

# Lymphocyte subsets in human tonsils: The effect of age and infection

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The aim of the present study was to determine the effect of repeated tonsillitis on the development of lymphocyte subsets in the tonsils and among peripheral blood lymphocytes (PBL) of children. Subsets of T- and B cells were analyzed in the tonsils and in PBL of patients undergoing tonsillectomy for idiopathic tonsillar hypertrophy, recurrent tonsillitis, or tonsillar hypertrophy and tonsillitis. The majority of the CD4<sup>+</sup> cells in the tonsils displayed the CD45RO<sup>+</sup> phenotype, while the majority of those in the PBL displayed the CD45RA<sup>+</sup> phenotype. Likewise, the proportion of CD45RO<sup>+</sup>CD8<sup>+</sup> cells was higher in the tonsils than among PBL. The proportion of CD4 cells expressing the CD45RO marker increased with age among PBL, but not in the tonsils. B cells, detected by their CD19, CD20, and CD21 markers, were three times more abundant in the tonsils than in the PBL. The proportion of CD38<sup>+</sup> cells showed a negative correlation with age, both in the tonsils and among PBL. Among PBL a striking age-related reduction was seen in the proportion of CD19<sup>+</sup>, CD21<sup>+</sup> and CD38<sup>+</sup>CD21<sup>+</sup> B cells. In contrast, in the tonsils age-related changes could be detected only in the proportion of CD21<sup>+</sup>CD38<sup>+</sup> cells. No difference among patients with various clinical diagnoses was detectable in any of the T- and B cell subsets in the tonsils and PBL. Thus, lymphocyte subsets evolve independently in the tonsils and peripheral blood, with the repeated antigenic challenge of tonsillar lymphocytes not influencing circulating memory cells.

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Naive T cells present among peripheral blood lymphocytes (PBL) differ from memory T cells in a number of cell surface markers (1–3). Naive T cells are characterized by the CD45RA<sup>+</sup>CD45RO<sup>−</sup> phenotype, while memory cells are CD45RA<sup>−</sup>CD45RO<sup>+</sup>. The proportion of CD45RO<sup>+</sup> CD4<sup>+</sup> memory T cells in the peripheral blood increases with age (4, 5). The processes that lead to the development of memory T cells during ontogeny are, however, far from clear. It is not clear to what extent repeated antigenic stimuli, such as frequent, recurring infections determine the appearance of the “memory” phenotype (6). In a previous study we noted that in children with acute gastroenteritis the proportion of memory CD4<sup>+</sup> cells in PBL was higher than in children with mild upper respiratory infections and in healthy controls (7). Recently, healthy children from a rural area, who had experienced recurrent gastrointestinal infections, were found to have an increased proportion of memory CD4 cells in comparison with chil-

dren from an urban area, which resembled the level of memory CD4 cells present in adults (Unpublished data).

The lymphocyte populations in palatine tonsils differ markedly from those found among PBL. In contrast to the peripheral blood, the number of B cells in the tonsils is higher than that of T cells (8–10). The tonsils contain various subsets of B cells (8–11) some of which correspond to those present in either the germinal center or the mantle zone (8–10). The majority of the T cells in the tonsils are CD4<sup>+</sup> (12–14) which, unlike their counterpart in the peripheral blood, bind peanut agglutinin lectin (13). A unique CD4<sup>+</sup>CD57<sup>+</sup> subset of germinal center T cells was described (15, 16). The responsiveness of tonsillar T cells to mitogens (14, 17, 18) and their distinct isoenzyme pattern of lactate dehydrogenase (14) indicate that they are in a state of activation, although they may be defective in IL-2 production (17).

In the present study the effect of repeated tonsillar infections on lymphocyte subsets in the tonsil and in PBL was determined by studying patients undergoing elective tonsillectomy for various indications. The patients were either individuals with repeated bouts of tonsillar infection such as recurrent tonsillitis (RT) or tonsillar hypertrophy and tonsillitis (HT), or individuals without overt tonsillar infection such as idiopathic tonsillar hypertrophy (ITH). Various subsets of T cells and B cells were analyzed in parallel in the tonsils and in PBL for each individual patient tested.

## Materials and methods

### Patients

Palatine tonsils and peripheral blood samples were obtained from patients undergoing elective tonsillectomy at the Department of Otorhinolaryngology/Head and Neck Surgery of the Hadassah University Hospital, Jerusalem. Written informed consent was obtained either from patients or from their legal guardians. The research proposal was in accordance with the institutional ethical guidelines, and was approved by the Scientific Council of the Israel Medical Association. Three groups of patients were included in the study: Patients with ITH presented symptoms of obstructive sleep apnea (OSA), patients with RT had at least five episodes of sore throat and fever per year, while patients with HT suffered from both recurrent infections and OSA. The mean age  $\pm$  SEM of the 31 individuals included in the present study was  $9.0 \pm 1.17$  years. The mean age of the 10 patients with idiopathic tonsillar hypertrophy was  $3.0 \pm 0.37$ , of the 8 patients with hypertrophic tonsillitis was  $7.25 \pm 1.5$  years, while that of 12 patients with recurrent tonsillitis was  $14.0 \pm 1.4$  years. The significance of the age differences between different groups of patients was: ITH vs. HT,  $P = 0.07$  (not significant), ITH vs. RT,  $P = 0.005$ , HT vs. RT, not significant.

### Cell suspensions

PBL were isolated from the venous blood obtained from each of the patients by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. The PBL were washed three times in phosphate buffered saline (PBS) and resuspended at a concentration of  $2 \times 10^7$  cells/ml in PBS supplemented with 0.1% bovine serum albumin.

Lymphocyte suspensions were prepared from freshly excised tonsils by first cutting the tonsils into small pieces and then passing through metal sieves and nylon filters to remove fibrous tissue and cell clumps. Cell suspensions were washed twice in

PBS and subjected to Ficoll-Hypaque density gradient centrifugation by a procedure similar to that used for the isolation of PBL.

### Immunofluorescent assays

The following monoclonal antibodies (mAbs) were used for the staining of PBL and tonsil cells: Unconjugated and fluorescent CD4, CD8, CD19 (B4), CD20 (B1), CD21 (B2), and CD45RA (2H4) mAbs were purchased from Coulter Immunology (Hialeah, FL). CD57 mAb was obtained from Becton-Dickinson (Mountain View, CA). CD45RA, CD45RO, and CD45RB mAbs were obtained from Serotec (Oxford, England). Cells of the OKT10 hybridoma clone, received from the American Type Culture Collection (Rockville, MD), grown in culture were used as a source for anti-CD38 mAb.

For immunofluorescent staining,  $0.5 \times 10^6$  cells in 0.05 ml of PBS were incubated with unconjugated mAb for 45 min, washed and incubated with 0.05 ml of either FITC-conjugated or PE-conjugated  $F(ab')_2$  fragments of anti-mouse IgG Ab (diluted 1:50) for additional 45 min, washed again and fixed in 1% paraformaldehyde in PBS. For two-color flow cytometry, 10  $\mu$ l of each of two fluorescent mAbs (PE and FITC labeled) mAbs were added to 50  $\mu$ l cell suspension containing  $10^7$  cells/ml. The mixture of lymphocyte suspension and mAbs was kept for 30 minutes at 4°C and was then centrifuged at  $200 \times g$  for 5 min. The pellet was washed twice with cold PBS containing 0.1% NaN<sub>3</sub> and 0.1% bovine serum albumin. Finally, the cells in the washed pellet were resuspended in 1% paraformaldehyde and kept at 4°C until they were analyzed by flow cytometry.

Acquisition and analysis were carried out in a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA) using the FACScan Research Software. For each test, ten thousand cells were acquired. The lymphocyte population was gated according to their typical forward light scatter and right-angle light scatter. The immunofluorescent staining was determined by two-color flow cytometry. The dual parameter program was used for analysis. The fluorescence of cells treated with fluorescent-anti-mouse  $F(ab)_2$  Ab alone and with fluorescent isotype controls was evaluated in each test to determine the level of background fluorescence of negative cells.

### Statistical analysis

The significance of differences between various groups was determined by the nonparametric Mann-Whitney test and by the Student's *t* test. The association between parameters was evaluated by regression analysis.

Table 1. Comparison of lymphocyte subsets in tonsils and PBL

| Antigenic marker                     | Tonsil          | PBL             | p        |
|--------------------------------------|-----------------|-----------------|----------|
| <b>T-cell markers</b>                |                 |                 |          |
| CD4                                  | 21.6 ± 1.2 (31) | 38.0 ± 1.6 (29) | < 0.0001 |
| CD8                                  | 5.0 ± 0.4 (26)  | 21.9 ± 1.3 (24) | < 0.0001 |
| CD4/CD8 ratio                        | 4.8 ± 0.3 (26)  | 2.0 ± 0.2 (24)  | < 0.0001 |
| CD4 <sup>+</sup> CD45RO <sup>+</sup> | 72.6 ± 1.9 (31) | 29.3 ± 2.1 (28) | < 0.0001 |
| CD4 <sup>+</sup>                     |                 |                 |          |
| CD4 <sup>+</sup> CD45RA <sup>+</sup> | 11.2 ± 1.0 (23) | 47.0 ± 3.0 (20) | < 0.0001 |
| CD4 <sup>+</sup>                     |                 |                 |          |
| CD4 <sup>+</sup> CD45RB <sup>+</sup> | 68.6 ± 3.3 (6)  | 94.8 ± 0.8 (6)  | < 0.0001 |
| CD4 <sup>+</sup>                     |                 |                 |          |
| CD8 <sup>+</sup> CD45RO <sup>+</sup> | 34.0 ± 2.3 (26) | 21.4 ± 1.3 (24) | < 0.0001 |
| CD8 <sup>+</sup>                     |                 |                 |          |
| CD8 <sup>+</sup> CD45RA <sup>+</sup> | 60.0 ± 3.2 (26) | 74.2 ± 1.9 (24) | < 0.0004 |
| CD8 <sup>+</sup>                     |                 |                 |          |
| CD4 <sup>+</sup> CD57 <sup>+</sup>   | 83.3 ± 2.3 (18) | 6.6 ± 1.1 (18)  | < 0.0001 |
| CD57 <sup>+</sup>                    |                 |                 |          |
| <b>B-cell markers</b>                |                 |                 |          |
| CD19                                 | 65.2 ± 1.8 (26) | 17.0 ± 1.5 (25) | < 0.0001 |
| CD20                                 | 71.6 ± 2.5 (24) | 14.2 ± 1.3 (24) | < 0.0001 |
| CD21                                 | 63.4 ± 1.8 (24) | 13.5 ± 1.4 (23) | < 0.0001 |
| CD21 <sup>+</sup> CD38 <sup>+</sup>  | 29.2 ± 2.7 (22) | 4.4 ± 0.7 (21)  | < 0.0001 |
| CD20 <sup>bright</sup>               | 50.5 ± 2.8 (24) | 60.9 ± 2.5 (24) | < 0.0072 |
| CD20                                 |                 |                 |          |
| <b>NK-cell markers</b>               |                 |                 |          |
| CD16                                 | 0.3 ± 0.04 (5)  | 15.3 ± 5.3 (5)  | < 0.025  |
| CD57                                 | 2.8 ± 0.6 (18)  | 8.5 ± 1.3 (18)  | < 0.0004 |
| <b>Activation marker</b>             |                 |                 |          |
| CD38                                 | 41.5 ± 3.0 (24) | 33.9 ± 1.9 (24) | < 0.0338 |

Suspensions of either PBL or tonsillar lymphocytes of the patients in the study were tested for their staining with various monoclonal antibodies. Shown are the means of the percentage of positive cells ± SEM. In brackets is the number of tests performed.

P = significance of difference between PBL and tonsil.

## Results

### Comparison of lymphocyte subsets in the tonsils and PBL

A comparison between the lymphocyte subsets in the tonsils and the PBL is shown in Table 1 in which results were pooled, regardless of the clinical diagnosis. The majority of the CD4<sup>+</sup> cells in the tonsils were CD45RO<sup>+</sup>CD45RA<sup>-</sup>, while the majority of CD4<sup>+</sup> cells among PBL were CD45RO<sup>-</sup>CD45RA<sup>+</sup> ( $P < 0.0001$ ). A similar difference between tonsils and PBL was seen for the CD45RA<sup>+</sup> ( $P = 0.0004$ ) and CD45RO<sup>+</sup> ( $P < 0.0001$ ) subsets of the CD8<sup>+</sup> cell population. The proportion of CD57<sup>+</sup> cells was higher among PBL than among tonsillar lymphocytes ( $P = 0.0004$ ), where the majority of the CD57<sup>+</sup> cells co-expressed CD4.

The proportion of B lymphocytes was over 3 times higher in the tonsils than in PBL. Among CD20<sup>+</sup> cells, the proportion of CD20<sup>bright</sup> cells was higher for PBL than for tonsillar lymphocytes ( $P = 0.007$ ). While the overall proportion of CD38<sup>+</sup>

Table 2. T-cell subpopulations in the tonsil and among PBL in various clinical conditions

| T-cell marker                        | Idiopathic hypertrophy |            | Recurrent tonsillitis |            | Hypertrophic tonsillitis |            |
|--------------------------------------|------------------------|------------|-----------------------|------------|--------------------------|------------|
|                                      | Tonsil                 | PBL        | Tonsil                | PBL        | Tonsil                   | PBL        |
| CD4 <sup>+</sup>                     | 19.1 ± 2.2             | 37.6 ± 4.3 | 23.4 ± 2.6            | 40.6 ± 2.6 | 22.0 ± 2.3               | 37.7 ± 2.2 |
| CD45RO <sup>+</sup> CD4 <sup>+</sup> | 76.1 ± 3.8             | 23.1 ± 3.9 | 70.0 ± 2.9            | 36.4 ± 4.8 | 73.8 ± 4.2               | 26.1 ± 2.3 |
| CD4                                  |                        |            |                       |            |                          |            |
| CD45RA <sup>+</sup> CD4 <sup>+</sup> | 10.4 ± 1.8             | 51.5 ± 3.9 | 13.9 ± 2.1            | 50.7 ± 9.1 | 12.3 ± 1.5               | 49.0 ± 3.4 |
| CD4                                  |                        |            |                       |            |                          |            |
| CD8 <sup>+</sup>                     | 4.4 ± 0.7              | 18.4 ± 1.7 | 5.8 ± 0.8             | 23.5 ± 2.6 | 4.7 ± 0.6                | 23.7 ± 2.3 |
| CD45RO <sup>+</sup> CD8 <sup>+</sup> | 38.4 ± 4.9             | 22.7 ± 2.4 | 33.0 ± 2.3            | 23.2 ± 2.0 | 30.9 ± 4.9               | 18.2 ± 1.9 |
| CD8                                  |                        |            |                       |            |                          |            |
| CD45RA <sup>+</sup> CD8 <sup>+</sup> | 56.1 ± 7.0             | 72.4 ± 3.6 | 60.9 ± 5.1            | 76.7 ± 3.8 | 62.9 ± 4.8               | 73.5 ± 2.4 |
| CD8                                  |                        |            |                       |            |                          |            |
| CD4/CD8                              | 4.8 ± 0.8              | 2.1 ± 0.3  | 4.6 ± 0.5             | 2.0 ± 0.4  | 5.0 ± 0.4                | 1.7 ± 0.2  |
| CD57 <sup>+</sup> CD4 <sup>+</sup>   | 85.7 ± 2.8             | 5.3 ± 1.0  | 81.3 ± 2.5            | 5.3 ± 0.9  | 81.5 ± 6.8               | 9.8 ± 3.4  |
| CD57 <sup>+</sup>                    |                        |            |                       |            |                          |            |

PBL and tonsils were obtained from 10 patients with idiopathic tonsillar hypertrophy, 12 patients with recurrent tonsillitis and 8 patients with hypertrophic tonsillitis. Shown are the means of the percentage of cells positive for each marker ± SEM.

cells was only slightly higher among tonsillar cells than among PBL ( $P < 0.034$ ), the proportion of CD21<sup>+</sup>CD38<sup>+</sup> cells was strikingly higher in the tonsils than among PBL ( $P < 0.0001$ ).

### The effect of tonsillitis on the level of lymphocyte subsets

**T cells:** In the tonsils, no significant differences in any of the T-cell subsets could be detected among patients with different clinical diagnoses. The level of CD45RO<sup>+</sup>CD4<sup>+</sup> cells was as high in the tonsils of patients with ITH as in the tonsils of patients with RT. Among PBL, the proportion of CD45RO<sup>+</sup>CD4<sup>+</sup> cells was higher in patients with RT than in those with ITH ( $P < 0.05$ ), which could be attributed to the older age of the RT patients.

**B-cells:** The proportion of CD20<sup>bright</sup> B cells was significantly lower in the peripheral blood of patients with RT and with hypertrophic tonsillitis than among those with ITH ( $P < 0.05$ ). No other difference in B cell populations could be detected.

### Correlation of age with the level of lymphocyte subsets

Age-related changes in the level of various subsets of PBL are amply documented (4, 5, 19, 20). In contrast, little is known of the age-related changes in lymphocyte subsets in the tonsils. In the present study, no significant differences in lymphocyte subsets were detected in the tonsils among patients with different clinical diagnoses. It was of interest, therefore, to determine whether age-related changes in lymphocyte subsets could be detected in the tonsils. Accordingly, for each individual in the study, the

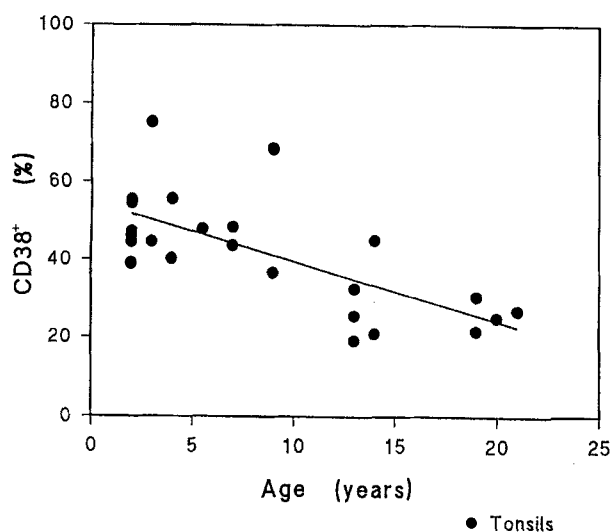


Fig. 1. Correlation between age and proportion of CD38<sup>+</sup> cells in the tonsils. Abscissa: Age in years, Ordinate: Percentage of CD38<sup>+</sup> cells in tonsils,  $r = -0.6927$ ,  $P = 0.0002$ .

age of the individual was correlated with the proportion of lymphocytes in the tonsils and among PBL which expressed various markers.

**CD38:** A highly significant negative correlation with age was seen in the proportion of CD38<sup>+</sup> cells in the tonsils (Fig. 1,  $r = -0.6927$ ,  $P = 0.0002$ ) and among PBL ( $r = -0.6067$ ,  $P = 0.0017$ ).

**CD45RO<sup>+</sup>CD4<sup>+</sup>/CD4<sup>+</sup>:** Among the subset of CD4<sup>+</sup> T cells in the peripheral blood, the proportion of cells which co-expressed the CD45RO marker increased in correlation with age (Fig. 2) ( $r = 0.6176$ ,  $P < 0.001$ ). In contrast, in the tonsils the proportion of CD45RO<sup>+</sup>CD4<sup>+</sup> cells among the CD4 population showed a slight, non-significant decrease with age (Fig. 2).

**CD45RO<sup>+</sup>CD8<sup>+</sup>/CD8<sup>+</sup>:** No correlation with age was seen in the proportion of CD8<sup>+</sup> cells which expressed CD45RO in the tonsils and among PBL.

**B cell markers:** A striking age-related reduction was seen among PBL in the proportion of CD19<sup>+</sup> cells ( $r = -0.4588$ ,  $P = 0.021$ ), and of CD21<sup>+</sup> cells ( $r = -0.4853$ ,  $P = 0.019$ ). Likewise, the proportion of the CD38<sup>+</sup>CD21<sup>+</sup> B cell subset declined with age ( $r = 0.5474$ ,  $P < 0.01$ ). While no age-related change was seen in the proportion of CD20<sup>+</sup> cells, the proportion of CD20<sup>bright</sup> cells among PBL decreased with age ( $r = -0.4789$ ,  $P = 0.018$ ).

Among tonsillar B cells no significant age-related changes could be detected in the expression of CD21 and of other B-cell markers. The proportion of CD21<sup>+</sup>CD38<sup>+</sup> tonsillar B cells declined with age ( $r = -0.5563$ ,  $P < 0.008$ ; Fig. 3). No age-related reduction of the proportion of CD20<sup>bright</sup> was noted among tonsillar B cells.

## Discussion

Previous studies presented conflicting data related to the question of whether tonsillar T lymphocytes are poorly differentiated or activated cells. The high proportion of tonsillar T cells that bind peanut agglutinin and express HLA-DR antigens suggested that tonsillar T cells are relatively immature (13). The enzyme makeup of tonsillar T cells and their responsiveness to stimulation with lectins and recombinant interleukin-2 indicated, however, that they constitute activated T cells (14, 17, 18). The elevated expression of CD38 (OKT10), CD71 (OKT9) and CD25 (Tac) on tonsillar T cells, as compared with peripheral blood T cells, supports the contention that these cells are in an activated state (14, 18). The relative weakness of the mitotic responsiveness

Table 3. B-lymphocyte subpopulations in the tonsil and among PBL in various clinical conditions

| B-cell marker                              | Idiopathic hypertrophy |             | Recurrent tonsillitis |            | Hypertrophic tonsillitis |            |
|--------------------------------------------|------------------------|-------------|-----------------------|------------|--------------------------|------------|
|                                            | Tonsil                 | PBL         | Tonsil                | PBL        | Tonsil                   | PBL        |
| CD19 <sup>+</sup>                          | 68.8 ± 2.1             | 21.0 ± 2.9  | 63.5 ± 3.0            | 15.5 ± 2.6 | 63.6 ± 3.9               | 14.7 ± 1.3 |
| CD20 <sup>+</sup>                          | 77.7 ± 3.4             | 17.7 ± 2.5  | 66.9 ± 4.2            | 13.3 ± 2.3 | 70.3 ± 4.8               | 11.5 ± 1.1 |
| CD21 <sup>+</sup>                          | 65.7 ± 2.3             | 18.1 ± 3.7  | 60.3 ± 3.6            | 10.7 ± 1.8 | 64.1 ± 3.6               | 12.3 ± 1.2 |
| CD20 <sup>bright</sup>                     | 40.6 ± 3.9             | 12.1 ± 1.9  | 33.7 ± 4.7            | 6.8 ± 1.2  | 36.5 ± 5.9               | 7.4 ± 0.8  |
| CD20 <sup>bright</sup>                     | 52.4 ± 4.55            | 66.4 ± 3.11 | 49.3 ± 4.5            | 51.7 ± 4.3 | 49.8 ± 5.7               | 64.6 ± 3.8 |
| CD20 <sup>+</sup>                          |                        |             |                       |            |                          |            |
| CD45RO <sup>+</sup> CD19 <sup>+</sup>      | 11.1 ± 1.1             | 19.2 ± 2.1  | 11.9 ± 1.0            | 15.9 ± 1.9 | 10.8 ± 0.9               | 17.7 ± 1.3 |
| CD19 <sup>+</sup>                          |                        |             |                       |            |                          |            |
| CD45RA <sup>+</sup> CD19 <sup>+</sup>      | 95.3 ± 0.7             | 94.7 ± 1.2  | 92.1 ± 3.0            | 93.9 ± 1.3 | 95.4 ± 0.7               | 95.6 ± 0.8 |
| CD19 <sup>+</sup>                          |                        |             |                       |            |                          |            |
| CD19 <sup>+</sup> CD45RA <sup>bright</sup> | 9.9 ± 1.9              | 46.7 ± 3.1  | 17.3 ± 3.6            | 49.6 ± 8.9 | 8.5 ± 2.0                | 43.0 ± 4.1 |
| CD19 <sup>+</sup>                          |                        |             |                       |            |                          |            |

PBL and tonsils were obtained from 10 patients with idiopathic tonsillar hypertrophy, 12 patients with recurrent tonsillitis and 8 patients with hypertrophic tonsillitis. Shown are the means of the percentage of cells positive for each marker ± SEM.

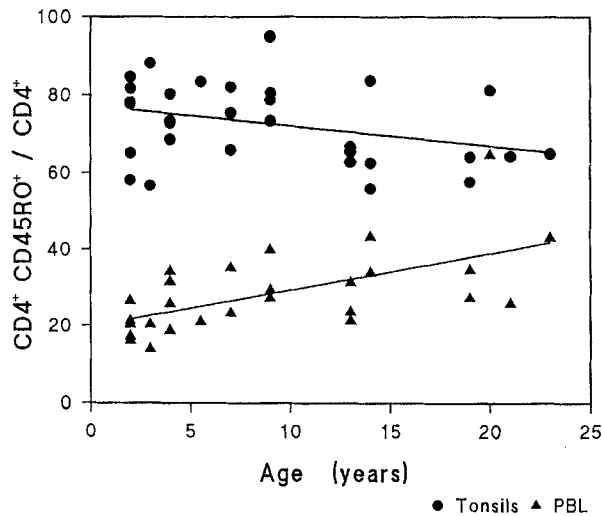


Fig. 2. Correlation between age and the proportion of CD45RO+ cells among CD4+ lymphocytes in the tonsils (●) and in PBL (▲),  $r = 0.6176$ ,  $P < 0.001$ .

of tonsillar cells could reflect the activity of suppressor T cells (18). Another indication for the relative maturity of tonsillar T cells is that tonsillar T cells do not display CD1 and do not co-express CD4 and CD8 (21).

In concordance with the young age of the patients tested in the present study, the majority of the CD4+ cells in the peripheral blood possessed the CD45RO-CD45RA+ "naive" phenotype. In contrast, the majority of the CD4+ T cells in the tonsils of these individuals displayed the CD45RO+CD45RA- "memory" phenotype. The expression of the CD45RO antigen by the majority of the CD4+ and CD8+ in the tonsils may indicate either that tonsillar T cells are strongly activated or alternatively that they are in an immature state of differentiation, similar to that of thymus cells (22). However, the fact that the proportion of CD45RB+CD4+ cells among the CD4 population is markedly reduced in the tonsils as compared with PBL argues for chronic antigenic stimulation of tonsillar cells, since among primed T cells the expression of CD45RB declines upon progressive differentiation (23). Striking age-related changes in lymphocyte populations were noted among PBL in previous studies (4, 5, 19, 20). In line with previous observations (4, 5), in the present study the proportion of CD45RO cells among peripheral CD4+ cells increased significantly with age. In contrast, no significant age-associated change in the proportion of CD45RO cells could be detected among CD4+ tonsillar cells, and if anything their proportion tended to decrease. Possibly, the proportion of CD45RO+ cells in the tonsils was so high at an early age that no further increase in their concentration could occur.

B cells in the germinal centers of tonsils are characterized by the IgD-, CD20<sup>high</sup>, CD38+ phenotype (8–10) and with maturation into memory cells, CD38+ B cells mature into CD38-negative cells (24). The proportion of germinal B cells in the tonsils decreases with age, while that of mantle zone B cells (IgD+, CD20<sup>low</sup>, CD38- cells) increases (8). In the present study, a highly significant age-related decline was noted in the level of CD38+ cells not only in tonsils but also in PBL. In particular, a significant age related decline was noted in the percentage of CD38+CD21+ B cells in both the tonsils and PBL.

In a number of studies tonsillar infections were associated with changes in lymphocyte subsets of the tonsils. Brodsky et al. (25) concluded that infections increased the number T cells and B cells in the tonsils, while Bernstein et al. (26) suggested that the percentage of T cells decreased in all compartments of the tonsils with increasing episodes of tonsillitis. In contrast, other studies failed to detect differences between lymphocytes from tonsils with either hypertrophy or recurrent overt tonsillitis (12, 14). No consistent difference between individuals with various diagnostic conditions could be documented.

The three conditions of tonsillar hypertrophy analyzed in the present study are well-defined clinically. The differences between idiopathic tonsillar hypertrophy and recurrent tonsillitis are particularly striking (25). Thus recurrent tonsillitis is defined by recurrent bouts of acute tonsillitis, occurring over four times a year, for more than a year. These bouts are associated with conspicuous pain and fever. In contrast, in idiopathic tonsillar hypertrophy no clinical signs of inflammation are obvious. Rather, the tonsils are enlarged to such a size that they cause nocturnal airway obstruction or dysphagia.

It should also be pointed out that the tonsils available for analysis were removed from patients with either RT or HT only after acute infection had subsided, so that possible alterations could have been missed. Moreover, it cannot be ruled out, a priori, that all clinical enlargements of the tonsils are elicited by either acute or chronic, insidious infections, and from this standpoint the distinction between the different clinical conditions of tonsillar hypertrophy may be artificial. In addition, irrespective of infection, tonsils are continually bombarded with antigens, which could lead to a constant pattern of lymphocyte activation.

In the present study the level of various lymphocyte subsets was remarkably similar in the three diagnostic groups of patients explored. No significant differences in lymphocyte subsets could be found in the tonsils of patients with different clinical diagnoses. Repeated episodes of tonsillitis also did not seem to affect the level of lymphocyte subsets in

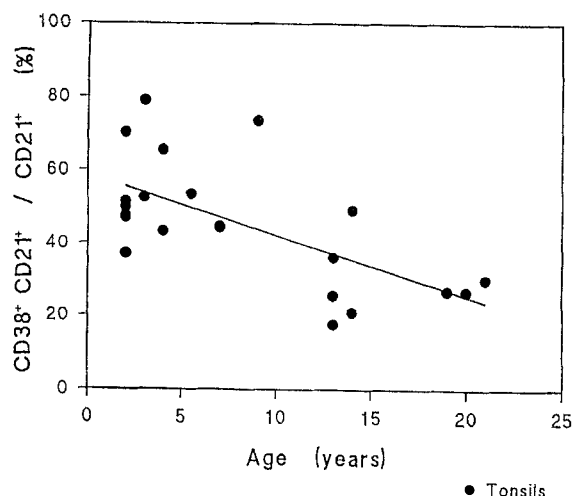


Fig. 3. Correlation between age and the proportion of CD21<sup>+</sup> CD38<sup>+</sup> lymphocytes in the tonsils  $r = -0.5563$ ,  $p < 0.008$ .

the peripheral blood. Among RT patients the level of CD45RO CD4<sup>+</sup> memory cells among PBL was higher than among ITH and HT patients while that of CD20<sup>bright</sup> B cells was lower in RT patients than in the other two groups of patients. These differences could, however, be attributed to age differences, since RT patients were the oldest group of patients in the present study. HT and ITH are both conditions that afflict relatively young children, but they differ in that repeated bouts of tonsillitis occur in HT. The fact that no differences were detected in lymphocyte subsets between HT and ITH underscores the failure of tonsillitis to affect the level of lymphocyte subsets in the tonsils and among PBL.

Agren et al. (27) showed that the proportion of cells producing IL-2 and  $\gamma$ -interferon was higher among cells in the tonsils of patients with recurrent tonsillitis than among patients with tonsillar hypertrophy, while the reverse was true for IL-4. This would favor the possibility that the T cells are in a TH1 state in RT as compared with a TH2 state in the tonsils of TH patients. The differences between the two groups of patients were, however, small and the pattern of secretion of most cytokines was similar in the two clinical conditions. On the basis of our findings it is tempting to suggest that the activated state of T cells in idiopathic tonsillar hypertrophy, which is similar to that found in the tonsils of patients with recurrent tonsillitis, may result from chronic antigenic stimulation by subclinical chronic infection. Similarly, Agren et al. (27) suggested that the production of numerous cytokines by tonsillar cells in TH could be explained by the fact that human tonsils persistently harbor microbial antigens intracellularly.

The proportion of the various lymphocyte subsets differed markedly between PBL and the tonsils and showed disparate changes in their level with age. Thus, the present study indicates that the development of lymphocyte subsets in the compartments of the tonsils and in the peripheral blood proceeds independently. Moreover, repeated antigenic challenges in the tonsils failed to affect the development of "memory" cells in the peripheral blood.

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#### References

1. SANDERS ME, MAKGOVA MW, SHAW S. Human naive and memory cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol Today* 1988; 9:195-9.
2. SANDERS ME, MAKGOBA MW, SHARROW SO, et al. Human memory T lymphocytes express increased levels of three adhesion molecules (LFA-3, CD2 and LFA-1) and three other molecules (UCHL1, CDw29, Pgp-1) and have enhanced IFN- $\gamma$  production. *J Immunol* 1988; 140: 1401-7.
3. SPRINGER TA. Adhesion receptors of the immune response. *Nature* 1990; 346: 425-34.
4. HAYWARD LA, LEE J, BEVERLEY PCL. Ontogeny of expression of UCHL1 antigen on TCR1<sup>+</sup> (CD4/8) and TcR $\delta$ <sup>+</sup> T cells. *Eur J Immunol* 1989; 19: 771-3.
5. PIRRUCELLO SJ, COLIINS M, WILSON JE, MCMANUS BM. Age-related changes in naive and memory CD4<sup>+</sup> T cells in healthy human children. *Clin Immunol Immunopathol* 1989; 52: 341-5.
6. BEVERLEY PCL. Is T-cell memory maintained by cross-reactive stimulation? *Immunol Today* 1990; 11: 203-5.
7. SCHLESINGER M, GRANOT E, RABINOWITZ R, DECKELBAUM RJ. Peripheral blood lymphocyte subsets in infants with diarrhea with or without *Giardia lamblia* infection. *Pediatric Research* 1993; 33: 15-8.
8. VALLES-AYOUB Y, GOVAN III HL, BRAUN J. Evolving abundance and clonal pattern of human germinal center B cells during childhood. *Blood* 1990; 76: 17-23.
9. GADOL N, PEACOCK M, AULT KA. Antigenic phenotype and functional characterization of human tonsil B cells. *Blood* 1988; 71: 1048-55.
10. LEDBETTER JA, CLARK EA. Surface phenotype and function of tonsillar germinal center and mantle zone B cell subsets. *Human Immunol* 1986; 15: 30-43.
11. DONO M, ZUPO S, MASANTE R, TABORELLI G, CHIORAZZI N, FERRARINI M. Identification of two distinct CD5<sup>+</sup> B-cell subsets from human tonsils with different responses to CD40 monoclonal antibody. *Eur J Immunol* 1993; 23: 873-81.
12. SUGIYAMA M, SAKASHITA T, CHO JS, NAKAI Y. Immunological and biochemical properties of tonsillar lymphocytes. *Acta Otolaryngol (Stockh)* 1984; 416 Suppl: 45-55.

13. ENNAS MG, MURRU MR, BISTRUSSO A, CADEDDU G, TORE G, MANCONI PE. Lymphocyte subsets in human adenoids and tonsils. Rosette formation, alpha-naphthyl acetate esterase cytochemistry, monoclonal antibodies and peanut lectin reactivity. *Immunol Lett* 1994; 8: 1-9.
14. PLUM J, VAN CAUWENBERGE P, DE SMEDT M. Human tonsillar T lymphocytes: an immature or activated T lymphocyte population. *Clin Immunol Immunopathol* 1986; 39: 14-23.
15. VELARDI A, MINGARI MC, MORETTA L, GROSSI CE. Functional analysis of cloned germinal center CD4<sup>+</sup> cells with natural killer cell-related features. Divergence from typical T helper cells. *J Immunol* 1986; 134: 2808-13.
16. BOWEN MB, BUTCH AW, PARVIN CA, LEVINE J, NAHM MH. Germinal center T cells are distinct helper-inducer T cells. *Human Immunol* 1991; 31: 67-75.
17. BERNSTEIN JM, RICH GA, ODZIEMIEC C, BALLOW M. Are thymus-derived lymphocytes (T cells) defective in the nasopharyngeal and palatine tonsils of children? *Otolaryngol Head Neck Surg* 1993; 109: 693-700.
18. TAKENAKA H, MIYOSHI S, MIZUKOSHI O, SAITO H. The effect of tonsillar T cell subpopulations in antigen-specific lymphocyte proliferative response. *Acta Otolaryngol* 1984; 416: 38-44.
19. ERKELLER-YUKSEL FM, DENEYS V, YUKSEL B, et al. Age-related changes in human blood lymphocyte subpopulations. *J Pediatr* 1992; 120: 216-22.
20. SCHLESINGER M, PETERS V, JIANG JD, ROBOZ JP, BEKESI JG. Increased expression of activation markers on CD8 lymphocytes in children with HIV-1 infection. *Pediatric Res* 1995; 38: 390-6.
21. SCHUURMAN HJ, BREKELMANS P, DAEMEN T, BROEKHUIZEN R, KATER L. T-cell maturation in the human thymus and tonsil: peanut agglutinin binding T lymphocytes in thymus and tonsils differ in maturation stage. *Clin Immunol Immunopathol* 1983; 29: 271-81.
22. JANOSSY G, TIDMAN N, PAPAGEORGIOU ES, KUNG PC, GOLDSTEIN G. Distribution of T lymphocyte subsets in the human bone marrow and thymus: An analysis with monoclonal antibodies. *J Immunol* 1981; 126: 1608-13.
23. SALMON M, PILLING D, BORTHWICK NJ, et al. The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis. *Eur J Immunol* 1994; 24: 892-9.
24. SILVY A, LAGRESLE C, BELLA C, DEFRANCE T. The differentiation of human memory B cells into specific antibody-secreting cells is CD40 dependent. *Eur J Immunol* 1996; 26: 517-24.
25. BRODSKY L, MOORE L, STANIEVICH JF, OGRA PL. The immunology of tonsils in children: The effect of bacterial load on the presence of B- and T-cell subsets. *Laryngoscope* 1988; 98: 93-8.
26. BERNSTEIN JM, SCHEEREN R, SCHOENFELD E, ALBINI B. The distribution of immunocompetent cells in the compartments of the palatine tonsils in bacterial and viral infections of the upper respiratory tract. *Acta Otolaryngol (Stockh)* 1988; 454 Suppl: 153-62.
27. AGREN K, ANDERSSON U, LITTON M, FUNA K, NORDLANDER B, ANDERSSON J. The production of immunoregulatory cytokines is localized to the extrafollicular area of human tonsils. *Acta Otolaryngol (Stockh)* 1996; 116: 477-85.