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Immunohistochemical Study of Dendritic Cells in Dental Pulps from Non-Carious And Carious Teeth.

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ABSTRACT

In order to elucidate the immune reaction in the dental pulp as a sequel of caries, we have studied the distribution of dendritic cells in association with the development of the carious lesion. In this study we have analysed 150 teeth with different stages of progression of the carious lesion. The condition of the pulp was classified into five groups according to the progression of the carious lesions from stages S0 to S4. Cells were identified immunohistochemically by the streptavidin-biotin complex immunoperoxidase method by using the monoclonal antibody HLA-DR for dendritic cells. The immune response in the unaffected pulp is linked with the presence of few anti-HLA-DR positive cells. Their number showed an increase in teeth with shallow dentinal caries with localized accumulation of cells beneath the dentinal tubules communicating with the superficial caries. This was followed by a caries depth related increase of the dendritic cells. The accumulation of these cells under the dentin was apparent with the progression of the caries toward the pulp. These findings suggest that the response of pulpal dendritic cells to carious irritants triggers the defense reactions of the pulp and respond promptly and actively to dentinal tubule derived carious stimuli.

Keywords: dental pulp, dental caries, immunohistochemistry, dendritic cells

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INTRODUCTION

In the oral cavity a number of elements are present which potentially can be injurious to the pulp [1,2,3]. It is now well documented that bacteria and bacterial by-products are associated with most pulpal disease processes, activating various forms of immune reactions by acting as antigens [4,5,6,7]. Manifestations of these responses are governed by a locally provided immune system which is specifically adapted to the anatomical features of the dental pulp.

In the dental pulp, dendritc cells are a major population of resident immune cells, predominantly located within the subodontoblast and paraodontoblast regions (Yoshiba *et al*., 1996; Jontell *et al*., 1998; Ohshima *et al.*, 1999) [8,9,10]. Dendritic cells are potent antigen-presenting cells that initiate and modulate immune responses against pathogen invasion, they are crucial for the preservation of dental pulp vitality during pathogenic injuries caused by caries infection (Hahn and Liewehr, 2007; Goldberg et al., 2008; Farges et al., 2013) [11,12,13].

Dental caries is a chronic infectious disease mediated by a complex and dynamic bacterial biomass that affects the mineralized tissues of the tooth. Bacterial invasion of dentinal tubules is a well-described phenomenon and the main cause of the inflammatory response of the dental pulp (Cooper *et al.*, 2010) [14]. Therefore, the detection of caries pathogens is crucial to neutralize and eliminate injurious stimuli, to preserve dentin-pulp complex functionality, and to promote the healing of the dental pulp.

The presence of immunosurveillance components in the pulp was unveiled by the recent discovery of antigen-presenting cells that are capable of inducing an immune response by presenting processed foreign antigens to T lymphocytes [15,16,17]. These cells are believed to be associated with recognition of foreign antigens in non-lymphoid organs [18,19,20]. Like professional dendritic cells (DC) in other peripheral tissues they play a fundamental role in the initiation of primary immune responses, they may capture the antigens, process them, and migrate to regional lymph nodes, where they can mature into potent antigen-presenting cells (APCs) and then present the antigens to naïve $CD4^+$ T lymphocytes [21,22]. Dendritic cells are not able to identify foreign antigens specifically; they provide necessary signals to activate T-lymphocytes which in turn will activate other immunocompetent cells to mount the local immune defense of the dental pulp [23,24]. We have studied the distribution and quantitative changes of dendritic cells in association with the development of the carious lesion in order to elucidate the initial response and the expansion of the immune reaction in the dental pulp.

MATERIAL AND METHOD

In this study we have examined 150 human teeth from patients at age of 9 to 14 years old. Teeth were extracted from various therapeutic reasons (mostly from orthodontic reason), immediately cut in two longitudinally and fixed in formalin for 48 hours at 4 °C. The specimens were immersed in xylol and embedded in paraffin. Sections were cut at 5 µm thicknesses and stained by the streptavidin-biotin complex immunoperoxidase method. Immediately after extraction, the teeth were fixed in 10% buffered paraformaldehyde, embedded in paraffine and sliced into 4-5µm sections. After deparaffinisation, immunoperoxidase staining was performed, with commercially available anti-HLA-DR monoclonal antibody (DAKO).

The condition of the pulp tissue was classified into the following groups: stage (S) 0 (n=30, pulp of non-carious teeth), S1 (n=30, pulp with enamel caries), S2 (n=30, pulp with caries in dentin, about 2-3mm from the pulp chamber), S3 (n=30, pulp with caries deep into the dentin, 0.5-1.5mm from the pulp chamber) and S4 (n=30, exposed pulp). The depth of the carious lesion was determined according to the pigmentation of hard tissues.

The main number of antibody positive cells in each stage was calculated and statistically analysed by means of the Mann-Whitney test.

RESULTS

The number of anti-HLA-DR positive cells at each stage is shown in Table 1.

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In healthy and carious teeth, dendritic cells are easily detected by HLA-DR positive staining. Dendritic cells are mainly distributed at the peripheral dental pulp within the subodontoblast and paraodontoblast region, a few anti-HLA-DR positive cells were present at S0 (Fig. 1) and S1 (Fig. 2). In carious teeth underneath moderate and severe dentin lesions, an increase of HLA-DR-positive immunolabeled cells within the dentin-pulp complex is evident. Their number was slightly increased in S2 (Fig. 3) while their number has increased significantly at S3 ($p<0.01$) (Fig. 4). The number of positive cells has markedly increased at S4 (Fig. 5), and a significant difference was demonstrated between S2 and S4 ($p<0.01$).

Table 1. Number of HLA-DR antibody-positive cells in the dental pulp with caries at various stages (S)

	S0	S1	S2	S3	S4
N	30	30	30	30	30
HLA-DR	1.1	2.2	5.0	22.1	39.4

Values are means ± SEM; N, number of samples

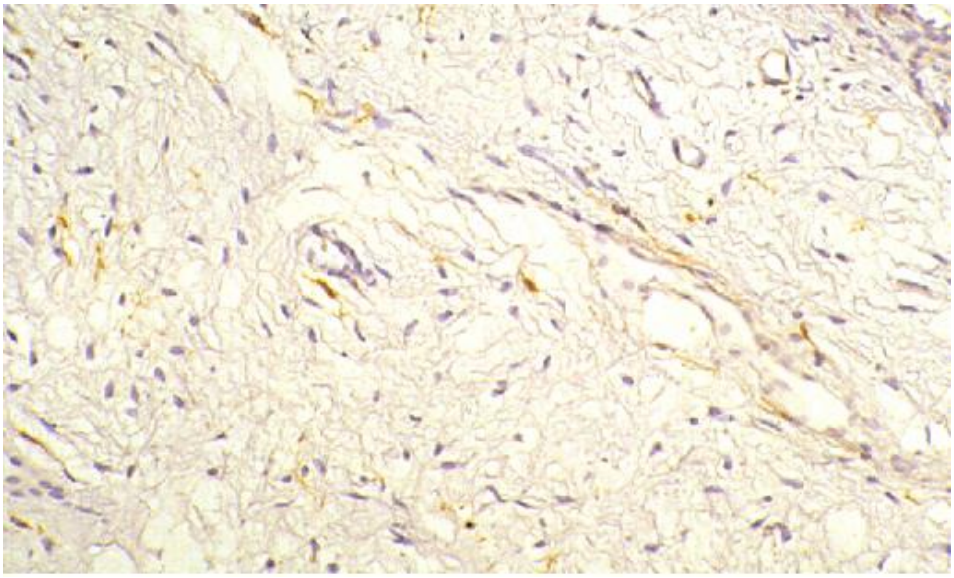

Figure 1. Immunoperoxidase staining of rare HLA-DR+ cells present in the dental pulp of a S0 sample

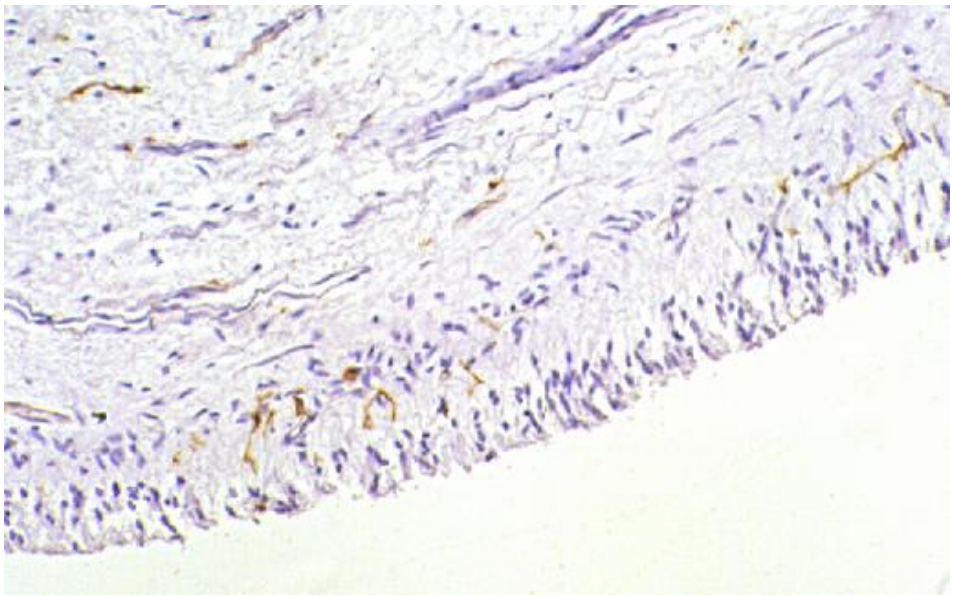

Figure 2. Immunoperoxidase staining of a few HLA-DR+ cells present in the dental pulp of a S1 sample

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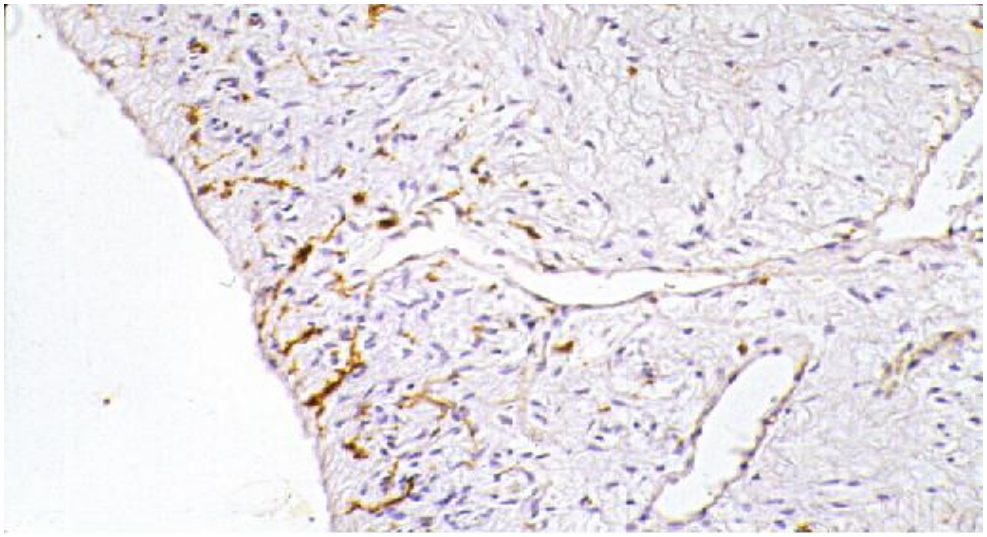

Figure 3. Immunoperoxidase staining of several HLA-DR+ cells present in the dental pulp of a S2 sample

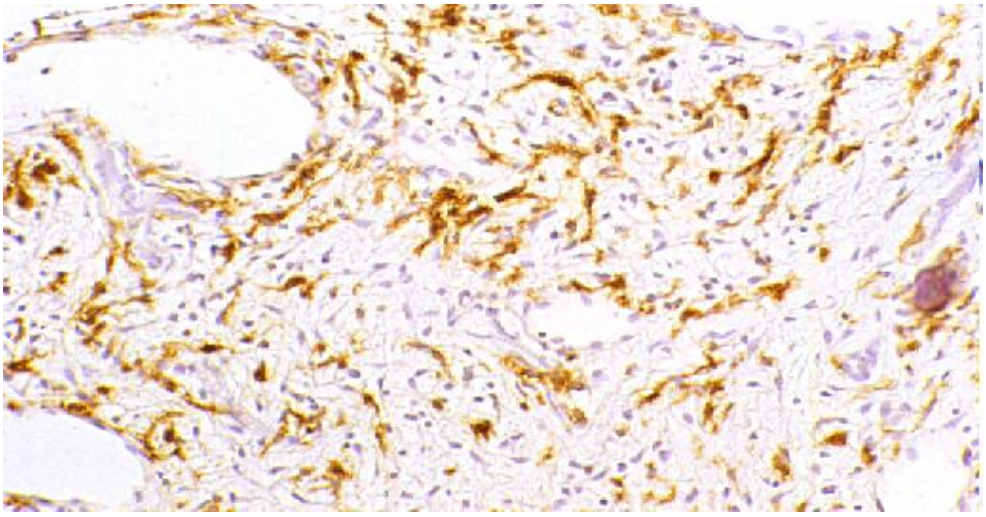

Figure 4. Immunoperoxidase staining of increased number of HLA-DR+ cells present in the dental pulp of a S3 sample

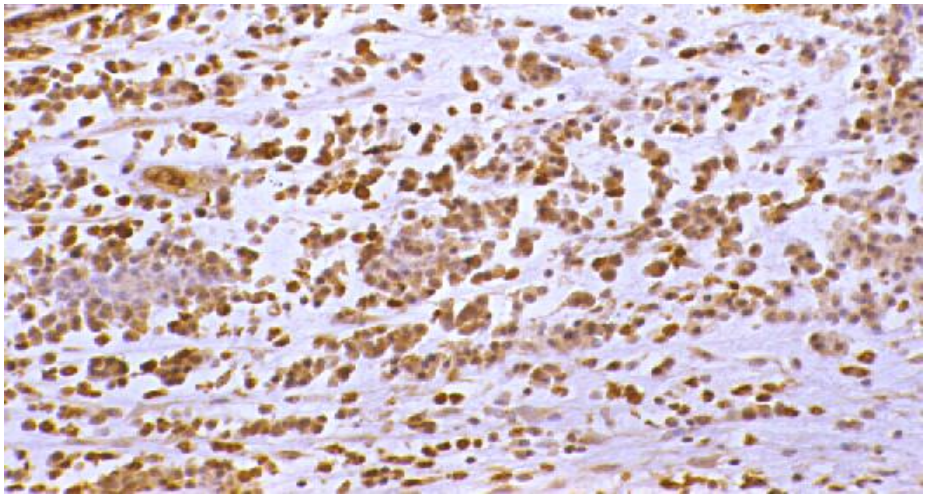

Figure 5. Immunoperoxidase staining of markedly increased number of HLA-DR+ cells present in the dental pulp of a S4 sample

DISCUSSION

Invasion of dentinal tubules by caries pathogens triggers defense and repair mechanisms within the dentin-pulp complex to contain pathogen spread and reduce dental pulp tissue damage. Defense reactions of the dental pulp involve a variety of biological reactions, in which the immune system plays a very important role. Identification of pulpal dendritic cells has led to a concept of how an antigenic challenge may evoke a pulpal inflammatory response. They are associated with the early phase of the immune response. Dendritic cells identify foreign antigens and provide necessary signals to activate T-lymphocytes which in turn will activate other immunocompetent cells to mount the local immune defense of the dental pulp.

Recent immunohistochemical and other morphological studies have actually provided evidence showing that the dental pulp contains a variety of indigenous and recruited immunocompetent cells. This cells seem to form a network that surveys invasion of noxious stimuli and, upon injury, establishes reaction pathways that characterize specificity of pulpal diseases [25,26,27]. The data obtained in this study suggest that regularly spaced immune-competent cells (*e.g.*, dendritic cells) act as sentinels against pathogen invasion at the dentin pulp interface. Once activated by diffusible bacterial compounds, dendritic cells initiate an innate immune response against caries pathogens and their migration into the predentin-dentin interface toward the bacterial aggressor. The activation of these cells is an early event in caries progression and implies changes at the odontoblast layer barrier to allow their passage into the predentin-dentin interface. Likewise, it has been demonstrated that dendritic cells open tight junctions between epithelial cells and send dendrites across the epithelial barrier to detect pathogens in gut and upper respiratory epithelium (Rescigno *et al.*, 2001; Kojima *et al.*, 2013) [28,29].

In healthy teeth only a few pulpal DC were scattered in the periphery of the dental pulp and along the vasculature where the pulp is initially subjected to noxious foreign stimuli. These cells are strategically concentrated at the site where the chance to encounter external antigens is the greatest. When caries lesion extended from the enamel just into the dentin, initial pulpal response was characterized by a localized accumulation of dendritic cells beneath the dentinal tubules communicating with the superficial caries. An aggregation of HLA-DR-positive cells was observed only in the subodontoblasic region. No distributive changes were observed in other areas. Progression of the lesion was followed by a caries depth related increase of the dendritic cells in the coronal pulp and they advanced toward the center of the pulp.

Antigen-presenting cells react with antigens that have reached the pulp tissue through the dentine tubules. 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 expansion of both the cell-mediated and the humoral immune reaction may occur in the dental pulp. Potential antigen-presenting cells in the pulp actually 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 activatate the pulpal immune defense reactions.

Our present results are in accordance with the accepted concept that pulp can start to react to caries long in advance of bacterial penetration into the pulp chamber and that the pulpal inflammatory status in response to caries maintains an inverse relation with the distance between the caries lesion and pulp chamber.

CONCLUSIONS

These findings suggest that the response of pulpal dendritic cells to carious irritants triggers the defense reactions of the pulp and respond promptly and actively to dentinal tubule derived carious stimuli. Anti-HLA-DR antibody positive cells participate in an efficient immune system in the human dental pulp. The intensity of the defense reactions may be correlated with the depth of the carious lesion.

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