

RESEARCH ARTICLE

Class II Antigen-presenting Dendritic Cells and Macrophages in Healthy and Carious Human Teeth

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ABSTRACT

Antigen-presenting cells are capable of participating in the stimulation of T cells by antigen presentation. Antigen-presenting cells are considered essential for the induction and expansion of the immune reaction because their interaction with antigen is the first step in immune induction.

We have studied the distribution of class II expressing cells in developing, healthy and carious human teeth to clarify when human pulp acquires an immunologic defense potential. Antigen-expressing cells were identified immunohistochemically with HLA-DR monoclonal antibodies (for dendritic cells) and CD68 monoclonal antibodies (for macrophages).

In the pulp of unerupted developing teeth, HLA-DR-positive cells were distributed mainly in and around the odontoblast layer. A few CD68 positive cells were located more coronary around the blood vessels. In erupted teeth, HLA-DR-positive cells were located, for the most part just beneath the odontoblast layer. CD68 positive cells were also located coronary mainly around the blood vessels. Superficial caries lesions caused aggregation of HLA-DR-positive cells and macrophages in the dental pulp corresponding to the lesion.

Our results showed that human teeth are already equipped with an immunological defense potential before the eruption. In the initial stage of caries infection, an immune response mediated by class II expressing cells is initiated in human dental pulp.

Keywords: Dendritic cells, Dental caries, Developing tooth, Human dental pulp, Macrophages, Immunohistochemistry.

How to cite this article: Ivkovska AS, Bilbilova EZ, Georgiev Z, Emilija BV, Ivkovski L. Class II Antigen-presenting Dendritic Cells and Macrophages in Healthy and Carious Human Teeth. *Int J Experiment Dent Sci* 2018;7(2):63-67.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Various types of immunocompetent cells are known to be present in normal dental pulp.¹⁻⁶ Antigen-presenting cells are considered essential for the induction and expansion of the immune reaction because their interaction with antigen is the first step in immune induction. One of their essential characteristics is to express MHC class II antigen in the lymphoid tissue. They can absorb and process complex antigens and present them to T lymphocytes, a function essential to the initiation of immune responses.⁷

The hard-tissue encasement provides a unique pathway of antigenic entry since the permeability of dentin seems to be critical in determining the number of incoming antigens and subsequently the magnitude of the reactions operational in the underlying pulp. There is some evidence showing that pulpal dendritic cells are actually able to respond to trans-dentinal antigen provocation and that the kinetics of these cells are strongly influenced by changes in dentin permeability. The initial response of pulpal dendritic cells may trigger the development of most pulpal pathosis of clinical importance. Upon antigenic challenge, these cells may not only interact locally with T lymphocytes as antigen-presenting cells but also communicate with nerves and the vascular systems which may modulate inflammatory reactions. Hence, pulpal dendritic cells appear to provide the pulpal connective tissue with potentials far beyond what has been previously anticipated.^{8,9}

Changes of the distribution of class II expressing cells have been shown in rat incisors during tooth development, as well as in molar pulp and the responses of class II-expressing cells to experimentally induced pulpitis have also been reported.¹⁰⁻¹² Izumi¹³ have shown that human dental pulp contains two kinds of class II MHC antigen-expressing cells: dendritic cells and macrophages.¹⁴ Both dendritic cells and macrophages are believed to participate in the immune defense system in the dental pulp. Class II molecule-expressing pulpal dendritic cells are of primary importance because of their critical role in pulpal immunosurveillance. In general, dendritic cells differ from the macrophages in their having little or no phagocytotic activity, in contrast to the macrophages, which possess a high phagocytotic activity.^{15,16} In intact teeth, enamel, and cementum protect

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dentinal pulp from external stimuli transmitted through dentinal tubules. Under various conditions, however, the pulp tissue is subject to constant exposure. These differences in the environment might affect the distribution of class II expressing cells in dental pulp.^{17,18}

The purpose of this study was to elucidate the distribution of class II expressing cells in developing, healthy and carious human teeth, to clarify when human pulp acquires an immunologic defense potential and how this reacts to dental caries.

MATERIALS AND METHODS

Four partially developed unerupted teeth were operatively extracted, four erupted developing intact premolars, and four premolars with closed apices were extracted. Ten premolars with various stages of decay were obtained from patients exhibiting no clinical symptoms. Teeth were obtained from a patient who needed orthodontic therapy and were from 11 to 18 years old.

Immediately after extraction, the teeth were cut longitudinally; the pulp was extirpated and fixed in 10% buffered paraformaldehyde, embedded in paraffin and sliced into 4–5 µm sections. After deparaffinization, immunoperoxidase staining was performed, with use of monoclonal antibodies. The commercially available primary antibodies were an anti-HLA-DR monoclonal antibody (CR3/43; DAKO Co. Ltd, Denmark) and rabbit anti-CD 68 polyclonal antibody (DAKO). The sections were further incubated with pre-formed ABC (Elite® ABC, Vector Laboratories, Burlingame, USA) for 30 minutes at room temperature. Finally, the localization of peroxidase activity was made visible by incubation of the sections with 3,3'-Diaminobenzidine (DAB) in PBS buffer for five to seven minutes and counterstained with hematoxylin. The depth of each caries lesion was determined by the pigmentation on the sections.

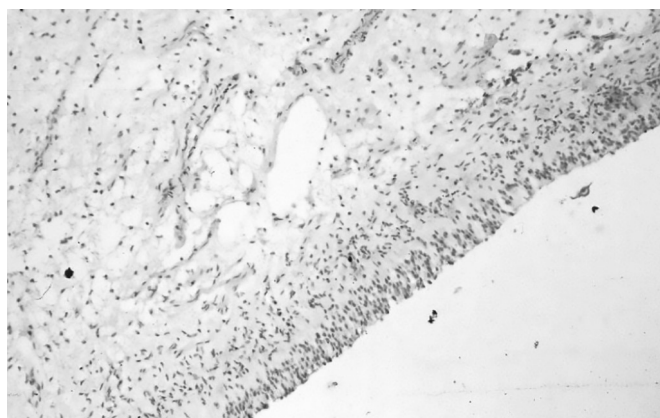


Fig. 1: Immunohistochemical localization of HLA-DR positive cells in unerupted developing teeth

RESULTS

Unerupted Developing Teeth

During the stage of root formation, numerous HLA-DR-positive cells and macrophages appeared in the coronal pulp. Distribution of HLA-DR-positive cells was especially dense in and around the odontoblast layer. Only a few HLA-DR-positive cells were scattered in the dental papilla (Fig. 1).

A few CD68 positive cells were located more coronary around the blood vessels (Fig. 2).

Erupted, Intact Teeth

Immunoperoxidase-labeled HLA-DR-positive cells were located, for the most part just beneath the odontoblast layer. In the connective tissue of the coronal and radicular pulp, they were distributed mainly around the blood vessels (Fig. 3).

CD68 positive cells were also located coronary mainly around the blood vessels (Fig. 4).

Carious Teeth

In teeth where caries lesions extended from the enamel into the dentin, an aggregation of HLA-DR-positive cells was observed in the subodontoblastic region. No distributive changes were noted in other areas (Fig. 5). Also, the number of the macrophages was increased in the area corresponding to the caries lesion (Fig. 6).

As the caries lesion advanced, the aggregated cells expanded along the odontoblast layer, and they advanced toward the center of the pulp (Figs 7 and 8).

DISCUSSION

The existence of two types of class II antigen-expressing cells-dendritic cells, which are characterized by poor

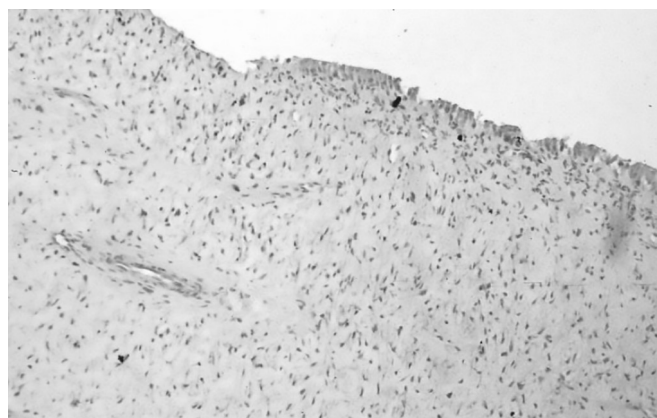


Fig. 2: Immunohistochemical localization of CD68 positive cells in unerupted developing teeth

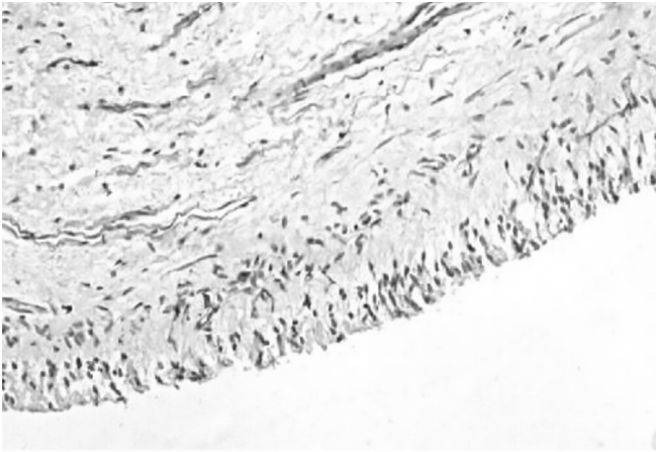


Fig. 3: Immunohistochemical localization of HLA-DR positive cells in erupted, intact teeth

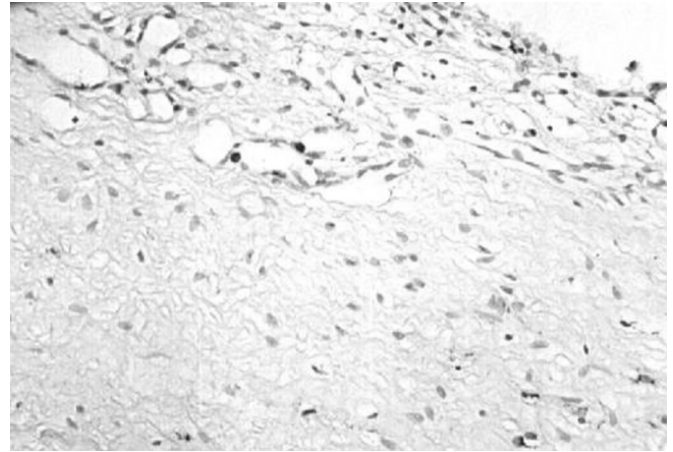


Fig. 4: Immunohistochemical localization of CD68 positive cells in erupted, intact teeth

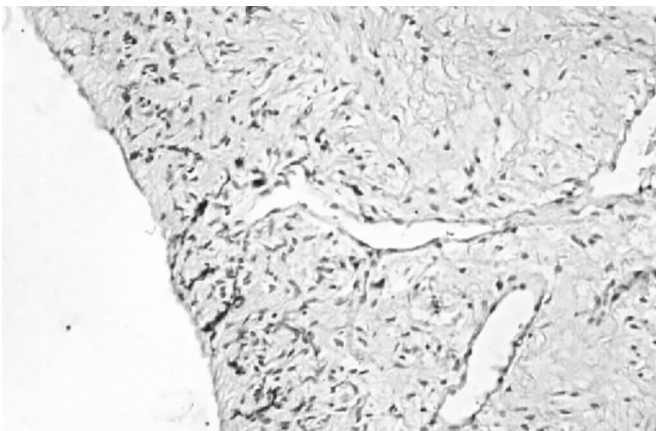


Fig. 5: Immunohistochemical localization of HLA-DR positive cells in the pulp affected by initial caries

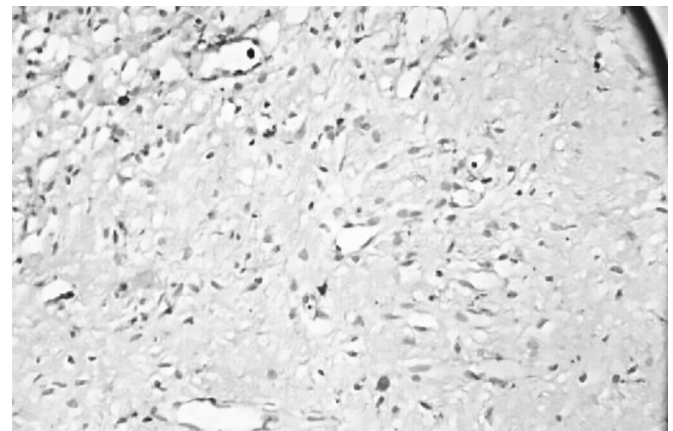


Fig. 6: Immunohistochemical localization of CD68 positive cells in the pulp affected by initial caries

phagocytic activity, and macrophages has been reported in the dental pulp of both rat and human teeth.^{6,19} Dendritic cells are localized to the periphery of the pulp, while macrophages tend to be more centrally located.^{2,3,20}

The HLA-DR-positive cells observed throughout the odontoblast layer are dendritic cells, and their location is ideal for capturing antigens invading the dental tubules.^{21,22} Previous studies of Jontell,¹⁰ Kosaka¹¹, and Yoshida²³ on rat dental pulp have shown that the majority of class II expressing cells occur only after the eruption, suggesting that there is a post-eruptive increase due to the invasion of bacterial antigens. Contrary, to the data obtained in rats, numerous HLA-DR-positive cells were found in the pulps of unerupted human teeth concentrated within the odontoblast layer, and macrophages more centrally located around the blood vessels. Thus, the results of the present study indicate that human teeth are already equipped with an immunological defense potential before they erupt.

Antigen-presenting cells react with antigens that have reached the pulp tissue through the dentine tubules.²⁴ Therefore, these cells in the early carious pulp may

migrate into the lymph node to present antigen to T cells. Initial antigen presentation followed by an expansion of both the cell-mediated and the humoral immune reaction may occur in the dental pulp. Potential antigen-presenting cells in the pulp respond to the carious attack at the very early stage of dentinal caries. Most likely these cells are functioning as sentinels of the pulpal immunological defense system to recognize externally derived carious antigens, which may activate the pulpal immune defense reactions.²⁵

The present study is also demonstrating distributive changes in class II expressing cells in the pulps of carious teeth. Changes were evident in teeth with early caries, and the aggregation of HLA-DR-positive cells and macrophages were observed in the restricted area corresponding to caries lesion, which is suggesting that antigenic materials have already spread into the pulp tissue through dentinal tubules. Similar lymphocyte infiltration was reported during enamel caries.²⁶⁻²⁸ The relationship between these data on carious teeth is suggested by the results of *in vitro* experiments which show that dendritic cells in dental pulp have the capacity to co-stimulate T

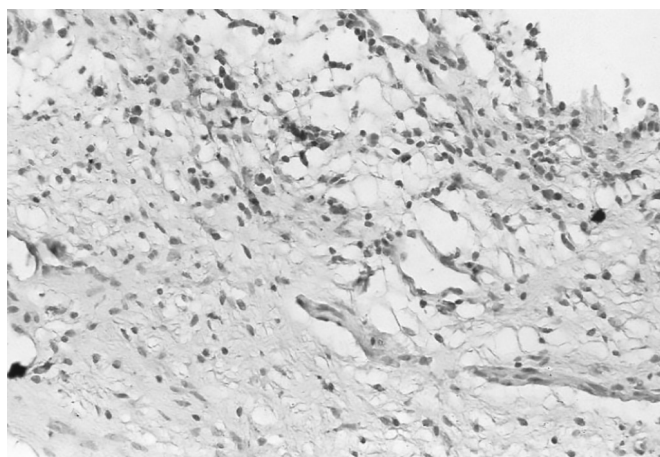


Fig. 7: Immunohistochemical localization of HLA-DR positive cells in the pulp affected by moderate caries

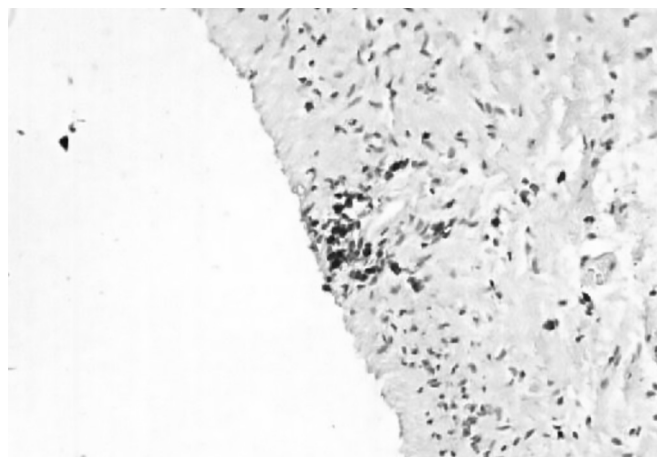


Fig. 8: Immunohistochemical localization of CD68 positive cells in the pulp affected by moderate caries

lymphocytes, a relationship of importance during the early stages of pulpal immune response.¹⁵

More advanced caries induced more expanded aggregations of HLA-DR-positive cells and macrophages along the odontoblast layer and subodontoblastic area of the pulp. This subodontoblastic zone is composed of the rich network of nerve fibers, capillaries, and a collagen matrix.^{29,30} The exact role of this zone remains unknown, but the present findings suggest its importance for accommodating immunoresponsive cells. HLA-DR-positive cells were observed along the dentin-pulp border corresponding to the caries lesion.

The mode of immunological reaction of the dental pulp seems to be strongly influenced by hard tissue encasement. Class II expressing cells can respond to trans dentinal antigen provocation, and the kinetics of these cells are strongly influenced by changes in dentin permeability.^{31,32} Upon antigenic challenge, these cells may interact locally with T lymphocytes and induce an immunological response to mount defense and repair of the dentin-pulp complex.

CONCLUSION

Our present findings indicate that human teeth are already equipped with an immunological defense potential before the eruption, even before they are submitted to antigenic challenges in the mouth. In the initial stage of caries infection, an immune response mediated by class-II-expressing cells is initiated in human dental pulp.

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