Lymphology 36 (2003) 74-83

THE ROLE AND EXPRESSION OF CD27 AND CD70 LYMPHOCYTES IN THE HUMAN TONSIL

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ABSTRACT

In this study, we investigated the expression and function of CD27 and CD70 in the human tonsil. We found that IgG production was significantly reduced after the administration of anti-CD27 and anti-CD70 antibodies in the T cell dependent B cell activation system, suggesting the involvement of CD27/CD70 in the production of IgG. In addition, the amounts of peripheral and tonsillar $CD4^+CD27^+$ cells were almost identical, whereas the expression of $CD4^+CD27^-$ cells was higher (4.9%) in tonsil than in peripheral blood (2.4%). Furthermore, anti-CD27 antibody and anti-CD4 antibody-positive cells were found mainly in the interfollicular region, and a small number of this cell population was observed in both germinal center (GC) and mantle. Double-staining showed that the germinal center was almost completely composed of $CD4^+CD27^+$ cells. In contrast to CD27 cells, only a small number of $CD70^+$ cells were present in the follicles. Moreover, anti-CD19 antibody, a B cell marker, was stained in the GC and mantle zone, and $CD19^+CD27^+$ cells were observed in the GC close to the mantle. The above findings favor the involvement and role of CD27/CD70 in the T-B cell interaction by inducing IgG production and increasing memory cell ($CD4^+$ $CD27^-$) number in the tonsil.

The tonsil is a lymphatic organ. Whereas lymph nodes process antigens via afferent lymph vessels, the tonsil is in direct contact with antigens via mucoepithelium; thus, it is considered as mucosa- associated lymphatic tissue (1). Because of its anatomical location, the tonsil is an immunological tissue responsible for the biological defense against bacteria, viruses, and oral and respiratory exogenous proteins such as dietary antigens. However, the tonsil is also thought to be a target organ of bacterial and viral infection. Furthermore, the tonsil has been reported to produce specific antibodies against pathogens and exogenous proteins (2).

Although many studies on the immunological function of the tonsil have been investigated, quite a few details remain unclear. Immune responses are regulated by direct or cytokine-mediated interactions among T cells, B cells, and macrophages. Recently, several lymphocyte-function molecules such as TNF receptor and its ligand family that are involved in cellular interactions have been identified (3,4). Based on their structural similarity, the lymphocyte-function molecules include CD40 ligand/ CD40 that plays a central role in T -B cell interaction *in vivo*, and CD27 and its ligand, CD70 (5-7). CD27 is expressed during the resting phase in T, B and natural killer (NK) cells, and is an activated- antigen promoted

by stimulation. In contrast to CD27, CD70 is only expressed on activated T and B cells (7).

Although the interaction between $CD27^+$ B cells and $CD70^+$ T cells has been reported to play an important role in T cell-dependent B cell activation (6,8), little is known about the role of CD27/CD70 in the human tonsil, which we investigated in this study. We provide support for the involvement and role of CD27/CD70 in T-B cell interaction.

MATERIALS AND METHODS

Preparation and Culture of Lymphocytes

Tonsillar and peripheral blood lymphocytes (PBL) were obtained from patients with chronic tonsillitis at the Department of Otolaryngology of Juntendo University Hospital. After tonsillectomy, the tonsil was minced and filtered through a gauze mesh. PBL from the same patients were obtained by Ficoll-Conray gradient centrifugation and used for flow cytometric studies. Tonsillar lymphocytes were aseptically collected and adjusted to $1x10^6$ cells/ml in RPMI-1640 (GIBCO, BRL, NY) supplemented with 5% newborn calf serum, 50 IU/ml penicillin and 50 µg/ml streptomycin (culture medium). The aliquots of 200µl were applied into 96-well microplates, and cells were divided into 1% pokeweed mitogen (PWM)-treated cells with and without 20µg/ml anti-CD27 antibody or anti-CD70 antibody, each. Each group, in triplicate, was cultured in 5% CO_2 incubator for 10 days. The culture medium alone was used as a negative control.

Antibodies and Reagents

Anti-human CD4 monoclonal antibody (mAb), RPA-T4 and anti-human CD19 mAb, HIB19 were obtained from Pharmingen (San Diego, CA). Anti-human CD27 mAb, 1A4 and anti-human CD70 mAb, 2F11 were kindly provided by Dr. I. Morimoto (Tokyo University, Tokyo, Japan). Anti-human CD70 mAb, HINE51 was purchased from Immunotech (Berkeley, CA), and anti-human CD70 mAb BU69 from Ancell from (Bayport, MN).

Flow Cytometry

Cells ($1x10^6$) were incubated for 30 min at 4°C with CD70 mAb, BU69 (with fluorescein isothiocyanate, FITC) or CD27 mAb, 1A4. After incubation, the cells were washed 3 times with phosphate-buffered saline (PBS), and those incubated with 1A4 were further stained with FITC-conjugated sheep anti-mouse immunoglobulin $F(ab')_2$ (Silenus, Australia) for 30 min on ice. For double staining, cells were incubated with phycoerythria (PE)-labeled CD4 mAb for 30 min. After washing, they were incubated with 1A4, and finally stained with FITC-conjugated sheep anti-mouse immunoglobulin $F(ab')_2$ for 30 min on ice.

After washing, the cells were analyzed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA) in accordance with the manufacturer's instructions.

Quantification of IgG

To calculate the amount of IgG in culture supernatant, sandwich enzyme-linked immunosorbent assay (ELISA) was performed using 2F11 (anti-CD70 antibody) or 1A4 (anti-CD27 antibody). The absorbance was measured according to the manufacturer instructions. The amount of antibody production (ng/ml) was calculated from the standard curve.

Immunohistochemistry

Four µm-sections were fixed in acetone for 10 min and blocked with avidin-biotin blocking kit (Vector Laboratories, Burlingane, CA). The sections were further blocked with 2% normal goat serum for 5 min and then incubated with mouse anti-CD27 (1:800) overnight at 4°C. After washing, sections were incubated with biotin-conjugated goat anti-mouse IgG (1:300) (DAKO A/S,

Glostrup. Denmark) for 60 min at room temperature and further incubated with FITC-conjugated streptavidine (DAKO) (1:50) for 30 min. For double staining, the sections were blocked with avidin-biotin blocking kit again. Then they were incubated with mouse anti-human CD4 (1:1), anti-human CD19 (1:1). or anti- human CD70 (1:10) mAbs overnight at 4°C. After washing, sections were incubated with biotin-conjugated goat anti-mouse IgG for 60 min at room temperature and further incubated with Cy-3 streptavidin (1:500) (Zymed, South San Francisco, CA). Slides were washed, mounted and then observed on fluorescence microscope (ZEISS, Germany).

Statistical Analysis

Statistical differences were analyzed by Student's t test, and $p<0.05$ was considered to be significant. The results are shown as mean ± SD.

RESULTS

Antibody Production by CD27/CD70 Interaction in Tonsil and Expression of CD27 and CD70 in the Tonsillar Lymphocytes and PBL

It has been reported that CD27/CD70 interaction enhances the IgG production in PBL (8). However, the effect of CD27/CD70 interaction on IgG production in tonsil has not been investigated. As shown in *Fig. 1A*, after the treatment of cells with anti-CD27 and anti-CD70 antibodies in the T cell-dependent B cell activation, the PWM-induced IgG production significantly decreased. Our results show that CD27/CD70 interaction increases IgG production not only in peripheral blood but also in tonsil.

Next, we performed a series of experiments using flow cytometry to evaluate whether CD27 and CD70 are differently expressed in tonsillar lymphocytes and PBL from a same patient. We found that there was no significant difference in the expression of CD27 or CD70 between the tonsil and PBL *(Fig. 1B)*. The expression of CD27 in PBL and tonsil was 54.3 ± 20.2% versus 52.1 ± 9.9%, respectively, whereas the expression of CD70 in PBL and tonsil was 7.4 ± 3.2% and 4.6 ± 1.5%, respectively, in eight separate experiments *(Fig. 1B)*.

Localization of $CD27^+$ and $CD70^+$ Cells in the Tonsil

Although the above results did not show a difference in expression of CD27 and CD70 between tonsil and peripheral blood, we wanted to know the localization of CD27 and CD70 in tonsil. A relatively high number of $CD27^+$ cells were observed in the interfollicular region, and a small and very small numbers of CD27 cells were observed in the follicles and mantles, respectively *(Fig. 2A)*. Furthermore, we examined the localization of $CD4^+$ cells, and found that many $CD4^+$ cells were observed in the interfollicular T cell regions and a small number was observed in the follicles *(Fig. 2B)*. Double-staining with anti-CD4 and anti-CD27 antibodies showed that most of cells in the germinal center (GC) were $CD4^+CD27^+$ cells *(Fig. 2C)*. In addition, antibody against CD19, which is a B cell marker, was abundantly positive in the mantles and the GC *(Fig. 3B)*. Double-staining with anti-CD19 and anti-CD27 antibodies showed that $CD19^+CD27^+$ cells were observed in GC close to the mantle *(Fig. 3C)*. Only a small number of $CD70^+$ cells were localized in the follicles *(Fig. 4B)*. The double-staining with anti-CD27 and anti-CD70 antibodies showed that $CD70^+$ cells in the GC were $CD27^+$ cells *(Fig. 4C)*.

Comparison of $CD4^+CD27^+$ and $CD4^+CD27^-$ Cells Between PBL and Tonsillar Lymphocytes

It has been reported that $CD4^+CD27^-$ cells were present only in the memory subset (9). Thus, to evaluate the differential expression of memory subset between

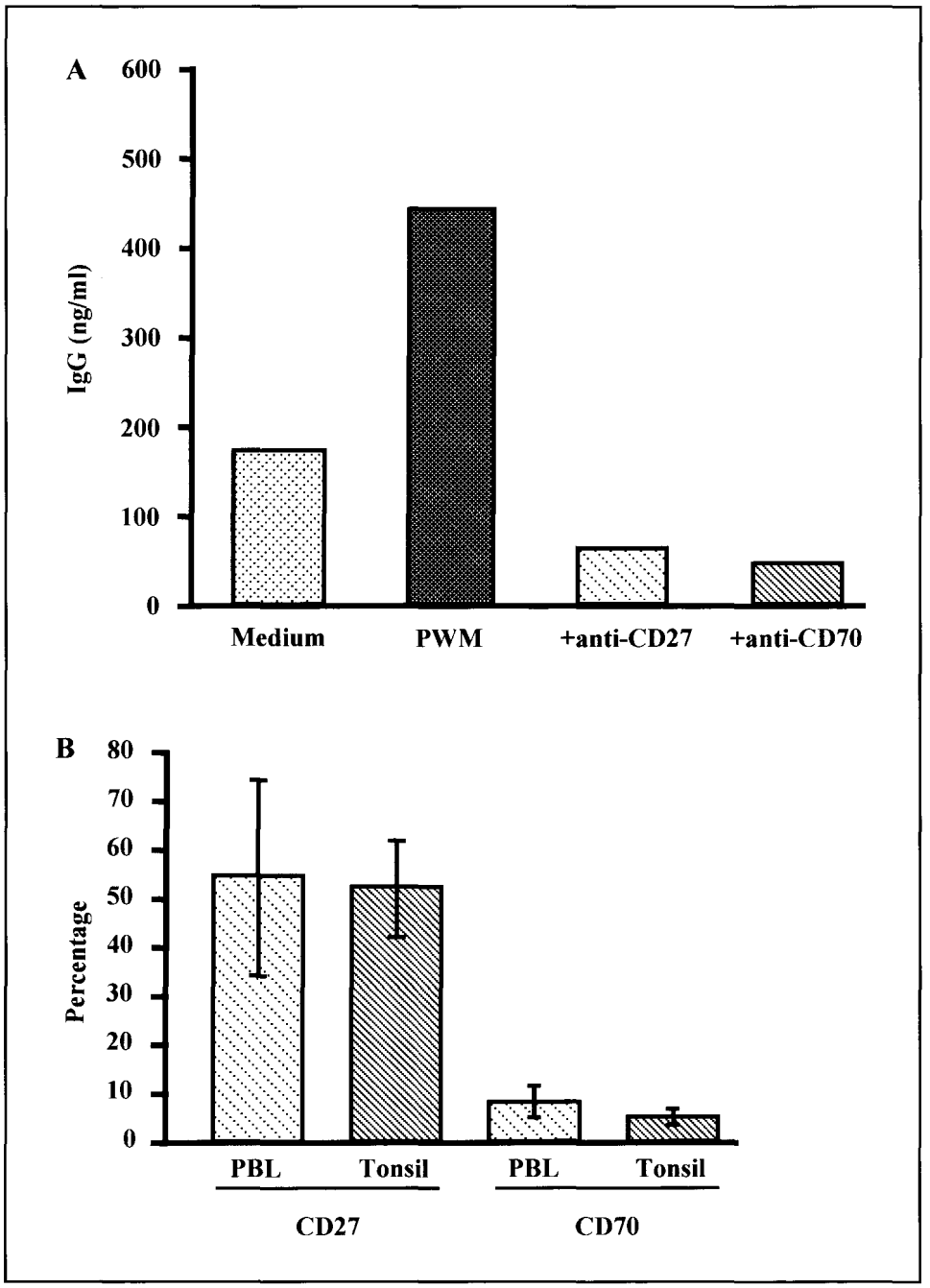

Fig. 1. Effect of CD27/CD70 interaction on IgG production and expression of CD27 and CD70 in tonsillar lymphocytes and PBL. (A) Tonsillar lymphocytes were aseptically cultured in the presence of PWM (1:100 dilution) (PWM) with anti-CD27 antibody (+anti-CD27) or anti-CD70 antibody (+anti-CD70). The medium alone (Medium) was used as a negative control. The IgG production was assayed by ELISA. (B) Tonsillar lymphocytes and PBL from a same patient were stained with anti-CD27 or anti-CD70 antibodies and the expression of CD27 or CD70 was assayed by FACS as described in Material and Methods section. Each bar represents the mean ± SD of eight separate experiments.

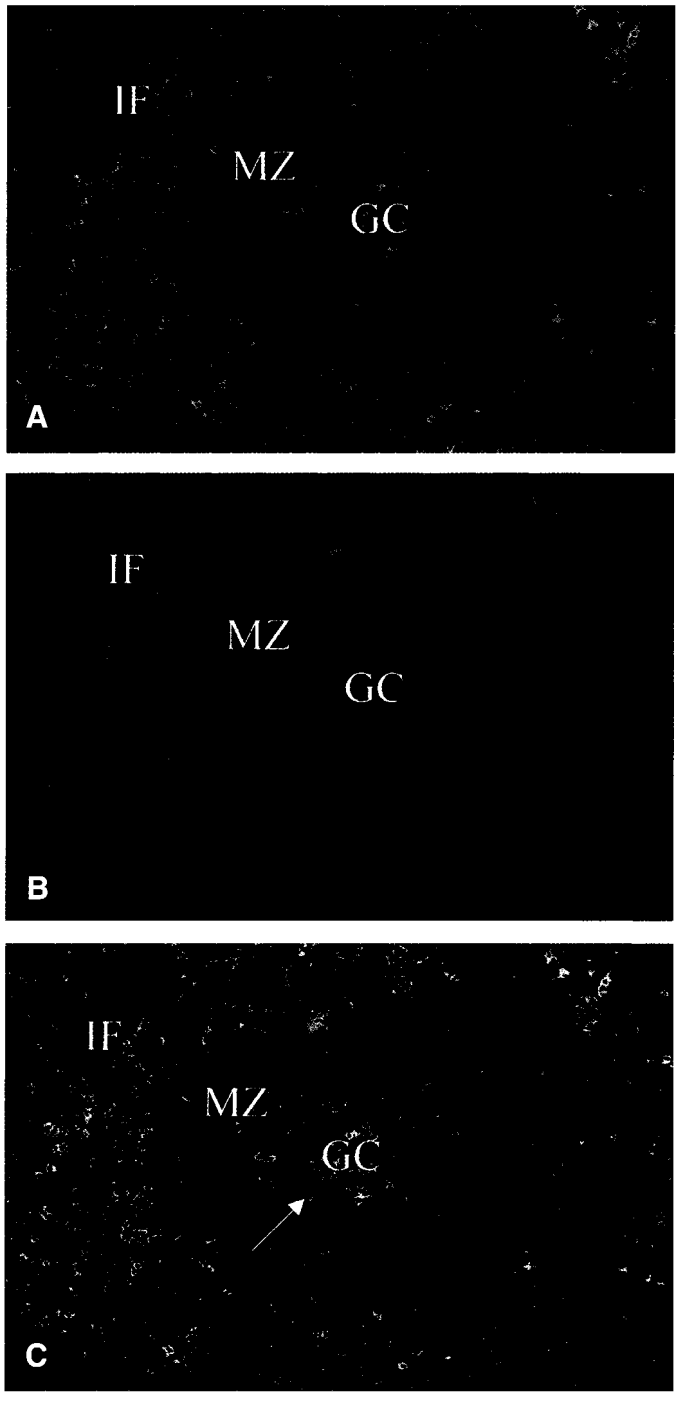

Fig. 2. Localization of CD27, CD4 and CD4 CD27 cells in tonsil. (A) The cryostat section of tonsil showing strong expression of CD27 in interfollicular T-cell areas and germinal center B-cell areas. (B) The cryostat section of the same tonsil as shown in A. A strong expression of CD4 was observed in the interfollicular T-cell regions and the germinal center B-cell areas. (C) Double staining of the same section as above with anti-CD4 and anti-CD27 antibodies showed that most of cells in the germinal center were $CD4^+CD27^+$ cells (arrow) (Magnification of x 200).

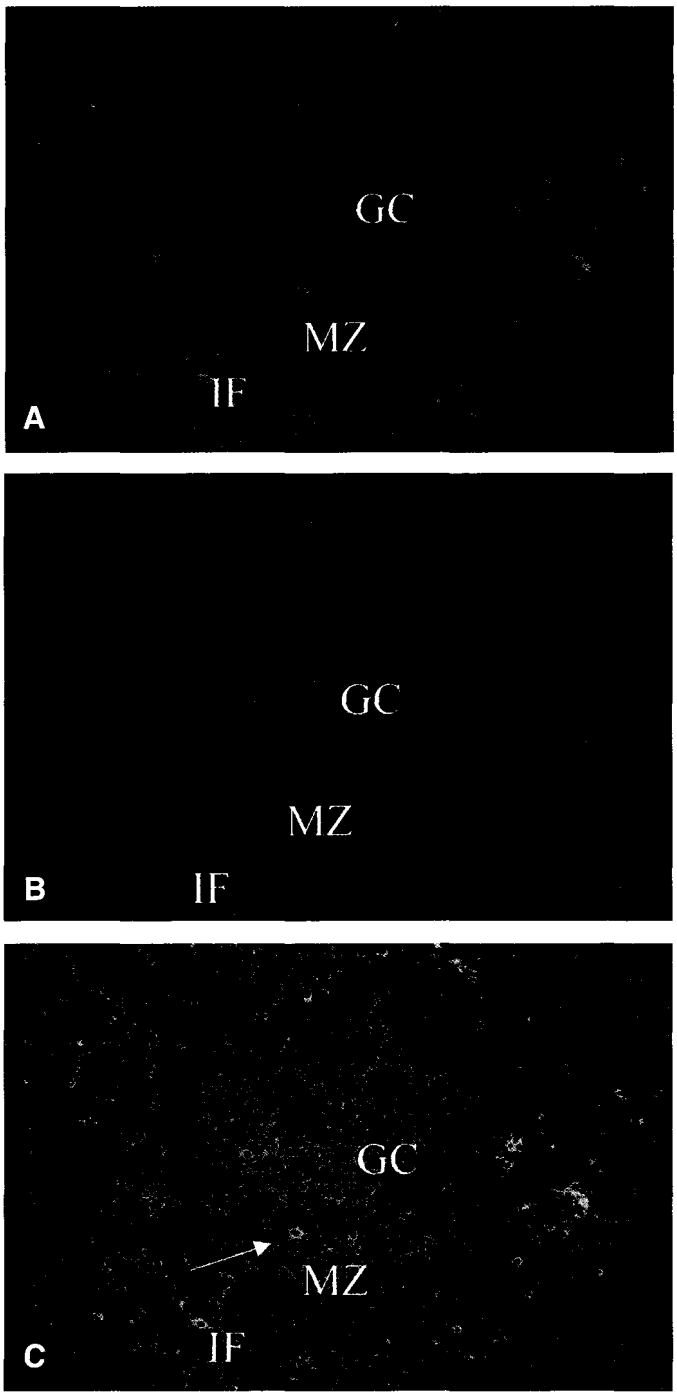

Fig. 3. Localization of CD19, CD27 and CD19 CD27 cells in tonsil. (A) The cryostat section of tonsil showing strong expression of CD27 in interfollicular T -cell areas and germinal center B-cell areas. (B) The cryostat section of the same tonsil as shown in (A). Antibody against CD19, a B cell marker, was abundantly positive in mantle zone and germinal center. (C) Double staining of the same section as above with anti-CD19 and anti-CD27 antibodies showed that $CD19^{+}CD27^{+}$ cells were in the germinal center close to mantle zone. (arrow) (Magnification of x 200).

tonsillar lymphocytes and PBL, we compared the content of $CD4^+CD27^-$ cells in both tonsil and peripheral blood. Most of peripheral $CD4^+$ T cells were CD27 cells, while in the tonsillar $CD4^+$ T cell fraction, $CD27^-$ cells tended to increase *(Fig. 5A)*. $CD4^+CD27^-$ cells were 2.4 ± 1.2% and 4.9 ± 2.3% in PBL and tonsillar lymphocytes, respectively, and their expression levels significantly differed ($P<0.05$) *(Fig. 5B)*. There was no statistical difference of the expression of $CD4^+CD27^+$ in both PBL and tonsillar lymphocytes (32.2 ± 10.6% versus 33.2 ± 4.8%) *(Fig. 5B)*.

DISCUSSION

In the present study, we found that the treatment of lymphocytes with anti- CD27 and anti-CD70 mAbs reduced IgG production. In addition, $CD4^+CD27^-$ cells were mainly found in tonsil rather than the PBL. Furthermore, a great amount of anti-CD27 antibody-positive cells was found in the interfollicular region, and a small number of this cell population was observed in both the GC and mantle zone. Moreover, most of $CD4^+$ $CD27^+$ cells were observed in the GC.

It has been reported that tonsillar lymphocytes possess the ability to produce antibody (10), and likewise are involved in autoimmune diseases (11). Soluble CD27 is increased in blood and body fluids in chronic and autoimmune diseases such as sarcoidosis, rheumatoid arthritis, multiple sclerosis, Hodgkin's disease, and chronic lymphatic leukemia (5). In this study, we focused on CD27 and CD70 expression in the tonsil. We compared the expression levels of CD27 as well as CD70 between tonsillar lymphocytes and PBL, and no significant difference was found. In T cell-dependent B cell activation, the amount of IgG production decreased significantly due to the treatment of cells with anti-CD27 or anti-CD70 antibodies, suggesting that CD27/CD70 was also involved in the interaction between T and B cells. Several studies have investigated the source of $CD27^-$ cells. For example, Hitzen and his group have documented that $CD27^-$ cells were present only in the memory subset $CD45RA^-$ $CD45RO^+$ in the $CD4^+$ T cell fraction (9), whereas Van Lier et al has shown these cells derive from $CD27^+CD45RA^-CD45RO^+$ T cells after long stimulation (12). Moreover, some investigators have suggested that most antigen reactive cells belong to the $CD27^-CD4^+CD45RO^+$ T cell subset in allergic reactions or in subjects after potentiation by recall antigens (13). In our work, we compared $CD27^-CD4^+$ and $CD27^+CD4^+$ fractions between tonsillar and peripheral lymphocytes in chronic tonsillitis. The $CD27^-CD4^+$ fraction increased in tonsillar lymphocytes compared with PBL, while there was no significant difference in the $CD27^+CD4^+$ fraction. It is possible that, after a prolonged local stimulation, memory cells appear in the tonsil and thus induce interaction between $CD27^+$ and $CD70^+$ T cells or $CD70^+$ B cells, or interaction between $CD27^+$ B cells and $CD70^+$ T cells. There are many GC that play an important role in the production of antibodies against T cell-dependent antigens. When antigen binds to immunoglobulin of naive B cells, these cells interact with T cells and interdigitating cells in lymph nodes as well as in the tonsil. Then, B cells proliferate and differentiate to antibody-producing cells that secrete IgM and IgG. Some of these differentiated cells migrate to follicles and proliferate, thereafter making a germinal center (14,15).

In this study, we showed that $CD4^+$ T cells were mainly $CD27^+$ cells, and that $CD70^+$ cells were present in GC and double-stained with $CD27^+$ cells. We also found that in GC, a few $CD19^+$ cells (B cells) could double-stain with $CD27^+$ cells. Thus, the interaction between $CD70^+$ cells and $CD27^+$ cells plays an important role in the production of antibody in GC. We suggest that local control or activation of these lymphocyte function molecules is clinically applicable for treatment of hypertrophy and infection of the human tonsil.

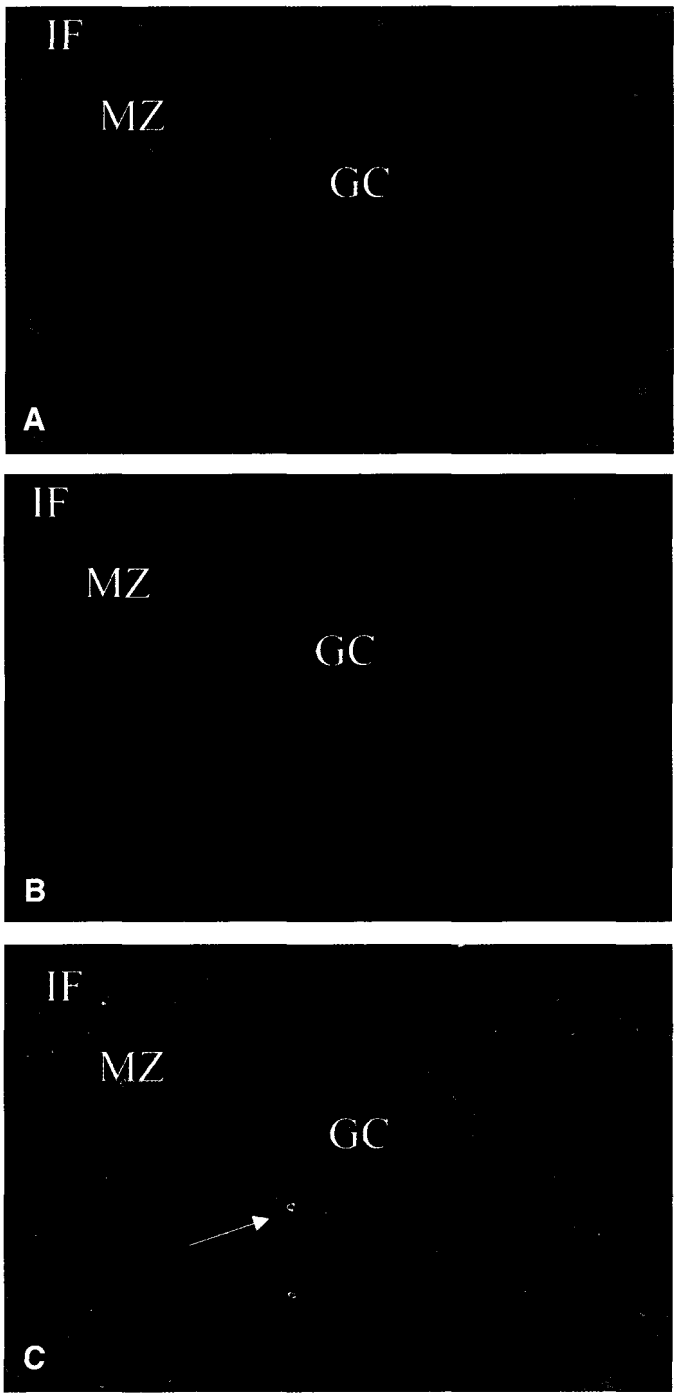

Fig. 4. Localization of CD27, CD70 and CD27 CD70 cells in tonsil. (A) The cryostat section of tonsil showing strong expression of CD27 in interfollicular T-cell areas and germinal center B-cell areas. (B) The cryostat section of the same tonsil as shown in A. Only a small number of CD70$^+$ cells were observed in germinal center. (C) Double staining of the same section as above with anti-CD27 and anti-CD70 antibodies showed that CD70$^+$ cells in germinal center were CD27$^+$ cells. (arrow) (Magnification of x 200).

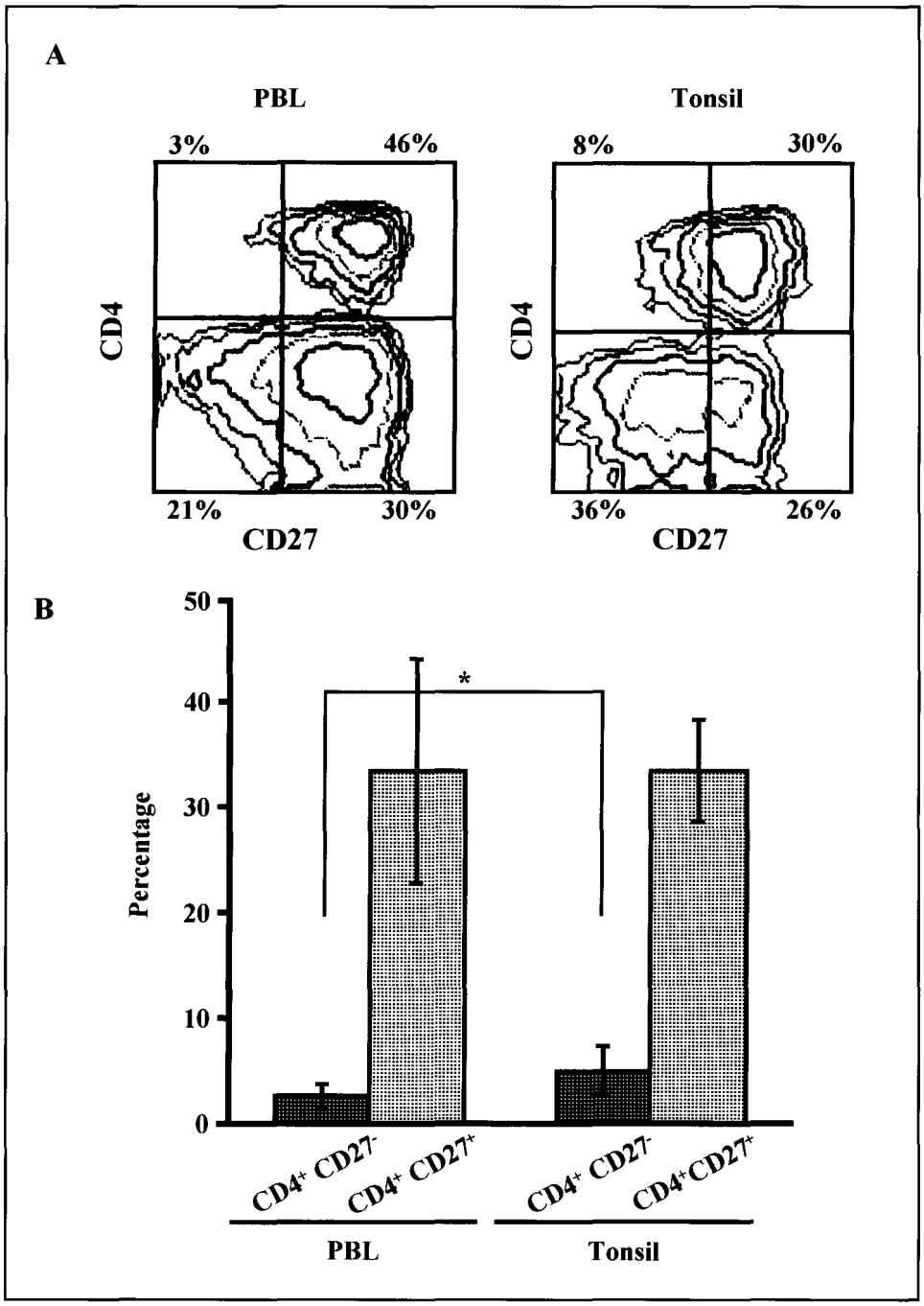

*Fig. 5. Content of CD4⁺CD27⁺ and CD4⁺CD27⁻ cells in PBL and tonsillar lymphocytes. The content of CD4+CD27+ and CD4⁺CD27⁻ cells in both PBL and tonsillar lymphocytes was evaluated by double staining cells with anti-CD4 and anti-CD27, the results were assayed by FACS. (A) Data are the representative of eight separate experiments, and the percentage of stained cells is given in each panel. (B) The average of eight experiments as above. Each bar represents the mean ± SD of eight separate experiments. Values are compared between the content of CD4⁺CD27⁻ cells in PBL and tonsillar lymphocytes. *P<0.05.*

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