., 1991). In the periodontal ligament continuous with gingival tissues, however, little attention has been focused upon macrophages and, hence, neither the precise location nor their role has been established in periodontal biology.

The present study was therefore undertaken to disclose the distribution of macrophages in the periodontal ligament and their relationship with dendritic cells using histochemical as well as immunohistochemical markers for macrophages and/or dendritic cells. The central one-third of the lingual periodontal ligament of the rat incisor was chosen for observations because the influence of exogenous antigenic stimuli could then be disregarded.

MATERIALS AND METHODS

Adult male rats of the Wistar strain weighing (260±5 g) were anesthetized by an intraperitoneal injection of
chloral hydrate (400 mg/kg body wt) and perfused either with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) or a mixture of 4% paraformaldehyde and 0.25% glutaraldehyde in the same buffer for 20 min. Some of the animals were perfused with 3% paraformaldehyde and 2.5% glutaraldehyde mixture in 0.06 M cacodylate buffer supplemented with 0.05% CaCl₂. Upper jaws were dissected and processed for decalcification with neutral 5% EDTA at 4°C. Following decalcification, the tissues were immersed in a 30% sucrose solution at 4°C overnight, and frozen in liquid nitrogen. Longitudinal cryosections of the upper incisors with adherent periodontal tissues, 20 μm in thickness, were made in a cryostat.

ACPase histochemistry

For histochemical demonstration of acid phosphatase (ACPase) activity, the azo-dye method (BURSTONE, 1961) and lead salt method (GOMORI, 1952) were used with slight modifications. The incubation medium for the azo-dye method comprised 0.01% naphthol AS-MX phosphate (Na salt) and 0.06% fast red violet LB salt in 0.1 M acetate buffer (pH 5.2). The modified Gomori's medium comprised 0.12% sodium-glycerophosphate, 0.1% lead nitrate, and 2% dextrose in 0.05 M acetate buffer (pH 5.2). In both methods, the cryosections were incubated for 30-45 min at 37°C. The sections incubated in the lead salt medium were treated with diluted ammonium sulfide for light microscopic identification of reaction sites. An incubation with the medium containing 10 mM NaF or with a substrate-free medium completely eliminated the reactions in both experiments.

Immunohistochemistry

Free floating sections were processed for immunohistochemistry by use of ED1- and OX6-monoclonal antibodies (Serotec, Oxford, England), which could recognize most of the monocyte-macrophage system cells (DIJKSTRA et al., 1985) and Ia antigens (McMASTER and WILLIAMS, 1979), respectively. A solution of 0.01 M phosphate-buffered saline (PBS, pH 7.4) was used to dilute the antibody and to rinse the sections. After blocking endogenous peroxidase with absolute methanol containing 0.3% H₂O₂, the sections were incubated for 48 h at 4°C with the primary antibodies, diluted to 1:500 (ED1), and 1:5000 (OX6). The sites of antigen-antibody reaction were made visible by an avidin-biotin peroxidase complex (ABC) method (HSU et al., 1981), using a commercially available ABC kit (Vector Lab. Inc., Burlingame, USA). Some of the sections were sequentially stained for ACPase histochemistry with the azo-dye method followed by immunostaining for Ia antigens with an OX6-monoclonal antibody.

RESULTS

ACPase activities

Intense reactions for ACPase were localized in macrophages located in the bone-related and shear zones of the lingual periodontal ligament. The reactive cells appeared to be concentrated along the shear zone, the border region between the tooth-related and bone-related zones (Fig. 1a). They were also seen in the periphery of the sinusoidal blood vessels in the bone-related zone. Enzymatic reactions were observed to be confined in the large sized lysosomal structures in the individual cells (Fig. 1b). Only a few cells in the tooth-related zone showed similar enzymatic reactions. Fibroblasts did not show any ACPase reactions as large granular structure but as fine fibrous structures; they could be easily distinguished from macrophages under the light microscope.

Immunohistochemistry

Immunostaining with an ED1-monoclonal antibody revealed distinct reactions in numerous cells possessing flamelike extensions throughout the periodontal ligament. A number of oval or spindle-shaped macrophages showing the ED1-immunoreactivity were also recognized, primarily associated with sinusoidal blood vessels in the bone-related zone of the ligament. Immunostaining with an OX6-monoclonal antibody that recognizes Ia antigens, a class of surface antigens encoded for the major histocompatibility complex (THOMAS and SHEVACH, 1978), revealed a similar distribution of reactive cells showing flamelike profiles as seen with an ED1-antibody. The immunoreactive cells with flamelike extensions to either ED1- or OX6-antibody were most highly concentrated in the shear zone of the lingual periodontal ligament (Figs. 2-4).

A double staining both with ACPase histochemistry and OX6-immunohistochemistry (Fig. 5a), allowed a clear-cut distinction between the two types of flamelike cells expressing Ia antigens: one with and the other without significant ACPase activity (Fig. 5b,c). The latter type of cell was distributed throughout the periodontal ligament. The OX6-positive flamelike cells showing intense ACPase activity were relatively small in number and were located primarily in the bone-related and shear zones where those
without ACPase activity were also located.

Two types of oval or spindle-shaped macrophages (ACPase positive) could further be distinguished by the presence or absence of OX6-immunoreactivity. These types of cells were mostly located in the periphery of the sinusoidal blood vessels.

The histochemical and immunohistochemical characterizations of these cells demonstrated in this study are summarized in Table 1.

DISCUSSION

Our data indicated the presence of numerous macro-
Fig. 3. Immunoreactions to an OX6-monoclonal antibody in the same region as in Figures 1 and 2. Most immunoreactive cells show typical flamelike profiles and are crowded at the border region between tooth-related (t) and bone-related zones (b). B alveolar bone, bv blood vessel, D dentin. ×330

Fig. 4. A close-up of flamelike cells in the ligament. b Bone-related zone, t tooth-related zone, * shear zone. ×635
phages and dendritic cells in the lingual periodontal ligament of rat incisors, and further could distinguish two types of macrophages with respect to the presence or absence of extensive flamelike cytoplasmic extensions.

Dendritic cells are irregular-shaped cells which were initially identified in vitro in the glass and plastic adherent population of cells from the mouse spleen (for review, STEINMAN and NUSSENZWEIG, 1980). Dendritic cells are clearly distinguishable from lymphocytes due to their lack of surface Ig, thy-1 and brain antigens, and also by differences in the properties of surface polysaccharides (STEINMAN et al., 1979). Dendritic cells can be also distinguished from

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<th>Macrophage</th>
<th>Regular-shaped</th>
<th>Flamelike</th>
<th>Dendritic cell</th>
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<tbody>
<tr>
<td>ED1</td>
<td>+</td>
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<tr>
<td>OX6</td>
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<td>ACPase</td>
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| Table 1. Histochemical and immunohistochemical characterization of macrophages and dendritic cells in the periodontal ligament of rat incisor. |
macrophages, since they show little or no endocytic activity in vivo and in vitro (STEINMAN and NUSSENZWEIG, 1980) and, hence, display low ACPase activity (STEINMAN et al., 1988) in contrast to macrophages that have a phagocytic activity and intense ACPase activity in lysosomal cytoplasmic vesicles.

Tissue macrophages generally appear oval or spindle in profile under the light microscope in vivo. As shown in Figure 1, however, histochemical reactions for ACPase alone do not allow the precise determination of the profiles of the reactive cells. Since an ED1-monoclonal antibody recognizes a monocyte-macrophage lineage as well as dendritic cells (DIJKSTRA et al., 1985) and since a large number of cells immunoreactive to ED1, including those located in the shear zone, have all displayed flamelike profiles in this study, it seems reasonable to infer the presence of macrophages with flamelike cytoplasmic extensions in the periodontal ligament.

It is well known that either dendritic cells or macrophages express I-region associated (Ia) antigens, and that dendritic cells lack ACPase activity, a marker enzyme for lysosomes rich in macrophages. The present double staining with ACPase histochemistry and Ia-immunohistochemistry serves a distinction between the two types of cells, both showing flamelike profiles. Current data indicate that, at least, some of the cells with flamelike extensions in the bone-related and shear zones of periodontal ligament of rat incisors can be regarded as macrophages from histochemical as well as immunohistochemical criteria.

The double staining of sections further allowed a distinction between the regular (oval or spindle) shaped macrophages located at the peripheral regions of sinusoidal blood vessels in the bone-related zone: one with and the other without Ia-immunoreactivity. Macrophages that lack Ia antigens may be of the monocyte-macrophage lineage having, directly or indirectly something to do with the osteoclasts that also lack Ia antigens.

Regarding the location of macrophages in the periodontal ligament, it is interesting to note that those without flamelike extensions (regular macrophages) are principally located in the periphery of blood vessels, whereas flamelike macrophages are distributed throughout the bone-related and shear zones of the ligament. Since macrophages have been thought to be differentiated from monocytes in the connective tissue (VAN FURTH et al., 1972), it is tempting to speculate that monocytes derived from local blood vessels may initially differentiate into regular macrophages in the periphery of blood vessels, and further into flamelike macrophages in the bone-related zone of the periodontal ligament. Some of the flamelike macrophages might migrate toward the shear zone where they differentiate into Ia-antigen-presenting flamelike cells (ACPase reactive flamelike cells). It should be noted that the tooth-related zone of the periodontal ligament is an avascular zone (BEERTSEN et al., 1974) where only dendritic cells (flamelike cells without ACPase activity) are located. It is assumed, therefore, that the dendritic cells in the periodontal ligament of rat incisors might be the derivative of the flamelike macrophages that obtained Ia antigens instead of a loss of ACPase by undefined mechanisms.

The correlation and functional significance of these cells shown in this study require future clarification.

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REFERENCES


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