

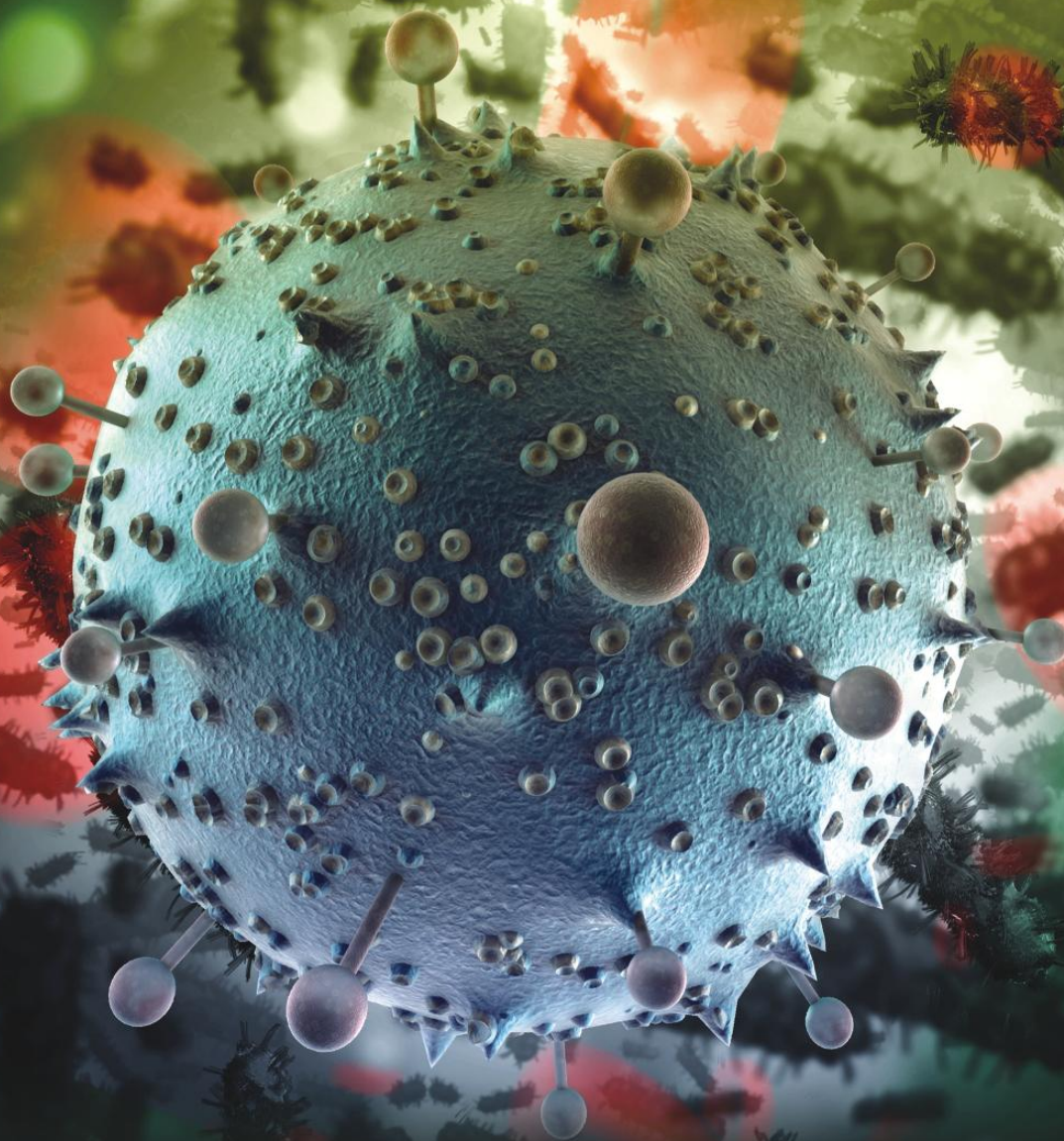
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- Distribution of class II major histocompatibility complex antigen-expressing cells in human dental pulp with carious lesions
- Maternal endotoxin-induced fetal growth restriction in rats: Fetal responses in toll-like receptor
- Novel development of carbonate apatite-chitosan scaffolds based on lyophilization technique for bone tissue engineering

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Case Report

Distribution of class II major histocompatibility complex antigen-expressing cells in human dental pulp with carious lesions

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ABSTRACT

Background: Dental caries is a bacterial infection which causes destruction of the hard tissues of the tooth. Exposure of the dentin to the oral environment as a result of caries inevitably results in a cellular response in the pulp. The major histocompatibility complex (MHC) is a group of genes that code for cell-surface histocompatibility antigens. Cells expressing class II MHC molecules participate in the initial recognition and the processing of antigenic substances to serve as antigen-presenting cells. **Purpose:** The aim of the study was to elucidate the alteration in the distribution of class II MHC antigen-expressing cells in human dental pulp as carious lesions progressed toward the pulp. **Methods:** Fifteen third molars with caries at the occlusal site at various stages of decay and 5 intact third molars were extracted and used in this study. Before decalcifying with 10% EDTA solution (pH 7.4), all the samples were observed by micro-computed tomography to confirm the lesion condition three-dimensionally. The specimens were then processed for cryosection and immunohistochemistry using an anti-MHC class II monoclonal antibody. **Results:** Class II MHC antigen-expressing cells were found both in normal and carious specimens. In normal tooth, the class II MHC-immunopositive cells were observed mainly at the periphery of the pulp tissue. In teeth with caries, class II MHC-immunopositive cells were located predominantly subjacent to the carious lesions. As the caries progressed, the number of class II MHC antigen-expressing cells was increased. **Conclusion:** The depth of carious lesions affects the distribution of class II MHC antigen-expressing cells in the dental pulp.

Key words: Class II major histocompatibility complex, dental pulp, caries

ABSTRAK

Latar belakang: Karies merupakan penyakit infeksi bakteri yang mengakibatkan destruksi jaringan keras gigi. Dentin yang terbuka akibat karies akan menginduksi respon imun seluler pada pulpa. Kompleks histokompatibilitas utama (MHC) merupakan sekumpulan gen yang mengkode histokompatibilitas antigen-antigen permukaan sel. Sel-sel yang mengekspresikan molekul-molekul ini berpartisipasi dalam pengenalan awal substansi-substansi antigenik untuk selanjutnya diproses dan dipresentasikan pada permukaan sel. **Tujuan:** Penelitian ini bertujuan untuk mengetahui perubahan distribusi sel-sel yang mengekspresikan molekul MHC kelas II pada pulpa gigi manusia dengan meningkatnya keparahan karies. **Metode:** Penelitian ini menggunakan 15 gigi molar ketiga yang mengalami karies pada permukaan oklusal dengan berbagai tingkat kedalaman dan 5 gigi molar ketiga normal (tidak mengalami karies). Sebelum didekalsifikasi dengan larutan EDTA 10% (pH 7,4), seluruh sampel diamati dengan menggunakan micro-computed tomography untuk mengetahui kedalaman lesi karies secara tiga dimensi. Spesimen kemudian diproses untuk dilakukan cryosection dan dilakukan imunohistokimia dengan menggunakan monoklonal antibodi anti MHC kelas II. **Hasil:** Ekspresi MHC kelas II oleh sel-sel pada ruang pulpa dijumpai disemua spesimen baik pada kondisi normal maupun karies. Pada gigi normal, sel-sel yang mengekspresikan MHC kelas II terletak terutama pada tepi pulpa. Pada gigi-geligi yang mengalami karies, agregasi sel-sel yang mengekspresikan MHC kelas II terutama terletak di bawah lesi karies. Semakin dalam lesi karies, jumlah sel-sel yang mengekspresikan MHC kelas II semakin meningkat. **Kesimpulan:** Kedalaman lesi karies berpengaruh terhadap distribusi sel-sel yang mengekspresikan MHC kelas II pada pulpa.

Kata kunci: Kompleks histokompatibilitas utama, pulpa gigi, karies

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INTRODUCTION

Dental caries is a bacterial infection which causes destruction of the hard tissues of the tooth. This disease remains the most prevalent infectious disease in the world. Among the oral bacteria, *Streptococcus mutans* and *Streptococcus sobrinus* are strongly associated with human dental caries. The ability of these bacteria to produce caries is directly related to their production of acid and their ability to tolerate large quantities of lactic acid. The acid will lead to the dissolution of the mineralized matrix of the teeth.^{1,2}

Dental pulp is a connective tissue resides in a rigid encasement of the mineralized dentin. When enamel is lost for any reason, the exposed dentinal tubules provide diffusion channels from the surface of the tooth to the pulp. It is known, however, that the pulp contains a variety of indigenous and recruited immunocompetent cells which have ability to initiate and maintain immune responses against pathogenic challenges that permeate the dentinal tubules. These cells seem to form a network that surveys invasion of noxious stimuli and, upon injury, establishes reaction pathways that characterize specificity of pulpal diseases. Exposure of the dentin to the oral environment as a result of caries inevitably results in a cellular response in the pulpo-dentinal organ.^{3,4}

Major histocompatibility complex (MHC) is a group of genes that code for cell-surface histocompatibility antigens. The function of the MHC molecules is to bind peptide fragments derived from the pathogens and display them on the cell surface for recognition by the appropriate T cells. The consequences are almost always deleterious of the pathogen.⁵ All cells in the body except erythrocytes express class I molecules, while only some cells such as dendritic cells and macrophages express class II molecules. Cells expressing class II molecules are called antigen presenting cell. These cells participate in the initial recognition and the processing of antigenic substances to serve as antigen-presenting cells. They migrate to lymphoid tissues via afferent lymph to induce T-cell proliferation and activation.⁶

Previous studies have revealed the presence of class II MHC antigen-expressing cells in normal dental pulp, both in permanent⁷ and deciduous teeth.⁸ However, the distribution of the class II MHC-expressing cells in the dental pulp remains obscure, and is not sufficiently understood. Changes in the distribution of class II MHC antigen-expressing cells have been shown during tooth development as well as cavity preparations.⁹ The present study aimed to elucidate the alteration in the distribution of class II MHC antigen-expressing cells in human dental pulp as carious lesions progressed toward the pulp.

MATERIALS AND METHODS

The protocol of this study was approved by ethical committee of Faculty of Medicine, Universitas Gadjah Mada. Twenty third molars from 20 to 40-years-old patients were used in this study. Informed consent was obtained from all patients who were enrolled in this study. Fifteen third molars with caries at the occlusal site at various stages of decay and 5 intact third molars were extracted for orthodontic or therapeutic reasons. The depth of the cavity judged by clinical examination was recorded. Under local anesthesia, the teeth were extracted and then immersed in 10% buffered formalin for 3 days. Before decalcifying with 10% EDTA solution (pH 7.4), all the samples were observed by micro-computed tomography to confirm the lesion condition three-dimensionally. The specimens were processed for cryosection. The tissues for cryosection were equilibrated in a 30% sucrose solution for cryoprotection. The specimens were then cut at thickness of 50 μ m on a freezing microtome, and processed for the avidin-biotin peroxidase complex (ABC) method using an anti-MHC class II (HLA-DR)-monoclonal antibody (Lab Vision, USA). Following incubation with the primary antibodies for 3 days in 4°C, the sections were reacted by two consecutive incubations with biotinylated anti-mouse IgG and ABC complex (Vector Lab. Inc., Burlingame, USA) for 2 hours each at room temperature. The sites of antigen-antibody reaction were then visualized by placing sections in 0.05 M TRIS buffer (pH 7.6) containing 0.04% 3–3'-diaminobenzidine tetrachloride and 0.002% H₂O₂. The immunostained sections were counterstained with methylene blue. Immunohistochemical controls were performed by: replacing the primary antibody with non-immune serum or PBS and omitting the anti-mouse IgG or the ABC complex. These immunocontrol sections did not show any specific immunoreactions.

RESULTS

An intense immunoreactive for class II MHC molecule was observed in the pulp tissue of the tooth in all cases. Class II MHC-immunopositive cells were distributed widely throughout the dental pulp. Some of them showed spindle-like or dendritic profiles, while some of them revealed macrophage morphology.

In normal intact tooth, the class II MHC-immunopositive dendritic cells were situated mainly at the cell-free zone and along the pulp-dentin border (Figure 1). Some of their cell processes were situated in the predentin and showed a close relationship to the odontoblast processes. They extended their thick cytoplasmic processes into the dentinal

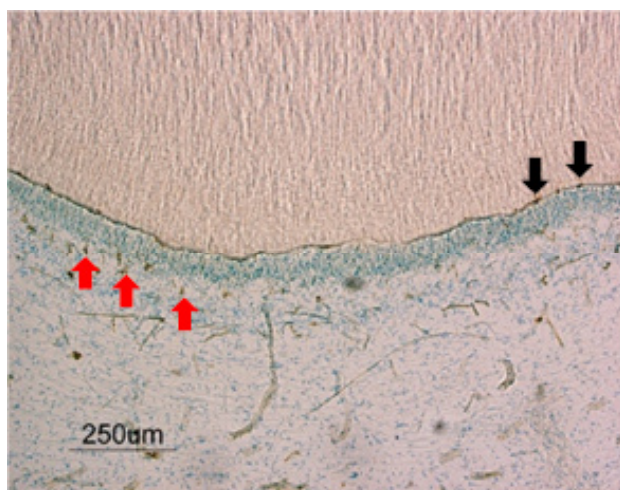


Figure 1. A specimen of normal intact tooth. The class II MHC-immunopositive dendritic cells are situated mainly at the cell-free zone (red arrows) and along the pulp-dentin border (black arrows).

tubules. Class II MHC-immunopositive macrophages were dispersed sparsely in the pulp tissue. The number of the class II MHC-immunopositive macrophages observed in normal pulps was fewer than the class II MHC-immunopositive dendritic cells.

In the cases of pulp affected by early caries, all specimens showed caries confined to the enamel. All specimens revealed small cluster of class II MHC-immunopositive cells in the pulpal restricted area beneath the pulpal ends of dentinal tubules communicating with the carious lesion (Figure 2). Numerous class II MHC-immunopositive dendritic cells extended their cytoplasmic processes into the affected dentinal tubules in the odontoblastic layer, while class II MHC-macrophage cells were seen distributed sparsely in pulp chamber. Compare to the intact teeth, the

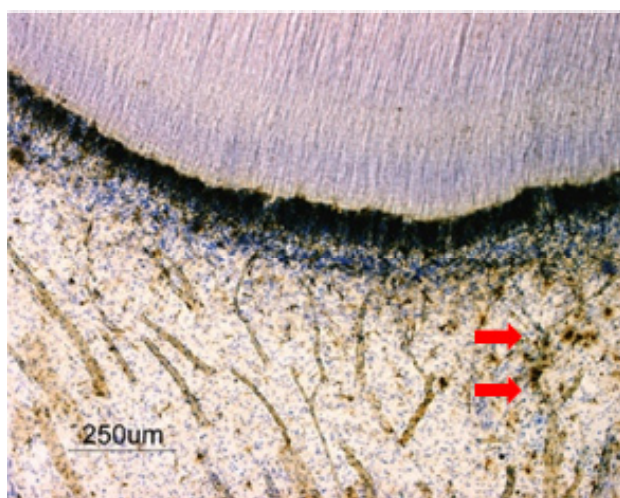


Figure 2. A specimen with early karies. A cluster of class II MHC-immunopositive cells are seen in the limited area subjacent to the pulpal ends of dentinal tubules communicating with the carious lesion (red arrows).

density of class II MHC-immunopositive cells in this case was higher.

In the cases of pulp affected by advanced caries, all teeth showed caries confined to the dentin. The aggregation of class II MHC-immunopositive cells was more densely gathered subjacent to the carious lesion than elsewhere. The specimens with advanced caries exhibited an expansion of the area of increased class II MHC-immunopositive cells; thus, the dense network of class II MHC-immunopositive cells was widely seen in the pulp chamber. The density of MHC-immunopositive cells was higher in the cases of carious lesion confined to dentin than enamel. Their expression became highest in the cases of carious lesion confined to dentin with pulp exposure. In advanced caries, lymphocytes and endothelial cells were also expressed class II MHC molecules in addition to dendritic cells and macrophages. Tertiary dentin was observed in all specimens with advanced caries. In the area beneath the tertiary dentin, limited presence of class II MHC-immunopositive cells were observed.

DISCUSSION

This study showed the alteration of class II MHC antigen-expressing cells distribution in human dental pulp as caries progressed toward the pulp. In normal tooth, the class II MHC-immunopositive cells were distributed sparsely throughout the dental pulp, and located predominantly at the periphery of the pulp tissue. As the caries progressed, the number of class II MHC-immunopositive cells was increased and distributed more widely in the pulp. They located mainly subjacent to the carious lesions.

The presence of class II MHC antigen-presenting cells in the normal dental pulp implied important roles played by these cells in maintaining physiological conditions of the pulp tissue. In the present study, the normal dental pulps were shown to contain two types of class II MHC-immunopositive cells: dendritic cells and macrophages. Dendritic cells are widely accepted to be the most potent and versatile antigen presenting cells in the immune system. These cells are believed to possess a more potent antigen-presenting capacity than macrophages. In peripheral tissues, dendritic cells are highly motile, possess superior capacity for acquiring and processing antigens for presentation to T lymphocytes, and within secondary lymphoid organs have the potential to express high levels of the co-stimulatory molecules that direct and fine-tune T cell activation.¹⁰ Dendritic cells are extremely efficient in stimulating naive T-lymphocytes.⁵

This study revealed that the dendritic cells were strategically positioned in the periphery of the pulp where foreign antigens are most likely to enter the tissue. Their primary function may be to monitor invasion of antigens. Numerous dendritic cells were situated in the predentin and extended their cytoplasmic processes into the affected dentinal tubules. These finding implying their high motility and support the concept that the dendritic cells play a critical

role in the initial immune defense of the dental pulp against trans-dentinal antigenic stimuli.¹⁰

Macrophages function as a first-line defense to detect and eliminate invading microbes and toxic macromolecules.¹¹ This study showed that macrophages were distributed sparsely in the pulp and seemed to form a network together with pulpal dendritic cells. There are three major biological functions of macrophages: phagocytosis, antigen processing and presentation, and cytokine secretion. In addition to its importance in defense against pathogenic bacteria, phagocytosis is an essential step in antigen processing. Following endocytosis, digestion, and degradation, peptide fragments from phagocytosed proteins are able to associate with class II MHC peptide within organelles of the endosomal pathway. The class II MHC-peptide complexes are then transported to the cell surface, where they interact with T lymphocytes. At the same time, the macrophages secrete interleukin (IL) 1, a necessary signal in antigen presentation which stimulates proliferation of the lymphocytes.¹²

In carious teeth, cluster of class II MHC-immunopositive cells were observed mainly beneath the lesion. The density of these cells in the area subjacent to the lesions was highest compare to other area in the pulp chamber. The dendritic cells which are class II molecule-expressing cells carry receptors on their surface that recognize common features of many pathogens. Cariogenic bacterial components will bind to these receptors, then stimulate the dendritic cells to engulf the cariogenic bacteria and degrade them intracellularly.⁵ After capture protein antigens, dendritic cells will migrate to the regional lymph nodes and present the peptide fragments in conjunction with class II MHC molecules to antigen-specific T lymphocytes (naïve T lymphocytes). Helper T lymphocytes will subsequently clonally expand and differentiate into effector T cells or memory T cells. Effector T cells and some memory T cells then enter into the vasculature and migrate to the pulp. Resident antigen-presenting cells such as macrophages will interact with and present antigen directly to the T cells, which are locally activated and trigger the effector phase of the immune response against bacterial antigens.¹³ Macrophages have a variety of receptors that recognize microbial surface components which are involved in the ingestion of bacteria by phagocytosis and in signaling for the secretion of proinflammatory cytokines, which then recruit and activate more phagocytes.¹¹

As the caries progressed, the number of the bacteria invaded the dentinal tubules increase.¹⁴ This study demonstrated that the number of class II MHC-immunopositive cells increased as the caries advanced and the lesions progressed toward the pulp. After capture the antigen, dendritic cells secrete certain chemokine namely IL-8, which then attract the inflammatory cells to the site of infection.¹⁵ Thus, as caries progressed, the inflammatory cells infiltration may increase as well. This finding was supported by the previous study¹⁶ which proved that the

number of inflammatory cells infiltration increased as the caries progressed toward the pulp.

When a carious lesion has invaded dentin, the pulp responds by depositing a layer of tertiary dentin over the dentinal tubules of the secondary dentin that communicate with the carious lesion. Compare to secondary dentin, tertiary dentin differs morphologically and less permeable to externally derived matter.¹⁷ This study revealed only few number of class II MHC-immunopositive cells presence beneath the tertiary dentin, implying its low permeability.

The dental pulp is known to have a high potential for participation in both defense against foreign stimuli and tooth repair. The class II MHC-immunopositive cells in the pulp-dentin border was assumed to have some unknown functions other than immunosurveillance. Kannari *et al.*,⁸ proposed an inductive role for these cells in the differentiation, migration and/or activation of odontoclasts and cementoblast-like cells during physiological root resorption. Thus, the predentinal class II MHC-immunopositive cells as shown in the present study might also exert some effects on the odontoblasts under physiological conditions. However, this hypothesis is needed to be tested in future studies.

Previous report¹⁸ has demonstrated a rich supply of pulpal nerve fibers beneath the odontoblast layer to form the subodontoblastic nerve plexus in the coronal dental pulp. The distribution pattern of the class II MHC antigen-expressing cells in the dental pulp of the teeth with carious lesions in this study was similar to the distribution pattern of the nerve fibers which have been shown in the previous study. This similarity in distribution patterns between the class II MHC antigen-expressing cells and neural elements in the tooth with carious lesions revealed an intimate functional correlation between them. Indeed, the author suggested the involvement of neural elements in the functional capacities of the class II MHC antigen-expressing cells. In conclusion, the depth of carious lesions affects the distribution of class II MHC antigen-expressing cells in the dental pulp.

REFERENCES

1. Marsh PD, Nyvad B. The oral microflora and biofilms on teeth. In: Fejerskov O, Kidd E, editors. Dental caries: the disease and its clinical management. 2nd ed. Oxford: Blackwell Munksgaard; 2008. p. 179–80.
2. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res* 2006; 85(1): 22–32.
3. Pashley DH, Liewehr FR. Structure and functions of dentin-pulp complex. In: Cohen S, Hargreaves KM, editors. Pathways of the pulp. 9th ed. Missouri: Mosby Elsevier; 2006. p. 461.
4. Yu C, Abbot PV. An overview of the dental pulp: Its functions and responses to injury. *Aus Dent J* 2007; 52(1 suppl): S4–16.
5. Murphy K, Travers P, Walport M. Janeway's immunobiology. 7th ed. New York: Garland Science; 2008. p. 331.
6. Hahn C, Liewehr FR. Update on the adaptive immune responses of the dental pulp. *J Endod* 2007; 33(7): 773–81.

7. Olgart L, Bergenholtz G. The dentine-pulp complex: responses to adverse influences. In: Bergenholtz G, Horsted-Bindslev P, Reit C, editors. *Textbook of endodontology*. Oxford: Blackwell Munksgaard; 2003. p. 26–7.
8. Angelova A, Takagia Y, Okiji T, Kanekoc T, Yamashita Y. Immunocompetent cells in the pulp of human deciduous teeth. *Arch Oral Biol* 2004; 49(1): 29–36.
9. Kawagishi E, Nakakura-Ohshima K, Nomura S, Ohshima H. Pulpal responses to cavity preparation in aged rat molars. *Cell Tissue Res* 2006; 326(1): 111–22.
10. Ricart BG, John B, Lee D, Hunter CA, Hammer DA. Dendritic cells distinguish individual chemokine signals through CCR7 and CXCR4. *J Immunol* 2011; 186(1): 53–61.
11. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* 2005; 23: 901–44.
12. Vega MA, Corbi AL. Human macrophage activation: too many functions and phenotypes for a single cell type. *Immunol* 2006; 25(4): 248–72.
13. Iwasaki A. Mucosal dendritic cells. *Annu Rev Immunol* 2007; 25: 381–418.
14. Love RM. Invasion of dentinal tubules by root canal bacteria. *Endod Top* 2004; 9: 52–65.
15. Haniastuti T. Histological evaluation of human pulp tissue in response to caries progression. *Dentika Dental J* 2010; 15(2): 165–9.
16. Oz-Arslan D, Ruscher W, Myrtek D, Ziemer M, Jin Y, Damaj BB, Sorichter S, Idzko M, Norgauer J, Maghazachi AA. IL-6 and IL-8 release is mediated via multiple signaling pathways after stimulating dendritic cells with lysophospholipids. *J Leukoc Biol* 2006; 80(2): 287–97.
17. Haniastuti T, Nunez P, Djais AA. The role of transforming growth factor beta in tertiary dentinogenesis. *Dent J (Majalah Kedokteran Gigi)* 2008; 41(1): 15–20.
18. Haniastuti T. Pulp nerve fibers distribution of human carious teeth: an immunohistochemical study. *Dent J (Majalah Kedokteran Gigi)* 2010; 43(4): 186–9.