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ヒト扁桃腺濾胞でのB細胞分化の単クローン抗体を用いる免疫組織学的検討

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Immunohistological Identification of B Cell Differentiation in Human Tonsillar Follicles by Using Monoclonal Antibodies

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HARABUCHI, Y., YAMANAKA, N. and KATAURA, A. *Immunohistological Identification of B Cell Differentiation in Human Tonsillar Follicles by Using Monoclonal Antibodies*. Tohoku J. exp. Med., 1985, **147** (1), 21 - 31 —— Using immunoperoxidase technique and various monoclonal antibodies, B1, B2, OKT9, OKT10 and Leu-7, we investigated B cell differentiation in the tonsillar follicle. Mantle zone was stained with B1 intensely and B2 faintly. In contrast, germinal center was stained with B1, OKT10 and OKT9, and more intensely with B2. In the intermediate part of the germinal center some large cells were stained with OKT10. With OKT9 antibody, most of cells in the lymphoepithelial symbiosis and some large dendritic cells considered to be tingible body macropharges or dendritic reticulum cells in the germinal center were intensely stained. Leu-7 positive cells were localized mainly in the intermediate part of the germinal center. Stages of B cell differentiation in the tonsillar follicle were discussed, considering these results. ———— immunohistological identification ; palatine tonsil ; monoclonal antibody ; B cell differentiation

The palatine tonsil is situated at the begining of both the respiratory tract and the gastrointestinal tract, and appears to play an important role in the immune defense against bacteria and other antigens. The structure of palatine tonsil is characterized by numerous crypts and lymphoid follicles. The tonsillar crypts have primary and secondary branches which markedly increase the surface of the tonsil, and appear to be the entrance of foreign antigens such as bacteria and virus which invade the tonsil and provoke an immunological response in the tonsil. The tonsillar follicle has an asymmetrical and cap-shape mantle zone which widens on the side facing the epithelium of the crypt, and has a germinal center in which active immune reactions of various immuno-competent cells occur.

In the previous study (Yamanaka et al. 1983), we investigated the distribution of T cell subsets in the palatine tonsil by using monoclonal antibodies which

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define T cell subsets.

In the present work, we investigated the distribution of the subpopulations of B cells and natural killer (NK) cells in the tonsillar follicles by using various monoclonal antibodies, B1, B2, OKT10, OKT9 and Leu-7, which define B cells generally, a subpopulation of B cells, activated cells and NK cells, in order to analysis B cell differentiation in the tonsillar follicles.

Materials and Methods

Tonsils. Ten human palatine tonsils were examined from surgical procedures for recurrent tonsillitis or simple tonsillar hypertrophy. The patients' ages were 3 to 23 years, mean age 8.9 years. The specimens were cut about $1 \times 1 \times 0.5$ cm and rapidly frozen and stored in hexan at $-80°C$.

Monoclonal Antibodies. A series of monoclonal antibodies employed in this study were B1 and B2 (Coulter Electronic, Hialaeh, Florida, U.S.A.), OKT10 and OKT9 (Ortho Diagnostic Systems, Raritan, New Jersey, U.S.A.) and Leu-7 (Becton Dickinson FACS Systems Monoclonal Antibody Center, Sunnyvale, California, U.S.A.). B1 antibody reacts with approximately 9% of the peripheral blood mononuclear cell fraction and over 95% of B cells from blood and lymphoid organs (Nadler et al. 1981 ; Stanshenko et al. 1980). B2 antibody is shown to be expressed exclusively on Ig^+B cells isolated from peripheral blood and lymphoid tissues (Nadler et al. 1981). OKT10 antibody reacts with 70-80% of thymocytes, and also with myeloblasts, immature B lymphocytes, immunoblasts and plasma cells (Janossy et al. 1981 ; Reinherz and Schlossman 1980, 1981). OKT9 antibody reacts with only 1-5% of resting peripheral blood cells, but 40-80% of mitogen stimulated ones, and recognizes the transferrin receptor on the proliferated cells (Goding and Burn 1981 ; Haynes et al. 1981 ; Suthernland et al. 1981). Leu-7 (HNK-1) antibody reacts with 20 ± 7% of adult peripheral blood mononuclear cells, and recognizes natural killer (NK)/killer cells (Abo and Balch 1981).

Immunoperoxidase Procedures. A four-step immunoperoxidase technique using avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981) was employed in this study. Four micrometer thick frozen sections were fixed in aceton for 10 min at 4°C and washed three times in cold phosphate buffered saline (PBS), pH 7.4, for 5 min each. Serial sections were incubated with mouse monoclonal antibodies, i.e., B1, B2, OKT10, OKT9 and Leu-7 (10 μl of 1 : 20-1 : 40 dilution), for 45 min at room temprature. After washing with PBS, all sections were incubated with biotin-labeled horse anti-mouse IgG antibody (Vector Labs, Burlingame, California, U.S.A.) diluted 1 : 200 for 45 min at room temprature. Sections were then washed with cold PBS, and the 1 : 200 diluted fresh ABC (Vector Labs, Burlingame, California, U.S.A.) was applied for 45 min at room temprature. After washing with PBS, all sections were reacted with a substrate 0.03% 3-3′ diaminobenzidine in 0.05 M tris buffer, pH 7.4, with 0.03% hydrogen peroxide for 2-5 min at room temprature. The sections were counterstained with 0.2% methylgreen, dehydrated, cleared in xylol and mounted in synthetic resin. Control for method specificity was performed by omission of primary antibody or replacement of primary antibody by non-immune mouse serum.

Results

In the present study, the tonsillar follicle was shown to have three distinct zones, expressing different distributions of cells which reacted with monoclonal antibodies, including B1, B2, OKT10, OKT9 and Leu-7 ; which consisted of the basal part and the intermediate part forming the germinal center, and the mantle

TABLE 1. *The distribution of cells which react with various monoclonal antibodies in the tonsillar follicle*

Antibodies	Mantle zone	Germinal center	
		Intermediate part	Basal part
B1	Most of cells with membrane staining	Most of cells with membrane and/or cytoplasmic staining	
B2	Most of cells with weak membrane staining	Most of cells with intense cytoplasmic and/or intercellular staining	
OKT10	Not stained	Most of cells with weak membrane staining Some large cells with intense membrane or cytoplasmic staining	
OKT9	Not stained	Most of cells with membrane staining	Some large dendritic cells with intense cytoplasmic staining
Leu-7	Not stained	Some cells with intense membrane staining	Not stained
IgM	Most of cells with membrane staining	Cytoplasmic and/or intercellular staining	Not stained
IgD	Most of cells with membrane staining	Almost not stained	

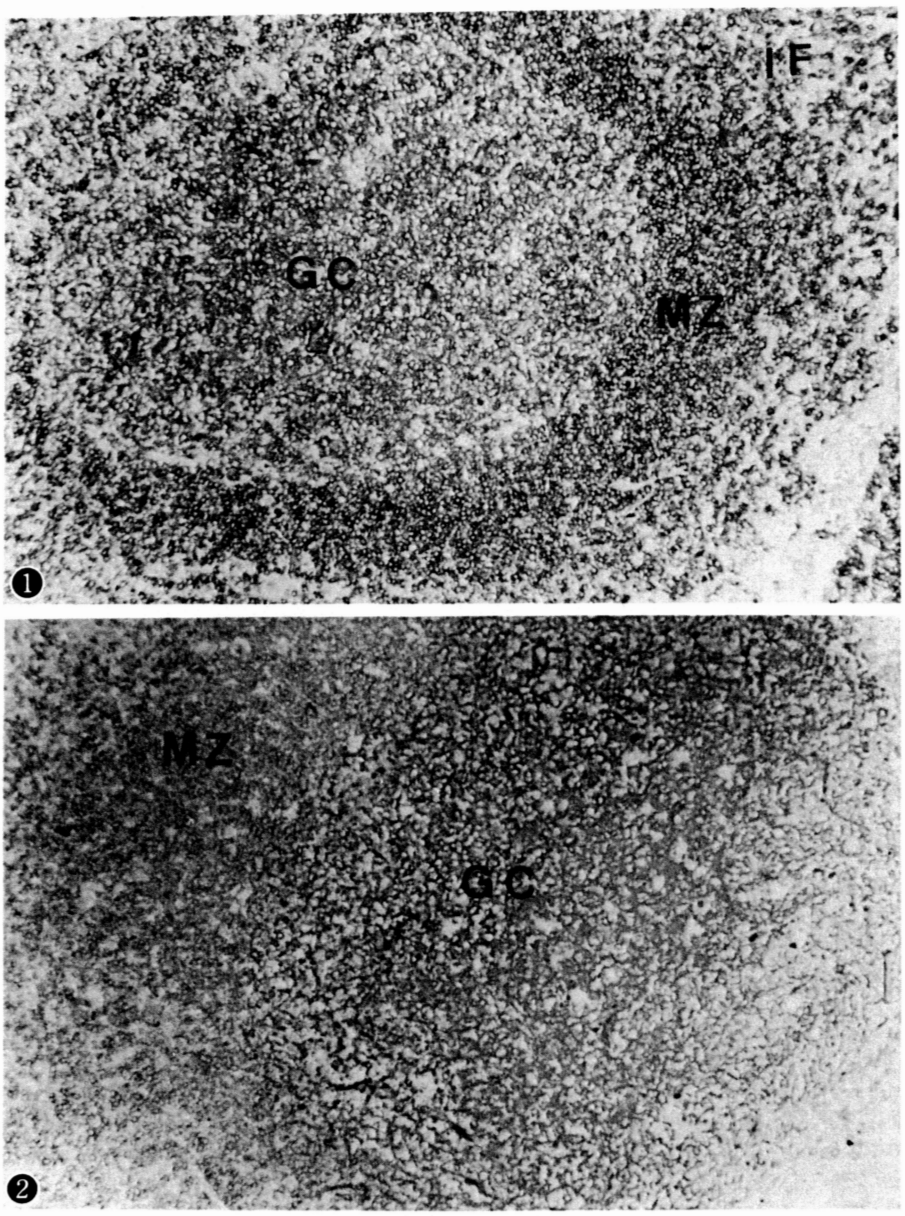


Fig. 1. Tonsillar section stained with B1 antibody (×100). Most of mantle zone (MZ) cells show membrane staining and majority of germinal center (GC) cells are represented with membrane or cytoplasmic staining. In the interfollicular area (IF), some positive cells are scattered.

Fig. 2. Tonsillar follicle stained with B2 antibody (×100). Small lymphocytes in the mantle zone (MZ) show faint membrane staining. In contrast there is an intense cytoplasmic and/or intercellular staining throughout the germinal center (GC).

zone (Table 1). Both B1 and B2 antibodies stained primarily tonsillar follicle and small number of lymphocytes in the interfollicular area, but there was a difference of the pattern of staining between B1 and B2 antibodies. With B1 antibody, there were membrane staining of small lymphocytes in the mantle zone and membrane and/or cytoplasmic staining in the germinal center, and both sites showed the same intense staining. On the other hand with B2 antibody, most of

small lymphocytes in the mantle zone showed weak membrane staining, in contrast majority of the germinal center cells were demonstrated with intense cytoplasmic and/or intercellular staining (Fig. 1 and 2).

OKT10 antibody positive (OKT10$^+$) cells were located both in the interfollicular area and in the germinal center. In the interfollicular area, especially besides the mantle zone, OKT10$^+$ cells were seen with intense cytoplasmic staining. Most of the germinal center cells showed faint membrane staining with OKT10 antibody, but in the intermediate part, especially on the basal side of the germinal center, some large OKT10$^+$ cells showed intense membrane or cytoplasmic staining (Fig. 3).

OKT9 antibody stained most of cells both in the lymphoepithelial symbiosis (LES) of the crypt and the germinal center, and small number of lymphocytes in the interfollicular area with membrane staining (Fig. 4). In the LES, there were a considerble number of cells which showed membrane or cytoplasmic staining with OKT9 antibody, especially intense staining on the crypt side (Fig. 5). Inside the germinal center, most of cells showed membrane staining with OKT9 antibody, in addition some large cells with dendritic cytoplasm showed intense cytoplasmic staining, especially in the basal part of the germinal center (Fig. 4 and 6).

Leu-7 antibody positive (Leu-7$^+$) cells were localized mainly in the intermediate part of the germinal center with intense membrane staining, in contrast these cells were scattered rarely in the interfollicular area (Fig. 7).

The number of both OKT9$^+$ cells and Leu-7$^+$ cells tended to be large in the hyperplastic follicles of simple hypertrophied tonsil in child patients, as compared with those in the lymphoid follicles of adult patients with recurrent tonsillitis.

Discussion

Curran and Jones (1977, 1978) described that tonsillar follicle consisted of three distinct zones by using heteroantisera against IgG, IgA, IgM, IgE and IgD. In addition, our previous results using anti-IgM and anti-IgD serum (Yamanaka et al. 1983) were consistent with their results, i.e., anti-IgM serum stained both the mantle zone and the germinal center, especially the intermediate part of the germinal center, whereas anti-IgD serum stained only the mantle zone (Fig. 8). The present results obtained by the immunoperoxidase method using various monoclonal antibodies may support these previous reports as shown in Table 1.

Most of small lymphocytes in the mantle zone were expressed with intense membrane staining with IgM, IgD and B1, and weak membrane staining with B2. In contrast, most of the germinal center cells were stained with B1, OKT9, OKT10, and intensely with B2. Furthermore the intermediate part of the germinal center were represented with intecellular and/or cytoplasmic staining for IgM, but not IgD. According to the previous reports, the majority of peripheral blood B cells express both IgM and IgD on their surface (Gathing et al. 1977;

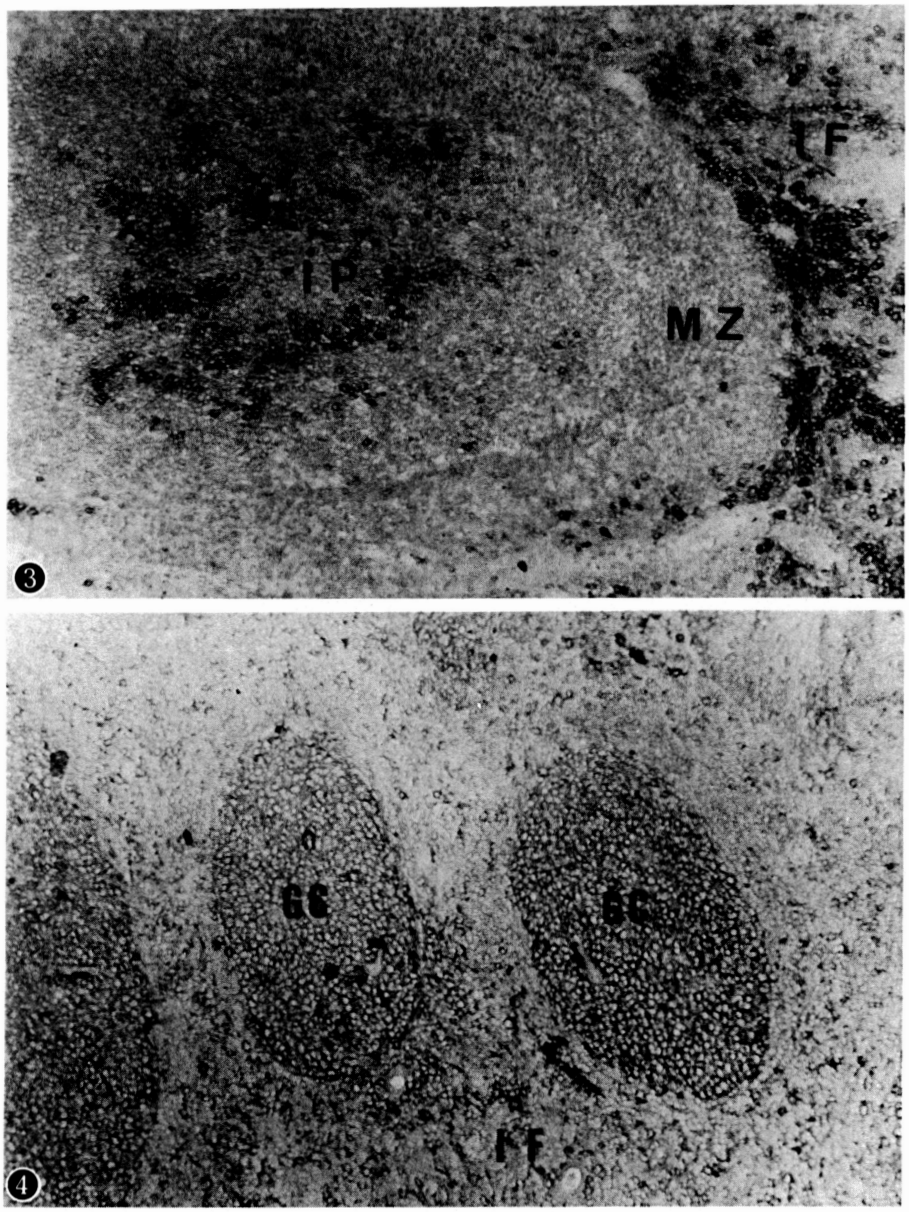
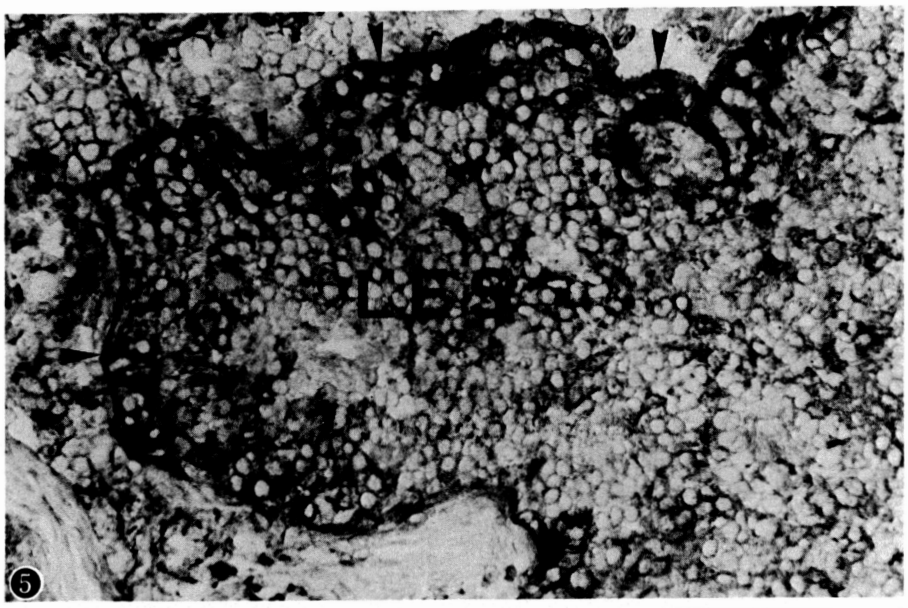


Fig. 3. Tonsillar section stained with OKT10 antibody (×100). OKT10$^+$ cells considered to be plasma cells are located in the interfollicular area (IF) besides the mantle zone (MZ). There is a weak membrane staining throughout the germinal center, in addition, large OKT10$^+$ cells are located in the intermediate part (IP) of the germinal center, especially on the basal side.

Fig. 4. A section of tonsil stained with OKT9 antibody (×100). Most of cells in the germinal center (GC) show membrane staining. Some positive cells are scattered in the interfollicular area (IF).

Keamy et al. 1977), but on the B cell ontogency, surface-IgD is lost soon after antigenic or mitogenic stimulation (Preud' homme 1977; Balch et al. 1980). Stanshenko et al. (1981) described that after mitogenic stimulation peripheral blood B cells expressed B2 intensely, OKT10, and acquired cytoplasmic IgM. Furthermore OKT9 antibody recognizes lymphoid cells stimulated by mitogens (Goding and Burn 1981; Haynes et al. 1981). Considering these previous reports



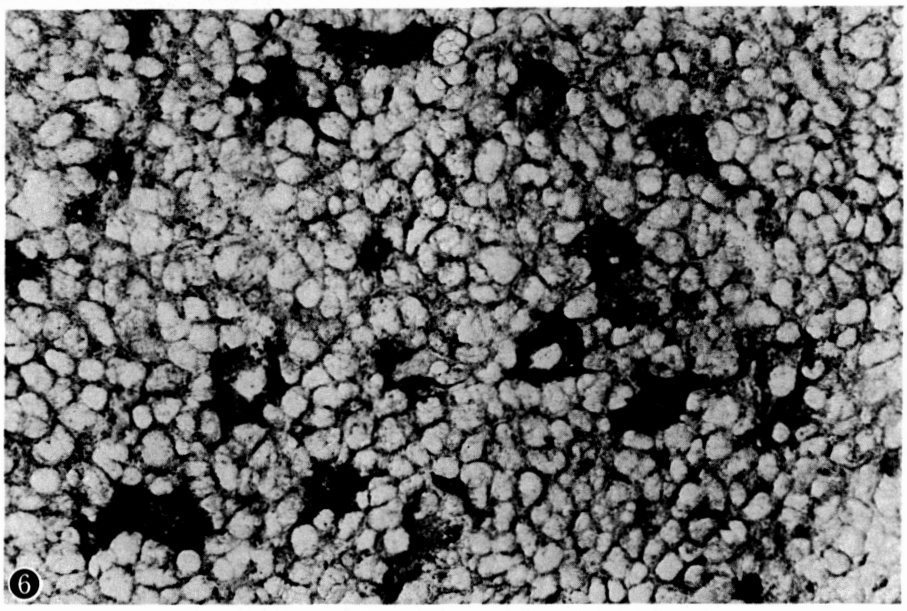


Fig. 5. A portion of the lymphoepitherial symbiosis (LES) stained with OKT9 antibody (×250). Majority of LES cells are with membrane or cytoplasmic staining, in addition, cells on the crypt side (arrow head) show intense staining.

Fig. 6. A higher magnification of the part of the germinal center stained with OKT9 antibody (×400). Some large dendritic cells considered to be tingible body macropharges or dendritic reticulum cells show intense cytoplasmic staining behind other germinal center cells with membrane staining.

and our present results, small lymphocytes in the mantle zone are mature B cells which are identical to peripheral B cells. On the other hand most of B cells in the germinal center differ from mantle zone cells in the maturation, i.e., after antigenic stimulations they are activated and differentiated into immunoglobulin-secreting cells.

OKT10$^+$ cells were located just in the outside of the lymphoid follicles with intense cytoplasmic staining. In the preious study (Curran and Jones 1977, 1978 ;

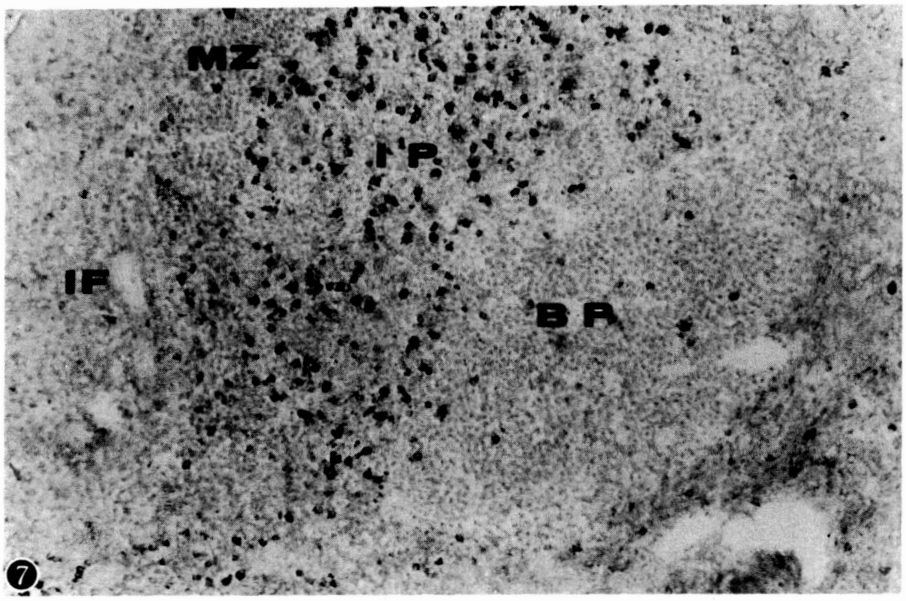


Fig. 7. Tonsillar section stained with Leu-7 antibody (×100). Leu-7$^+$ cells are localized mainly in the intermediate part (IP) of the germinal center with intense membrane staining. A few positive cells are seen in the interfollicular area (IF), but the mantle zone (MZ) and the basal part (BP) of the germinal center are almost not stained.

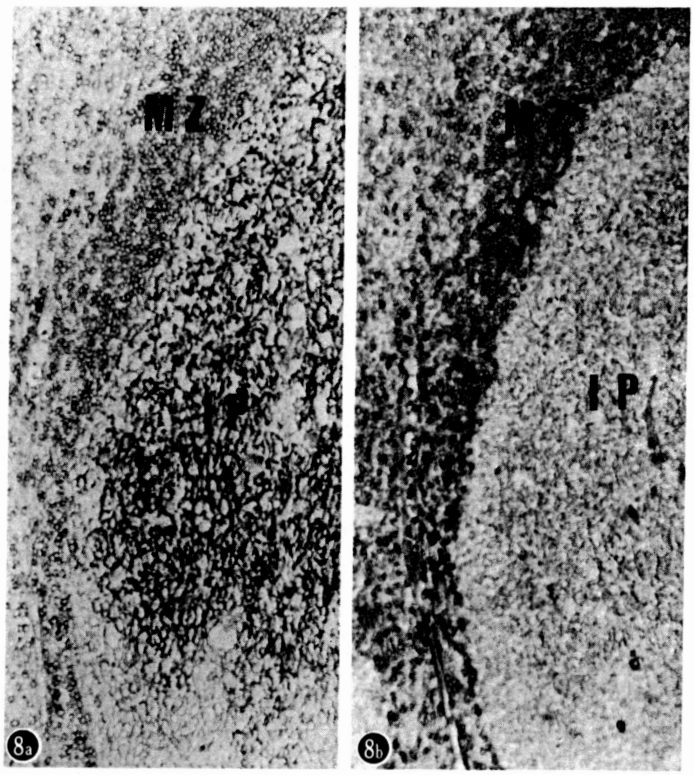


Fig. 8. Tonsillar follicle sections stained with anti-IgM serum (a) and anti-IgD serum (b). (×100) Anti-IgM serum stains both the mantle zone (MZ) and the intermediate part (IP) of the germinal center, however, anti-IgD serum stains only the MZ.

Matthews and Basu 1983) and our present results, cells in this portion are not stained with B1 and Leu-1 antibodies, but stained intensely with anti-immunoglobulin sera. Therefore, OKT10$^+$ cells in this portion may represent plasma cells. In addition, some large cells in the intermediate part of the germinal center stained intensely with OKT10 antibody, but not Leu-1 antibody. These results suggest that OKT10$^+$ cells in the intermediate part of the germinal center are probably cells in the B cell lineage, i.e., immature B cells, immunoblasts or plasma cells, but neither thymocytes nor activated T cells.

The LES in the crypt is a characteristic structure of the palatine tonsil, and foreign antigens may be getting into the tonsillar tissue via this site. Majority of cells in this site showed membrane or cytoplasmic staining with OKT9 antibody, especially more intense staining on the crypt side. This fact may indicate that most of LES cells are in activated state, especially cells on the crypt side contacting with foreign antigens are in more activated state, and active reactions of LES cells against antigens are performed. In addition there were some dendritic large cells with intense cytoplasmic staining with OKT9 antibody in the germinal center, especially in the basal part. These cells are considered morphologically to be tingible body macropharges (TBM) or dendritic reticulum cells (DRC). The functions of both cells have not been clearly understood yet, however, TBM are described to phagocytize lymphocytes and gammaglobulins (Swartzendruber and Congdon 1963), and DRC are demonstrated to be antigen-presenting cells which hold antigens and transfer them and/or antigen informations to helper T cells (Nossal et al. 1968 ; Kamperdijk et al. 1978). The present results with OKT9 antibody show that TBM or DRC are in the activated state and play actively an important role in the immune response in the germinal center.

Leu-7$^+$ cells, i.e., natural killer (NK) cells, are localized mainly in the intermediate part of the germinal center where B cell differentiation is performed. In our recent study using two-color immunoperoxidase technique (unpublished data), these Leu-7$^+$ cells showed the cross-reaction with Leu-1 antibody. Abo et al. (1982) suggested that Leu-7$^+$OKT3$^+$ cells had low NK activity by two-color immunofluorescene assay. In vitro study, Nabel et al. (1981) described that B cell differentiation was regulated by a cloned cell line of NK cell fraction. Considering these findings, Leu-7$^+$ cells in the intermediate part of the germinal center may be immature NK cells with low NK activity, and may play a role for the regulation of B cell differentiation.

In hypertrophied tonsil of young children there were relatively large number of OKT9$^+$ cells and Leu-7$^+$ cells in the germinal center as compared with tonsils of adult with recurrent tonsillitis. These results support that germinal centers of hypertrophied tonsils with child patients are immunologically more active than those of recurrent tonsillitis in adult patients.

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