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## Characterization of Pulpal Responses To Caries In Human Teeth.

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### ABSTRACT

This study investigated the responses of the immune system in 3 different clinical conditions: shallow and deep cavities and treated caries. Cells were identified immunohistochemically by using the following monoclonal antibodies: HLA-DR, CD45RO and CD20. Initial pulpal response was characterized by a localized accumulation of HLA-DR antibody-positive cells in the pulp tissue beneath the dentinal tubules communicating with the caries lesion. In the pulp of progressed caries, a large number of HLA-DR-positive cells were observed with a marked increase of other kinds of immunocompetent cells. In treated carious teeth, clusters consisting of HLA-DR-positive cells and CD45-positive T lymphocytes were recognized locally in the pulp tissue, regardless of cavity depth. CD20-positive B cells were seen only under the deeper cavities. The results of this study demonstrated that dental pulps respond to cavity preparation and restoration, and antigen presentation and cellular or humoral immunoresponses persist for many months, even after caries treatment.

**Keywords:** human dental pulp, MHC class II molecule-expressing cells, lymphocytes, dental caries, caries treatment, adhesive system, immunohistochemistry

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## INTRODUCTION

It is well documented that bacteria and bacterial by-products are associated with most pulpal disease processes. Dental pulp is a specialized connective tissue occupying the narrow pulp chamber of the tooth, containing various kinds of immunocompetent cells. Like any other part of the organism, dental pulp is constantly submitted to a multitude of immune reactions. These occur in response to a great variety of antigenic factors, which in most cases, are caused by direct pulp contact through dentinal tubules (1,2,3,4).

Pulpal cell immunity has been investigated by Jontell et al. who has used the peroxidase-conjugated biotin-avidin complex technique and demonstrated the presence of T lymphocytes in pulps from human teeth. The relation between the depth of carious lesions and inflammatory cell infiltration in human dental pulp has been studied in detail (5,6,7,8).

Caries removal, cavity preparation, and restoration with adhesive systems are generally conducted in dental practices. There has been no shortage of papers published on the subject of histological evaluations of pulpal responses to cavity preparation in animals, but very little attempts to evaluate those in human teeth.

Dental pulp is equipped with major histocompatibility complex (MHC) class II molecule-expressing cells for initiating immune responses to exogenous antigenic stimuli. In intact teeth, they are distributed mainly in and around the layer of odontoblasts and are called pulpal dendritic cells. Drastic changes in their localization are induced by human dental caries (9,10,11,12,13), and after cavity preparation in rats (14,15,16,17). Analysis of these data suggests that class II molecule-expressing cells are highly sensitive to antigenic stimuli penetrating dentinal tubules (18,19,20,21).

Caries attack also induces changes in the distribution of lymphocytes; they become concentrated beneath the carious lesions (22,23). Following the exogenous invasion of microorganisms, host defence reactions, such as inflammatory and immunological reactions, take place in the pulp in order to eliminate the foreign pathogens and to maintain the local homeostasis in the pulp. Interactions between lymphocytes and MHC class II molecule-expressing cells have been shown in pulpal inflammation (24).

The focus of this paper is the influence of an operative procedure upon the distribution of MHC class II molecule-expressing cells and lymphocytes. We have investigated pulpal responses in untreated carious teeth compared with carious lesions treated with an adhesive system. We postulated that pulpal responses for cavities with caries and that with treated carious teeth would no longer have their responses after 6 months.

## MATERIAL AND METHOD

We have examined 60 human teeth from patients at the age of 9 to 18 years. Teeth were extracted from various therapeutic reasons (mostly from orthodontic reason), and immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 hours at 4°C. The specimens were embedded in paraffin, according to standardized laboratory procedure. Sections were cut at 5 µm thickness and stained by the streptavidin-biotin complex immunoperoxidase method. Cells were identified immunohistochemically by using the following monoclonal antibodies: HLA-DR (for dendritic cells), CD45RO (for memory T lymphocytes) and CD20 (for B-lymphocytes).

To verify our hypothesis, we analyzed pulpal responses in 3 different clinical conditions: shallow (n=20, pulp with caries in dentin, about 2-3mm from the pulp chamber), deep cavities (n=20, pulp with caries deep into the dentin, 0.5-1.5mm from the pulp chamber) and treated caries (n=20). Treatment of caries lesions was carried out on occlusal surfaces. The distance between cavity floors and pulpal walls varied from 0.5 to 3 mm. The Uni Fil Bond dental adhesive system and GC Gradia composite resin were applied to the prepared teeth.

The depth of the carious lesion was determined by the pigmentation of hard tissues.

The main numbers of dendritic cells, T-cells and B-cells in each group were statistically analysed with ANOVA.

## RESULTS

The number of antigen-presenting and immunocompetent cells in each group is shown in Table 1 and Fig.1.

**Table 1. Number of HLA-DR, CD45RO and CD20 antibody-positive cells in dental pulp in shallow, deep cavities and treated caries**

	Group 1	Group 2	Group 3
N	20	20	20
HLA-DR	5.0	22.1	2.2
CD45RO	19.5	89.6	7.1
CD20	4.7	51.2	1.5

Values are means  $\pm$  SEM; N, number of samples

In shallow dentinal lesions a few HLA-DR cells were present and they were distributed mainly around an odontoblast layer and along the dentin-pulp border (Fig. 2).

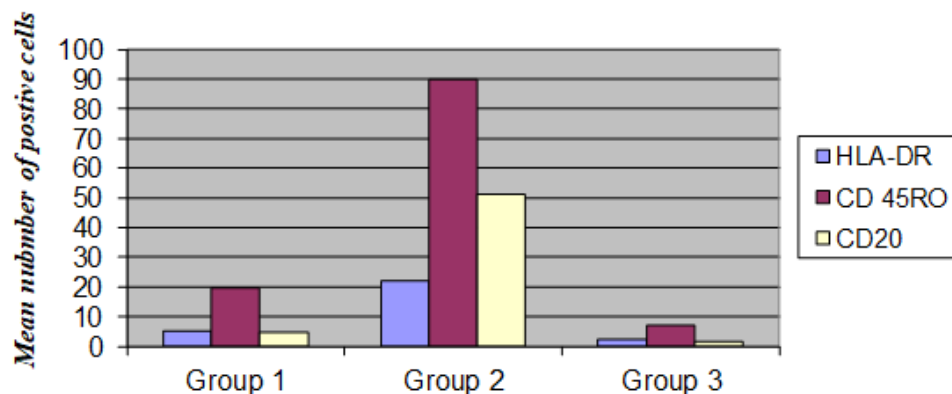
As the caries lesion advanced, cells expanded toward the center of the pulp. Under deeper cavities HLA-DR-positive cells were dispersed among affected odontoblasts and they have displaced odontoblasts below the cavities. Cells were markedly increased, but not significantly (Fig.3).

An increase of CD45RO-positive cells T-lymphocytes was observed in majority of specimens in teeth with moderate to deep caries. These cells were concentrated below the para-odontoblastic region, forming an aggregation. The number of T-cells was markedly increased in deep cavities and significant differences were evident between group 1 and 2 ( $p < 0.01$ ), (Fig.4, Fig.5).

The number of CD20-positive B-lymphocytes was much smaller than that of T-lymphocytes in most specimens. A considerable number of CD20-positive cells was detected among lymphocytes forming clusters in deeper cavities, with significant differences between group 1 and 2 ( $p < 0.01$ ), (Fig.6, Fig.7).

Treatment of caries lesions showed that beneath 9 of the 10 cavities samples, aggregations of HLA-DR-positive cells were recognized locally (Fig.8), and always followed the accumulation of CD45RO-positive cells T-lymphocytes (Fig.9). CD20-positive B-cells were seen only under deeper cavities (Fig.10). There were significant differences in the number of T-cells between group 3 and 1 ( $p < 0.05$ ), and between group 3 and 2 ( $p < 0.01$ ), and in the number of B cells between group 3 and 2 ( $p < 0.01$ ).

**Fig. 1. Mean number of HLA-DR, CD45RO and CD20 antibody positive cells in human dental pulp in shallow and deep cavities and treated caries**



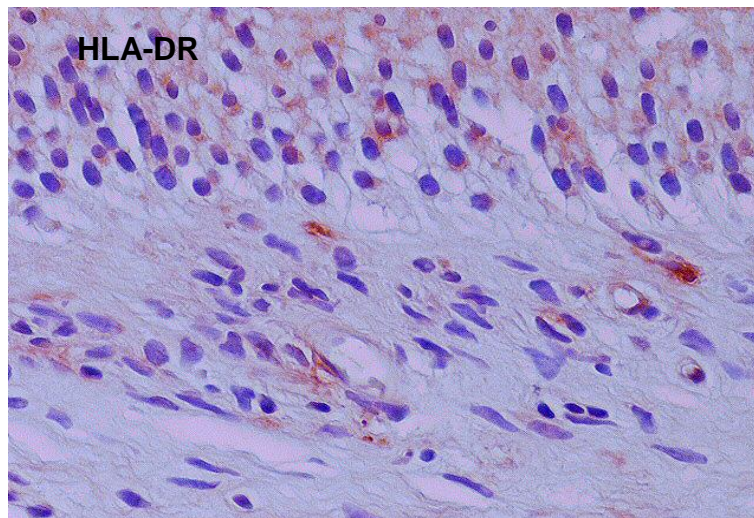


Fig. 2. Immunohistochemical localization of HLA-DR positive cells in the pulp with shallow cavities

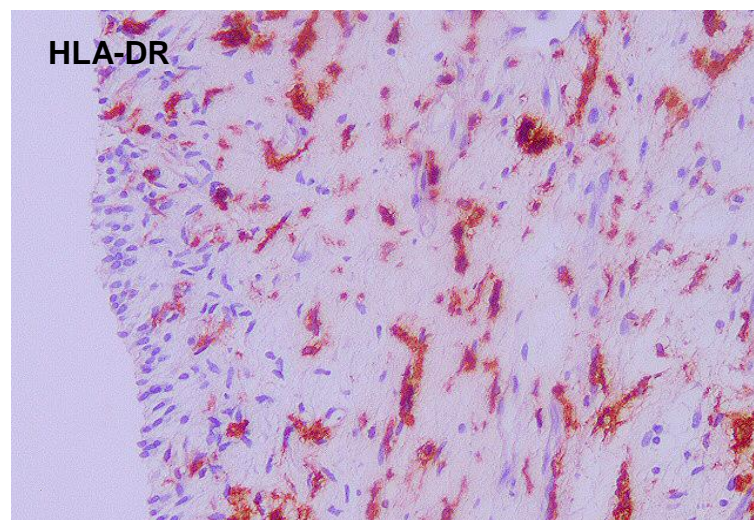


Fig. 3. Immunohistochemical localization of HLA-DR positive cells in the pulp with deep cavities

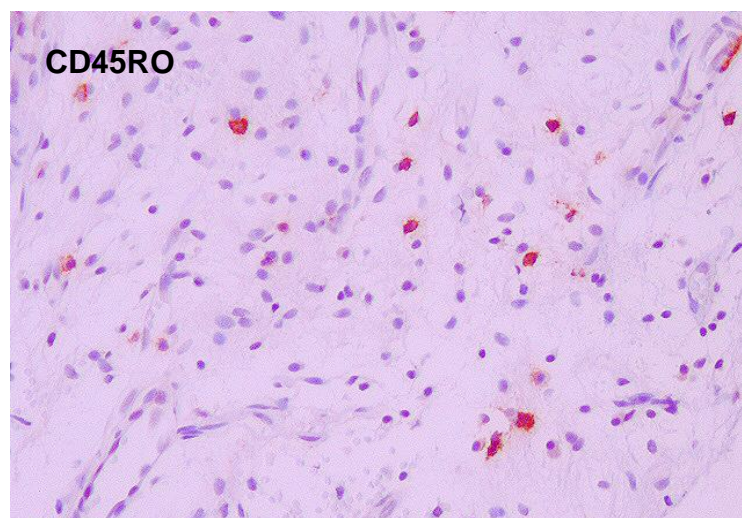


Fig. 4. Immunohistochemical localization of CD45RO positive cells in the pulp with shallow cavities



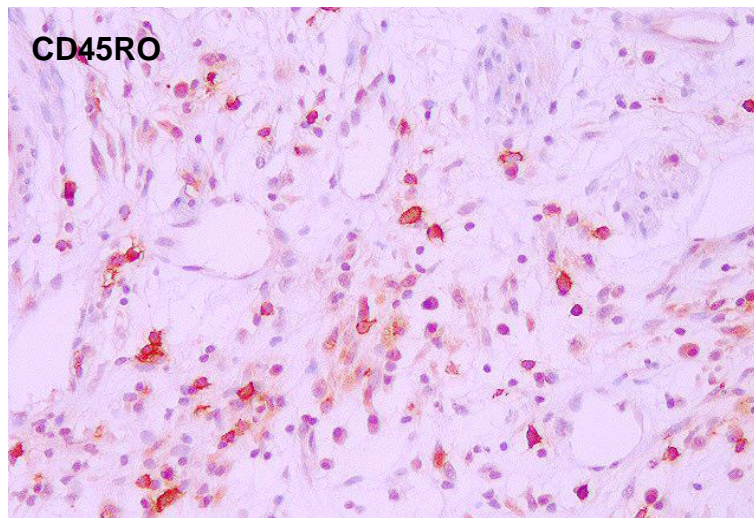


Fig. 5. Immunohistochemical localization of CD45RO positive cells in the pulp with deep cavities



Fig. 6. Immunohistochemical localization of CD20 positive cells in the pulp with shallow cavities

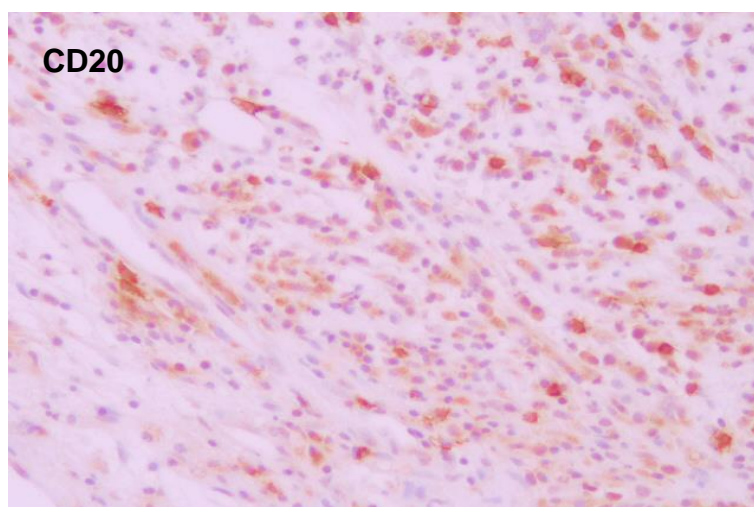


Fig. 7. Immunohistochemical localization of CD20 positive cells in the pulp with deep cavities

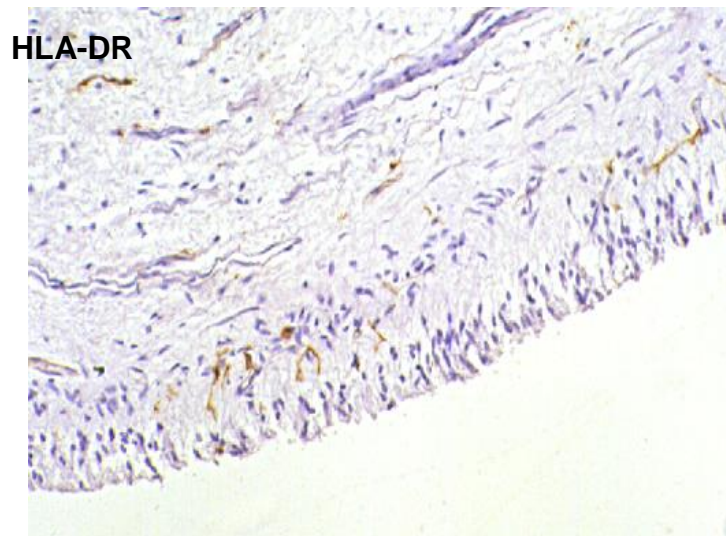


Fig. 8. Immunohistochemical localization of HLA-DR positive cells in the pulp of treated caries

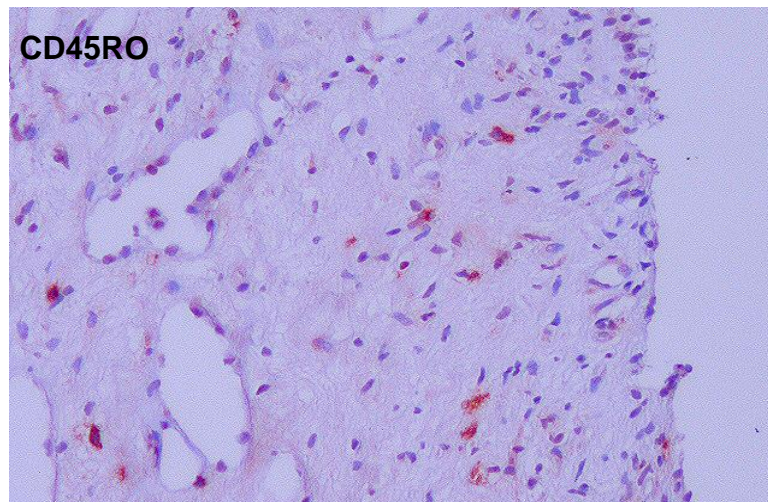


Fig. 9. Immunohistochemical localization of CD45RO positive cells in the pulp of treated caries

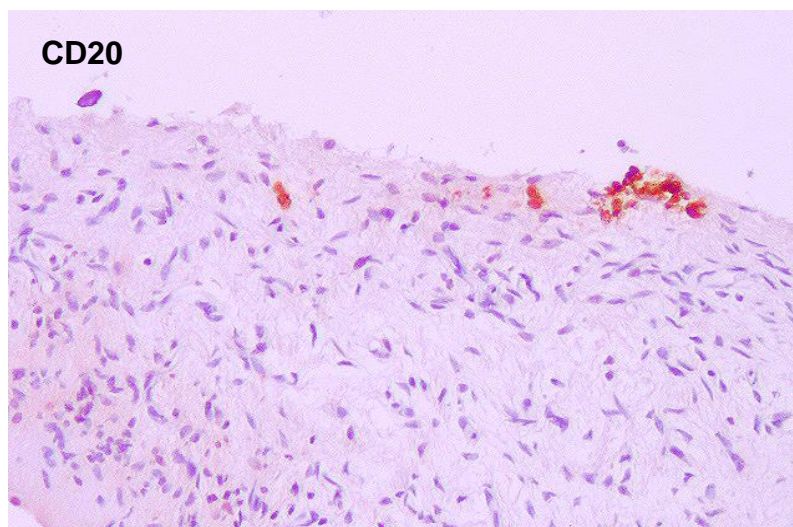


Fig. 10. Immunohistochemical localization of CD20 positive cells in the pulp of treated caries



## DISCUSSION

Defence reactions of the dentin/pulp complex involve a variety of biological systems, in which the immune system plays a very important role. The recognition of B and T lymphocyte subpopulations and antigen-presenting cells in tissues was made possible by the advent of immunohistological staining techniques using monoclonal antibodies against specific surface molecules on lymphocytes. Three monoclonal antibodies directed to immunocompetent cells and antigen-presenting cells were used to study pulpal tissues.

This study provides evidence that cavity depth influences the distribution of HLA-DR-positive dendritic cells and lymphocytes. Reduction in the thickness of residual dentin had an impact on the distribution of the cells. These changes are in agreement with findings from studies on the distribution of dendritic cells and lymphocytes in human teeth. Early pulpal response to bacterial diffusion of bacterial products through dentinal tubules elicits the influx of dendritic cells, T-lymphocytes and rare B-lymphocytes. As the infection is coming closer to the pulp, the response assumes a typical mixed character, consisting of T-cells and B-cells.

On the other hand, most components of adhesive systems and composite resins are able to diffuse through the dentinal tubules and reach the pulp tissue producing noxious effects on odontoblasts (25,26) and influence the function of pulpal immunocompetent cells (27,28,29,30). In meanwhile, no aggregations of dendritic cells were recognized under prepared cavities, and no aggregations of CD45-positive lymphocytes were detected in any sample of the cavity prepared teeth. Thus, the materials used here provided excellent sealing characteristics, and they effectively prevented the ingress of noxious substances to the dentin-pulp complex.

Decrease in the number of dendritic cells and lymphocytes were recognized under prepared cavities, with a statistical significance between caries-affected teeth and after treatment. Against our expectation, even after caries treatment, small aggregations of HLA-DR-positive dendritic cells, accompanied by CD45-positive cells T-lymphocytes, were left behind in 17 out of 20 samples. The inflamed lesions would be the result of activities by bacteria, which had existed locally deep in the dentinal tubules and survived even after the removal of caries. Presence of dendritic cells and lymphocytes after removal of the carious lesion shows that local antigen presentation and cellular and/or humoral immunoresponses persist even after careful treatment of caries.

## CONCLUSION

Our study demonstrated that dental pulps have different response to cavity preparation and restoration, and that antigen presentation and immunoresponses persist for many months, even after caries treatment. Further investigations are needed to ascertain how to control the bacterial activities that might have remained deep in the dentinal tubules.

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