Pathogens of otitis media originate from the adenoid (11), and colonization of the pathogens is facilitated in the adenoids of children with otitis (5, 6, 10). However, the underlying mechanism which facilitates the adenoidal colonization still requires clarification. Because the anti-infective local immune response may be associated with prevention of colonization, some studies on the immunological character of the adenoids have been conducted. In the adenoids of children with otitis, immunoglobulin A (IgA)-positive cells show a significant decrease (8). Adenoidal lymphocytes from children with otitis fail to recognize a specific immunogen of pathogen (9), produce lower IgG and IgA than those from children without otitis media (7) and produce significantly less T helper type 1 (Th1) cytokines than peripheral blood lymphocytes (PBL) (2, 3). However, as far as we know, only a limited number of studies, as mentioned above, concerning the immunological character of the adenoid from children with otitis are available. In the present study, to investigate the immunological potential of the adenoid from children with otitis, we compared adenoidal lymphocytes and PBL. IL-4 release was significantly increased after stimulation with PMA and ionomycin, in PBL as well as in adenoidal lymphocytes (Fig. 1B). And there was no difference in the amount of IL-2 release between adenoidal lymphocytes and PBL. IL-4 release was significantly increased after stimulation with PMA and ionomycin in PBL as well as in adenoidal lymphocytes. Although the significance in adenoidal lymphocytes was lower than that in PBL, the difference between adenoidal lymphocytes and PBL was not significant. After the lymphocytes were stimulated with S. aureus strain Cowan I, gamma IFN release was significantly increased after stimulation with PMA and ionomycin in PBL as well as in adenoidal lymphocytes. However, the difference between adenoidal lymphocytes and PBL was not significant. After the lymphocytes were stimulated with S. aureus strain Cowan I, gamma IFN release was significantly enhanced in PBL but not in adenoidal lymphocytes. As a negative control, incubation with medium alone was performed. Assays for cytokines in the culture supernatants were performed by using commercially available enzyme-linked immunosorbent assay kits. For flow cytometry analysis, lymphocytes were stained with fluorescent-conjugated monoclonal antibodies (MAbs). Then, two- or three-color dot plots were performed on a FACScan flow cytometer and CellQuest software (Becton Dickinson). Cells were analyzed in duplicate for each assay.

Firstly, we evaluated the levels of gamma interferon (IFN) and interleukin-2 (IL-2) as Th1 cytokines and also evaluated the level of IL-4 as a Th2 cytokine. As shown in Fig. 1A, gamma IFN release was significantly increased after stimulation with PMA and ionomycin in PBL as well as in adenoidal lymphocytes. Although the significance in adenoidal lymphocytes was lower than that in PBL, the difference between adenoidal lymphocytes and PBL was not significant. After the lymphocytes were stimulated with S. aureus strain Cowan I, gamma IFN release was significantly enhanced in PBL but not in adenoidal lymphocytes. However, the difference between adenoidal lymphocytes and PBL was not significant (Fig. 1A). IL-2 release was significantly increased after stimulation with PMA and ionomycin, but not with S. aureus Cowan I, in PBL as well as in adenoidal lymphocytes (Fig. 1B). And there was no difference in the amount of IL-2 release between adenoidal lymphocytes and PBL. IL-4 release was significantly increased after stimulation with PMA and ionomycin, in PBL as well as in adenoidal lymphocytes (Fig. 1C). However, the difference in the amount of IL-4 release between adenoidal lymphocytes and PBL was statistically significant.
We next investigated the activation potential of lymphocytes by analyzing CD69 expression. As shown in Fig. 2, CD69 expression of T cells was enhanced in PBL but not in adenoidal lymphocytes. When the results were expressed as stimulation indices, the difference between PBL and adenoidal lymphocytes was significant (Fig. 3A). In contrast, CD69 levels of expression of CD3$^+$ cells after stimulation of adenoidal lymphocytes and PBL were similar (Fig. 2). Because most CD3$^+$ cells consist of B cells, we also investigated CD69 expression of CD19$^+$ cells (B cells). As shown in Fig. 3B, no difference was found between PBL and adenoidal lymphocytes.

Then, CD69 expression on each subset of T cells was investigated. CD69 expression of CD4$^+$ T cells was enhanced in PBL but not in adenoidal lymphocytes (Fig. 4). And the difference between PBL and adenoidal lymphocytes was significant (Fig. 5A). Similarly, CD69 expression of CD4$^+$ T cells was enhanced in PBL, while it was not so enhanced in adenoidal lymphocytes (Fig. 4). Because most CD4$^-$ T cells consist of CD8$^+$ T cells, we investigated CD69 expression of CD8$^+$ T cells. As shown in Fig. 5B, the difference between the CD69 expression levels of CD8$^+$ T cells of PBL and adenoidal lymphocytes was also significant.

In the present study, we investigated cytokine release from PBL and adenoidal lymphocytes of children with otitis. As far as we know, with the exception of this study, only two studies on the difference between cytokine release of adenoidal lymphocytes of children with otitis and that of PBL of children with otitis exist, both by Bernstein et al. (2, 3). Their studies showed that adenoidal lymphocytes had a lower potential to produce Th1 cytokines than PBL, whereas adenoidal lymphocytes produced Th2 cytokines in amounts equal to those of PBL (2, 3). Conversely, in this study, adenoidal lymphocytes could generate Th1 cytokines, but not Th2 cytokines, equally as well as PBL. This inconsistency may be due to a difference in the ratios of atopic patients between their studies and ours, because adenoidal lymphocytes of atopic children show an increased proportion of IL-4-positive CD4$^+$ T cells (1). While there were no atopic children in our study, 7 of 22 children (2) and 4 of 12 children (3) were atopic in the studies of Bernstein et al. These atopic patients might have caused a bias in the results of their studies. More additional study is necessary to resolve this hypothesis. However, because IL-4 is associated with the production of immunoglobulin (13), our result of low IL-4 production in adenoidal lymphocytes is consistent with studies showing low immunoglobulin production in adenoidal lymphocytes of children with otitis (7–9).

We next investigated the difference in the activation potential between adenoidal lymphocytes and PBL by analyzing
CD69 expression. CD69 reaches a maximum level within several hours to a few days after stimulation (4, 12), and expression of CD69 is believed to be integral to the activation process in lymphocytes (12). Our results suggest that T cells (both CD4⁺ T cells and CD8⁺ T cells) of the adenoid have less activation potential than those of PBL. Because IL-4 is produced by T cells (13), the result of attenuated IL-4 release from adenoidal lymphocytes might be associated with the re-

FIG. 2. CD69 expression of CD3⁺ cells and CD3⁻ cells in PBL and in adenoidal lymphocytes after stimulation. The figure shows representative data of flow cytometry analysis. After incubation of lymphocytes for 18 h with S. aureus strain Cowan I (SAC) or with PMA and ionomycin (Io), the cells were stained with anti-CD3 MAb and anti-CD69 MAb. Then, −10,000 events were collected with CellQuest software by using a fluorescence or forward scatter threshold. Acquired data were displayed as two-color dot plots as shown and were analyzed by using CellQuest. The values represent percentages of CD69-positive CD3⁺ cells (upper right) and CD69-positive CD3⁻ cells (upper left). FL1-H, expression of CD3; FL2-H, expression of CD69.

FIG. 3. CD69 expression of T cells and B cells in PBL and in adenoidal lymphocytes after stimulation. After incubation of lymphocytes for 18 h with S. aureus strain Cowan I (SAC) or with PMA and ionomycin (Io), the cells were stained with anti-CD3 MAb in addition to anti-CD69 MAb for analysis of T cells (A) or anti-CD19 MAb for B cells (B). Then, −10,000 events were collected with CellQuest software by using a fluorescence or forward scatter threshold. Acquired data were displayed as two-color dot plots and were analyzed using CellQuest. The percentages of CD69-positive CD3⁺ cells or CD69-positive CD19⁺ cells were calculated. The results were expressed as stimulation indices. Values represent means ± standard deviations. Results were compared among the groups by using the unpaired Student t test.

A  CD69 expression of T cells

B  CD69 expression of B cells
result of attenuated activation in adenoidal T cells. Our study suggests that antibody production which is induced by the Th2 immune response (IL-4) may be attenuated in the adenoids of children with otitis.

In summary, we showed that adenoidal lymphocytes produced less IL-4 and expressed the CD69 activation antigen in smaller quantities than PBL. Our results suggest that there may be a difference between the immunological potential of

FIG. 4. CD69 expression of CD3⁺CD4⁺ cells and CD3⁺CD4⁺ cells in PBL and in adenoidal lymphocytes after stimulation. The figure shows representative data of flow cytometry analysis. After incubation of lymphocytes for 18 h with *S. aureus* strain Cowan I (SAC) or with PMA and ionomycin (Io), the cells were stained with anti-CD3 MAb, anti-CD4 MAb, and anti-CD69 MAb. Then, ~10,000 events were collected with CellQuest software by using a fluorescence or forward scatter threshold. The data were gated by CD3-positive cells and were displayed as two-color dot plots as shown. The percentages of CD69-positive CD3⁺CD4⁺ cells or CD69-positive CD3⁺CD4⁺ cells were calculated using CellQuest. The values represent percentages of CD69-positive CD3⁺CD4⁺ cells (upper right) and CD69-positive CD3⁺CD4⁺ cells (upper left). FL2-H, expression of CD69; FL3-H, expression of CD4.

FIG. 5. CD69 expression of CD4⁺T cells and CD8⁺T cells in PBL and in adenoidal lymphocytes after stimulation. After incubation of lymphocytes for 18 h with *S. aureus* strain Cowan I (SAC) or with PMA and ionomycin (Io), the cells were stained with anti-CD69 MAb in addition to anti-CD3 MAb and anti-CD4 MAb for CD4⁺T cells (A), or anti-CD3 MAb and anti-CD8 MAb for CD8⁺T cells (B). Then, ~10,000 events were collected with CellQuest software by using a fluorescence or forward scatter threshold. The data were gated by CD3-positive cells and were displayed as two-color dot plots. The percentages of CD69-positive CD3⁺CD4⁺ cells or CD69-positive CD3⁺CD8⁺ cells were calculated by using CellQuest. The results were expressed as stimulation indices. Values represent means ± standard deviations. Results were compared among the groups by using the unpaired Student *t* test.
adenoidal lymphocytes and that of PBL. Our results help us to understand the anti-infective immune response in adenoids of children with otitis.

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