Proliferating Active Cells, Lymphocyte Subsets, and Dendritic Cells in Recurrent Tonsillitis

Their Effect on Hypertrophy

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**Objective:** To investigate the causes of hypertrophy in recurrent tonsillitis with hypertrophy (RTTH).

**Design:** An immunohistochemical study of recurrent tonsillitis with or without hypertrophy and adenoid tissue.

**Setting:** Academic medical center.

**Patients:** The study population comprised 15 patients with RTTH, 15 patients with recurrent tonsillitis (RT), 9 patients with adenoid vegetation, and 6 controls.

**Main Outcome Measures:** The following outcome measures were investigated: follicle number and follicular area and circumference; degree of papillary arrangement and keratin cyst in crypts; fibrosis; and density of S-100\(^+\) cells, CD20\(^+\) cells, CD45RO\(^+\) cells, lymphocytes and plasma cells, and cyclin D1\(^+\) cells in surface epithelium, crypt epithelium, extrafollicular area, and follicles.

**Results:** In the RTTH group, follicle number and follicular area and circumference, S-100\(^+\) cell density in crypt and surface epithelium, and CD20\(^+\) cell density in crypt epithelium were higher than in the RT group. The other measures were lower in the RTTH group than in the RT group. In patients with RTTH, the increase in follicle number and S-100\(^+\) cell density in surface epithelium and decrease in cyclin D1\(^+\) cell density in surface epithelium were significant. The number of CD45RO\(^+\) cells was unchanged, while S-100\(^+\) cell density increased in surface epithelium; however, in the RTTH group, CD20\(^+\) cell density, together with cyclin D1\(^+\) cell density, decreased in surface epithelium when compared with the RT group. Also, there was a 50% decrease in cyclin D1\(^+\) cell density in follicles, but CD20\(^+\) cell density decreased minimally in follicles. In the RTTH group, the increase or decrease in the number of cyclin D1\(^+\) expressing cells was parallel with the increase or decrease in the number of CD20\(^+\) cells in the areas without follicles.

**Conclusions:** Tonsil hypertrophy occurred with follicular hyperplasia and hypertrophy. There is a deficiency of proliferating active cells in response to mitogenic stimulation in RTTH. New follicles might have formed with B cells supplied from other sites because of the deficiency of proliferating active cells.

In the present study, we investigated the causes of tonsil hypertrophy in recurrent acute tonsillitis (RT) and composition of immune cells in RT, recurrent acute tonsillitis with tonsil hypertrophy (RTTH), adenoid vegetation (AV), and controls.

STUDY POPULATION

The present immunohistochemical study was performed in tonsils surgically obtained from 15 patients with RTTH (5 girls and 10 boys; mean [SD] age, 13.9 [4.01] years; range, 6-18 years), 15 patients with RT (7 girls and 8 boys; mean [SD] age, 12.5 [3.69] years; range, 7-18 years), and 9 patients with AV without tonsillectomy (4 girls and 5 boys; mean [SD] age, 9.0 [3.96] years; range, 5-14 years) at the Ear-Nose-Throat Clinic, Harran University Hospital, Sanliurfa, Turkey. The controls comprised 6 patients who underwent diagnostic tonsillectomy (3 girls and 3 boys, mean [SD] age, 18.33 [1.32] years; range, 17-20 years) with asymmetric tonsil enlargement and suspected neoplasia without RT (a case of tonsillitis for 2 years), malignancy, and known allergy. All procedures were performed in accordance with the ethical guidelines of the Helsinki Declaration, and it had been approved by the ethics committee of Harran University Medical Faculty. All patients presented with a history of tonsillitis, more than 4 episodes per year (mean [range], 6 [3-7]) for more than 1 year. Patients with a known history of allergies, who had been receiving steroids for 3 months or antibiotic or antihistaminic drugs for at least 1 month before surgery, were not included in the study. All of the patients had no evidence of a clinical infection at the time of surgery.

CLINICAL CLASSIFICATION OF TONSIL AND ADENOID SIZE

A standardized grading classification is based on the ratio of the tonsils to the oropharynx (in medial to lateral plane) as measured between the anterior pillars (0, tonsils completely in fossa; size +1, tonsils occupy <25% of oropharynx; size +2, tonsils occupy between 25% and <50% of oropharynx; size +3, tonsils occupy between 50% and <75% of oropharynx; and size +4, tonsils occupy >75% of oropharynx).7 According to the distance between vomer and adenoid tissue, the relative size of adenoid size is classified into 3 categories: size 0, adenoid tissue is <50% of area; size 1, adenoid tissue occupies 50%-75% of oropharynx; and size 2, adenoid tissue occupies >75% of oropharynx.8

GROUPS

The patients were classified into the following 3 groups according to clinical diagnosis and a group of controls:

- Group 1: Patients with RTTH. These children presented with very large tonsils (size +3 and +4) (n=15).
- Group 2: Patients with RT. These children presented with size 0, +1, and +2 tonsils (n=15).
- Group 3: Patients with AV who had obviate mouth breathing, snoring, hyponasal speech, and size +3 adenoids (n=9).
- Group 4: Controls with size +3 tonsils (n=6).

HISTOPATHOLOGICAL SPECIMENS

The tonsils were routinely sectioned longitudinally through the median, toward the tonsil hilus. The sections were cut from different parts of the tonsil, covering the peripheral part and central part. The tonsils and adenoid tissues were fixed in 10% buffered formalin, routinely processed in automatic tissue processor, and embedded in paraffin blocks. Four-micrometer-thick sections were prepared and then stained with hematoxylin-eosin.

IMMUNOHISTOCHEMICAL ANALYSIS

CD45RO and CD20 markers for T and B cells were selected because the majority of the CD4+ cells in the tonsils displayed the CD45RO+ cells and CD20+ cells were abundant in the tonsils and age-related changes could not be detected.9 The sections were stained according to the following techniques of each stain’s procedure data sheet:

- Examination of cyclin D1+ cells: Sections were stained using cyclin D1 (SP4; Neomarkers, Fremont, California). Human breast cancer tissues were used as positive controls.
- Examination of S-100+ cells: Sections were stained using an S-100 kit (polyclonal rabbit antico; Dako, Glostrup, Denmark).
- Examination of B lymphocytes: Sections were stained using antihuman B-cell monoclonal antibody CD20 (monoclonal, mouse antihuman; Dako).
- Examination of memory T lymphocytes: Sections were stained using CD45RO (clone UHCL-1; SCYTEK Laboratories, Logan, Utah).

HISTOLOGICAL EVALUATION

Each section was examined with a Nikon Optiphoto-2 microscope (Nikon Optical Co, Tokyo, Japan) with original magnifications of ×400 and ×100. The tissues were evaluated according to cytologic contents of surface epithelium, crypt epithelium, peripheral-central extrafollicular area, and follicles, with the following outcome measures: density of lymphocyte and plasma cells, CD45RO+ cells, CD20+ cells, S-100+ cells, cyclin D1+expressing cells; degree of papillary arrangement in crypt epithelium; intensity of fibrosis; presence of keratinous material in crypt epithelium; and the relative amount of follicular lymphoid tissues. All slides were coded before evaluation, and positive cells were counted blindly by an uninformed pathologist. Mean cell counts for each compartment were found by counting the cells at 10 consecutive high-power fields (original magnification, ×400; field area of 0.152 mm²) on light microscopy. Follicle diameter and number were measured by means of calibrated eyepiece reticle at 10 consecutive fields (original magnification ×100; field area of 0.608 mm²). Follicular area was calculated by first measuring longitudinal (a) and horizontal (b) diameters, and then follicular area and circumference were calculated using the following formulas: πab and π(a+b), respectively.

SCORING BY ROUTINE HISTOPATHOLOGICAL ASSESSMENT

We performed a semiquantitative analysis using the following scale: absence, mild intensity (<25% positive cells), moderate (between >25% and <50% positive cells), and severe (>50% positive cells).

STATISTICAL ANALYSIS

In Table 1 and Table 2, we present (1) the mean (SD) age, tonsil weight, and follicle number and follicular area and circumference and (2) the percentage of patients who had moderate or severe fibrosis and severe infiltration of S-100+ cells, CD20+...
In the RTTH group, degree of crypt epithelial papillary structure, follicle number and follicular area and circumference, tonsil weight, S-100\(^+\) cell density in crypt and surface epithelium, and CD20\(^+\) cell count in crypt epithelium were higher than in the RT group. Also, CD20\(^+\) cell density in surface epithelium, follicular and central extrafollicular areas, CD45RO\(^+\) cell density in both central and peripheral extrafollicular areas, cyclin D1\(^+\) cell density in surface epithelium and follicles, and lymphocyte and plasma cells in crypt epithelium were lower than in the RT group. Intensity of parenchymal fibrosis and presence of keratinous material in crypt epithelium were the same in both groups.

There were statistically significant differences in tonsil weight (\(P = .001\)), mean follicle number, S-100\(^+\) cell count in surface epithelium, and cyclin D1\(^+\) cell expression in crypt epithelium and extrafollicular area of all groups and controls.

### RTTH COMPARED WITH RT

In the RTTH group, degree of crypt epithelial papillary structure, follicle number and follicular area and circumference, tonsil weight, S-100\(^+\) cell density in crypt and surface epithelium, and CD20\(^+\) cell count in crypt epithelium were higher than in the RT group. Also, CD20\(^+\) cell density in surface epithelium, follicular and central extrafollicular areas, CD45RO\(^+\) cell density in both central and peripheral extrafollicular areas, cyclin D1\(^+\) cell density in surface epithelium and follicles, and lymphocyte and plasma cells in crypt epithelium were lower than in the RT group. Intensity of parenchymal fibrosis and presence of keratinous material in crypt epithelium were the same in both groups.

### AV COMPARED WITH RTTH AND RT

In the AV group, degree of crypt epithelial papillary structure, follicle number and follicular area and circumference, CD20\(^+\) cell density in extrafollicular area and surface epithelium, S-100\(^+\) cell density were lower than in the RTTH and RT groups. The results are presented in Table 1.

#### Table 1. Cellular and Structural Differences Between the RTTH and RT Groups, the RTTH and AV Groups, and the RT and AV Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>RTTH Group</th>
<th>RT Group</th>
<th>RTTH Compared With RT</th>
<th>AV Group</th>
<th>RTTH Compared With AV</th>
<th>AV Compared With RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD), y</td>
<td>13.9 (4.01)</td>
<td>12.5 (3.69)</td>
<td>.87 (2.20-4.98)</td>
<td>9.0 (3.96)</td>
<td>.048 (2.42-7.95)</td>
<td>.23 (1.28-8.28)</td>
</tr>
<tr>
<td>Body weight (mean ± SD), kg</td>
<td>45.56 (14.1)</td>
<td>36.83 (10.3)</td>
<td>.23 (2.85-20.29)</td>
<td>31.00 (16.7)</td>
<td>.23 (5.30-34.40)</td>
<td>.93 (13.52-25.20)</td>
</tr>
<tr>
<td>Follicle (mean ± SD), No/ct</td>
<td>4.78 (1.26)</td>
<td>3.25 (1.25)</td>
<td>.01 (0.26-2.80)</td>
<td>4.07 (0.26)</td>
<td>.30 (0.34-1.78)</td>
<td>.24 (0.37-2.00)</td>
</tr>
<tr>
<td>Follicular area (mean ± SD), mm(^2)</td>
<td>2.2 (0.8)</td>
<td>1.7 (1.0)</td>
<td>.92 (0.6-1.2)</td>
<td>3.7 (1.0)</td>
<td>.055 (0.5-2.8)</td>
<td>.001 (0.7-3.2)</td>
</tr>
<tr>
<td>Follicular circumference (mean ± SD), mm</td>
<td>0.16 (0.3)</td>
<td>0.14 (0.4)</td>
<td>.77 (0.02-0.05)</td>
<td>0.22 (0.3)</td>
<td>.003 (0.02-0.10)</td>
<td>.001 (0.03-0.12)</td>
</tr>
<tr>
<td>Tonsil weight (mean ± SD), g</td>
<td>4.09 (0.94)</td>
<td>1.69 (0.51)</td>
<td>.001 (1.69-3.12)</td>
<td>1.23 (0.29)</td>
<td>.001 (2.16-3.52)</td>
<td>.04 (0.89-1.30)</td>
</tr>
<tr>
<td>Fibrosis, %</td>
<td>33.3</td>
<td>33.3</td>
<td>&gt;.99</td>
<td>0</td>
<td>.07</td>
<td>.07</td>
</tr>
<tr>
<td>Surface epithelium, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S-100(^+) cells</td>
<td>55.6</td>
<td>16.7</td>
<td>.04</td>
<td>66.7</td>
<td>.69</td>
<td>.03</td>
</tr>
<tr>
<td>CD20(^+) cells</td>
<td>22.2</td>
<td>16.2</td>
<td>.16</td>
<td>100</td>
<td>.001</td>
<td>.01</td>
</tr>
<tr>
<td>CD45RO(^+) cells</td>
<td>22.2</td>
<td>66.7</td>
<td>.02</td>
<td>66.7</td>
<td>.04</td>
<td>&gt;.99</td>
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<tr>
<td>Crypt epithelium, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lymphocytes and plasma cell</td>
<td>77.8</td>
<td>100</td>
<td>.10</td>
<td>66.7</td>
<td>.65</td>
<td>.03</td>
</tr>
<tr>
<td>Papillary structure</td>
<td>55.6</td>
<td>33.3</td>
<td>.32</td>
<td>66.7</td>
<td>.69</td>
<td>1.3</td>
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<td>Keratin cyst</td>
<td>66.7</td>
<td>66.7</td>
<td>&gt;.99</td>
<td>33.3</td>
<td>.13</td>
<td>.13</td>
</tr>
<tr>
<td>S-100(^+) cells</td>
<td>44.4</td>
<td>33.3</td>
<td>.73</td>
<td>33.3</td>
<td>.69</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>CD20(^+) cells</td>
<td>44.4</td>
<td>16.7</td>
<td>.15</td>
<td>NS</td>
<td>.03</td>
<td>.53</td>
</tr>
<tr>
<td>Follicles, %</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>CD20(^+) cells</td>
<td>88.9</td>
<td>100</td>
<td>.49</td>
<td>100</td>
<td>.54</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>CD45RO(^+) cells</td>
<td>33.3</td>
<td>66.7</td>
<td>.09</td>
<td>100</td>
<td>.001</td>
<td>.07</td>
</tr>
<tr>
<td>Extrafollicular area, central, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20(^+) cells</td>
<td>44.4</td>
<td>66.7</td>
<td>.32</td>
<td>100</td>
<td>.009</td>
<td>.07</td>
</tr>
<tr>
<td>CD45RO(^+) cells</td>
<td>88.9</td>
<td>100</td>
<td>.49</td>
<td>100</td>
<td>.54</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Extrafollicular area, peripheral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45RO(^+) cells</td>
<td>75.0</td>
<td>83.3</td>
<td>.68</td>
<td>33.3</td>
<td>.09</td>
<td>.03</td>
</tr>
</tbody>
</table>

Abbreviations: AV, adenoid vegetation; CI, confidence interval; NS, not severe infiltration; RT, recurrent tonsillitis; RTTH, recurrent tonsillitis with hypertrophy.

A The percentage of only severe (>50% positive cells) infiltration of S-100\(^+\), CD20\(^+\), and CD45RO\(^+\) cells and the percentage of cyclin D1\(^+\)–expressing cells are presented. Boldface values are statistically significant.

The patients with RTTH and RT and the controls had a mean (SD) tonsil weight of 4.09 (0.94) g, 1.69 (0.51) g, and 3.45 (0.24) g, respectively. The patients with AV had a mean (SD) adenoid tissue weight of 1.23 (0.29) g. The mean (SD) age in the patients with RTTH, RT, AV and controls were 13.9 (4.01) years, 12.5 (3.69) years, 9.0 (3.96) years, and 18.33 (1.32) years, respectively.

The results are presented in Table 1.

#### Results

The patients with RTTH and RT and the controls had a mean (SD) tonsil weight of 4.09 (0.94) g, 1.69 (0.51) g, and 3.45 (0.24) g, respectively. The patients with AV had a mean (SD) adenoid tissue weight of 1.23 (0.29) g. The mean (SD) age in the patients with RTTH, RT, AV and controls were 13.9 (4.01) years, 12.5 (3.69) years, 9.0 (3.96) years, and 18.33 (1.32) years, respectively.
cell density in surface epithelium, and cyclin D1–expressing cell density in follicles were the highest in both the RTTH and RT groups. Also in the AV group, density of CD20\(^+\) cells in follicles, CD45RO\(^+\) cells in central extrafollicular area, and cyclin D1–expressing cells in surface epithelium were higher than in the RTTH group. Follicle number in the AV group was higher than in the RT group. Density of S-100\(^+\) cells in crypt epithelium, cyclin D1–expressing cells in surface epithelium, and CD20\(^+\) cells in follicles were the same in both the AV and RT groups. There was no parenchymal fibrosis in the patients with AV.

Between the AV and RTTH groups, there were statistically significant differences in age (P = .001), tonsil weight (P = .001), follicular area (P = .005), and circumference (P = .003); density of CD20\(^+\) cells in extrafollicular area (P = .009) and surface area (P = .001), and crypt epithelium (P = .03); and cyclin D1–expressing cell density in surface epithelium (P = .04) and follicles (P = .001). Between the AV and RT groups, there were statistically significant differences in tonsil weight (P = .04); follicular area (P = .001) and circumference (P = .001); and density of CD20\(^+\) cells in surface epithelium (P = .01), S-100\(^+\) cells in surface epithelium (P = .03), and CD45RO\(^+\) cells in peripheral extrafollicular area (P = .03) (Table 1).

### Table 2. Cellular and Structural Differences Between the Control and RTTH Groups, the Control and RT Groups, and the Control and AV Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>P Value (95% CI), Controls Compared With RTTH</th>
<th>P Value (95% CI), Controls Compared With RT</th>
<th>P Value (95% CI), Controls Compared With AV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>18.33 (1.32)</td>
<td>.001 (x^2 = 20.555)</td>
<td>.02 (0.54-8.34)</td>
<td>.001 (3.02-8.63)</td>
</tr>
<tr>
<td>Body weight, mean (SD), kg</td>
<td>64.66 (2.17)</td>
<td>.001 (x^2 = 28.150)</td>
<td>.001 (0.09-29.13)</td>
<td>.001 (20.42-35.24)</td>
</tr>
<tr>
<td>Follicle, mean (SD), No.</td>
<td>2.8 (0.79)</td>
<td>.001 (x^2 = 20.962)</td>
<td>.001 (0.73-2.23)</td>
<td>.001 (0.85-1.70)</td>
</tr>
<tr>
<td>Follicular area, mean (SD), mm(^2)</td>
<td>1.01 (0.2)</td>
<td>.001 (x^2 = 20.570)</td>
<td>.002 (0.35-1.70)</td>
<td>.01 (0.06-1.40)</td>
</tr>
<tr>
<td>Follicular circumference, mean (SD), mm</td>
<td>0.11 (0.1)</td>
<td>.001 (x^2 = 20.242)</td>
<td>.001 (0.02-0.07)</td>
<td>.00 (0.00-0.06)</td>
</tr>
<tr>
<td>Tonsil weight, mean (SD), g</td>
<td>3.45 (0.24)</td>
<td>.001 (x^2 = 36.981)</td>
<td>.06 (1.12-1.30)</td>
<td>.001 (1.40-2.11)</td>
</tr>
</tbody>
</table>

The FINDINGS IN CONTROLS

Follicle number and follicular area and circumference, lymphocyte and plasma cell infiltration in crypt epithelium, and density of CD20\(^+\) cells in follicles of controls were the lowest. There were statistically significant differences between the control and the RTTH groups and between the control and AV groups for follicle number and follicular area and circumference, between the control and RTTH groups and between the control and RT groups for lymphocyte and plasma cell infiltration in crypt epithelium, and between the control and RT groups for density of CD20\(^+\) cells in follicles. In controls, there were no cyclin D1–expressing cells in surface epithelium, no S-100\(^+\) cells in crypt epithelium, and no CD20\(^+\) cells in extracellular area and surface and crypt epithelium. Parenchymal fibrosis and density of S-100\(^+\) cells in surface epithelium were the highest in controls. There was a statistically significant difference in the density of S-100\(^+\) cells in surface epithelium between the control and RTTH groups and between the control and RT groups (P < .05) (Table 2).

Cyclin D1–expressing cells in surface epithelium in a patient with RT are shown in the Figure A; cyclin D1–expressing cells in adenoid follicles in a patient with AV are shown in the Figure B; and S-100\(^+\) cells in crypt epithelium in a patient with RTTH are shown in the Figure C.
The palatine tonsils and adenoid tissues are primary sites of bacterial- and viral-induced B- and T-cell activation and generate the specific type of polarized immune reaction. Immune responses are divided into 2 types: (1) humoral immune response, which is dependent on B cells, plasma cells, and antibodies, and (2) cellular immune response, which is dependent on T cells and cytokines. The tonsils and adenoid tissues are highly organized into T- and B-cell areas and possess the cellular prerequisites for antigen uptake, processing, presentation, and T- and B-cell cooperation, maturation, and differentiation. Tonsils appear to be able to raise both primary and secondary T-cell responses. The adenoids play a more active role than the tonsils in specific immune response to respiratory antigens.

In this study, we investigated the causes of tonsil enlargement and differences in the numbers of memory T cells (CD45RO\(^+\) cells), memory B cells (CD20\(^+\) cells), dendritic cells (S-100\(^+\) cells), and proliferating active cells (cyclin D\(_1\)-expressing cells) in patients with RTTH, RT, and AV and controls. We showed that tonsil enlargement occurred by follicular hyperplasia in the patients with RT. In this process, increase of follicle number, increase of S-100\(^+\) cell density in surface epithelium, and decrease of cyclin D\(_1\)-expressing cell density in surface epithelium were statistically significant. In addition, there were increases in papillary arrangement, S-100\(^+\) cell density, and CD20\(^+\) cell density in crypt epithelium and decreases in CD20\(^+\) cell density in surface epithelium, extrafollicular area, and follicles, CD45RO\(^+\) cell density in central extrafollicular area, and cyclin D\(_1\)-expressing cell density in follicle in RTTH when compared with RT.

Furthermore, degree of papillary arrangement of crypt epithelium, CD20\(^+\) cell density in surface epithelium and extrafollicular area, follicular area and circumference, and follicular cyclin D\(_1\)-expressing cell density in adenoid tissue were the highest. Density of S-100\(^+\) cells in surface epithelium and peripheral extrafollicular CD45RO\(^+\) cells in adenoid tissue were higher and lower, respectively, than tonsil tissues except in controls. Surface epithelium of adenoids and crypt epithelium of tonsils were more active on immune response. Interestingly, the increase or decrease of cyclin D\(_1\)-expressing cell density was parallel with increase or decrease of CD20\(^+\) cells in cyclin D\(_1\)-expressing areas.

Rosenmann et al detected no significant differences in any of the T- and B-cell subsets in tonsil tissues among patients with the idiopathic tonsillar hypertrophy RT and RTTH. We also determined no significant difference in T- and B-cell subsets between the RT and RTTH groups; however, different from their study, we investigated lymphocyte subsets in different compartments of tonsil tissue. Particularly, the differences in the number of CD20\(^+\) cells in different lymphoid compartments between the RT and RTTH groups were striking, although this was a statistically insignificant difference.

Lymphoid dendritic cells play an essential role for antigen presentation in primary immune responses and are believed to be important in normal healthy responses of the mucosal immune system. Dendritic cells function to capture and process antigen and present it to T and B lymphocytes. S-100\(^+\) dendritic cells were found in the surface epithelium and the crypt epithelium in tonsils, and dendritic cell distribution in adults was not different from that in children. Dense infiltrates of S-100\(^+\) dendritic cells were noted in the majority of hyperplastic tonsils, while they were fewer in nonhyperplastic tonsils. Differences at distribution, quantity, and phenotype of dendritic cells in the surface and crypt epithelium in both normal and diseased tonsils were observed. During disease, dendritic cells decreased in the surface epithelium and increased in the crypt epithelium and
than in the RTTH group. The ratios of S-100 filamentous epithelium. The epithelial area exposed to antigen is ent compartments: surface epithelium and crypt epithelium. The ratio of S-100+ cells in the surface epithelium of the RT group was significantly lower than in the RTTH group. The ratios of S-100+ dendritic cells in surface to crypt epithelium were 0.5:1 in the RT group, 1.0:8 in the RTTH group, 1.0:5 in the AV group, and 1.0:0 in the control group. There was high-density antigenic stimulus in the children with RTTH and AV.

Human palatine tonsil epithelium consists of 2 different compartments: surface epithelium and crypt epithelium. The epithelium of tonsils is characterized as lymphoepithelium. The epithelial area exposed to antigen is maximized by 10 to 30 blind-ending crypts, extending deeply into the tonsil tissue. The integrity of cryptal epithelium is important for the immunological functioning of palatine tonsil as a defense organ against infections. Crypt epithelium may have an epithelial barrier different from surface epithelium. Also, the level of barrier function of the epithelium differs between the adenoid and palatine tonsils. Furthermore, an increase in lymphoid elements was reported in tonsil hypertrophy: there was an increase in the number of B cells, helper T cells, and suppressor T cells when compared with controls.

We showed differences in density of various immune active cells in surface epithelium and crypt epithelium among all groups. The degree of papillary structure of crypt epithelium was highest in the AV group and second highest in the RTTH group. S-100+ cell density in surface epithelium was high in both AV and RTTH groups, while it was the lowest in the RT group. CD20+ cell density in the surface epithelium of adenoids was the highest, while CD45RO+ cell density in the peripheral extrafollicular area was the lowest. CD20+ cell density in surface epithelium was the second highest in the RT group and the lowest in the RTTH group. CD45RO+ cell density was high in the peripheral extrafollicular area of both tonsil groups. In the surface epithelium of adenoids, the increase in S-100+ cell density was parallel to the increase in the number of CD20+ cells, but in tonsils, there was an increase in S-100+ cell density with a decrease in the number of CD20+ cells or a decrease in S-100+ cell density with an increase in the number of CD20+ cells. S-100+ and CD20+ cell densities in crypt epithelium were the highest, and an increase in these cell densities was parallel in the RTTH group. Crypt epithelium in the case of RTTH and surface epithelium in the case of AV have the most active role in specific immune response. Interestingly, there was low S-100+ cell density and high CD20+ cell density in the surface epithelium and low CD20+ cell density and relatively high S-100+ cell density in crypt epithelium in the RT group. However, S-100+ cell density in crypt and surface epithelium was lower than in the RTTH group. These findings show that the level of barrier function of the lymphoid epithelium differs among the patients with AV, RT, and RTTH.

Histological analysis of the epithelial component in 44% of cases demonstrated the presence of a hyperparakeratosis in the base of the crypts in chronic inflammation of tonsils. In our study, the presence of keratin cysts was the same in all tonsil groups and was demonstrated in 66.7% of cases.

In previous studies, high follicular area and low follicle number was determined in patients with tonsil hypertrophy when compared with those with RT. Zhang et al found that the mean (SD) follicular circumference in patients with tonsillar hypertrophy (0.23 [0.02] mm2) was significantly larger than in those with RT (0.15 [0.02] mm2). The mean (SD) number of follicles in the RT group (4.5 [2.1]) was greater than in the tonsil hypertrophy group (3.8 [0.5]). Olofsson et al found that the mean (SD) number of follicles per millimeters squared was 1.8 (0.1) (range, 1.2-2.4) in patients with RT (n=4) and 1.3 (0.4) (range, 0.8-2.0) in patients with tonsil hypertrophy (n=4). In our study, we found that the mean (SD) follicular area and circumference (per ×100 field) was larger in the RTTH group (2.2 [0.8] mm and 0.16 [0.3] mm, respectively) than in the RT group (1.7 [1.0] mm and 0.14 [0.4] mm, respectively). However, this difference was not statistically significant. The mean (SD) follicle number in the RTTH group (4.78 [1.26]) was significantly higher than in the RT group (3.25 [1.25]). Follicle number in AV (4.07 [0.26]) was lower than in the RTTH group, but follicular area and circumference (3.7 [1.0] mm2 and 0.22 [0.3] mm, respectively) was significantly higher than in all palatine tonsil groups. Our results were different from previous studies, but none of these studies had RT in tonsil hypertrophy groups. We determined that hypertrophy in RT and AV occurred because of follicular hyperplasia and hypertrophy.

Koch and Brodsky suggested that local lymphocyte dysfunction may be central to the etiology of RT with hyperplasia. Persistent local inflammatory reactions in adenotonsillitis may lead to histomorphological changes and functional deficiencies in defense barriers. In our study, we investigated the entity of proliferating active cells by the presence of cyclin D1-expressing cells. The cell cycle comprises 4 major phases, described as gap 1 (G1), DNA synthesis (S), gap 2 (G2), and mitosis (M). In mammalian cells, proliferation control is primarily achieved in the G1 phase of the cell cycle. During the G1 phase, cells respond to extracellular signals by either advancing toward another division or withdrawing from the cycle into a resting state (G0). Cyclins are the key group of regulatory proteins in the cell. In the G1 phase, cyclins are in their inactive form. They only become activated when they bind to their associated cyclin-dependent kinase (CDK) protein. It is the cyclin-CDK active complex that stimulates the cell to undergo mitosis. Cyclin D-CDK4 and cyclin E-CDK2 are activated during the G1 phase. The cyclin D-CDK4 complex assembles and accumulates through the G1 phase. The catalytic activities of the assembled holoenzymes are first manifested by a mid-G1 increase to a maximum near the G1-S transition and persist through the first and subsequent cycles as long as mitogenic stimulation continues. Conversely, mitogen withdrawal leads to the cessation of cyclin D synthesis.

Particularly, we detected the presence of cyclin D1-expressing cells in surface epithelium and follicles. There...
was no expression in crypt epithelium and extrafollicular area in all groups and controls. Cyclin D1–expressing cells were found in surface epithelium in 66.7% of patients with RT and AV and in 22.2% of patients with RTTH, and they were found in follicles in 100% of patients with AV, in 66.7% of patients with RT and controls, and in 33.3% of patients with RTTH. Presence of cyclin D1–expressing cells in patients with RTTH was the lowest in both surface epithelium and follicles. Interestingly, in patients with RTTH, the increase or decrease in the number of cyclin D1–expressing cells was parallel with the increase or decrease in the number of CD20+ cells in cyclin D1–expressing areas without follicles. We suggest that there may be 2 different answers: (1) Cyclin D1–expressing cells are ready for mitosis, and their numbers are decreased by consumption during follicular hypertrophy and the increase in follicle number. (2) There may be a deficiency of proliferating active cells in the tonsils, and an increased need of immune cells may be supplied from other sites.23 With these immune cells, follicular hypertrophy and the formation of new follicles occur in RTTH. Deficiency of CD20+ cells (or B cells) and proliferating active cells in the tonsils may be the main cause in the etiology and pathogenesis of tonsil hypertrophy.

We suggest that there is an increase of antigenic stimulus in children with AV and RTTH and controls because of the increase in S-100+ cell density in the surface epithelium. These children may possess chronic insidious infections or they may continually be bombarded with many unknown foreign antigens in the upper airway, which is different from RT. Now, these antigenic stimuli for controls are not foreign; they are more likely related to a previous condition. In conclusion, we determined that tonsil hypertrophy occurred by follicular hyperplasia and hypertrophy. In this process, we showed that the number of CD45RO+ cells had not remarkably changed in response to increased antigenic stimulus; however, the number of CD20+ cells and cyclin D1–expressing cells decreased in surface epithelium in patients with RTTH. Also, there was a 50% decrease in the number of cyclin D1–expressing cells in follicles in the RTTH group when compared with the RT group, but the decrease in the number of CD20+ cells was less remarkable than the decrease in the number of cyclin D1–expressing cells in follicles. The decrease in the number of proliferating active cells was not parallel with the decrease in the number of CD20+ cells in follicles in the RTTH group. We suggest that CD20+ cells (or B cells) may be supplied from other sites for the formation of new follicles because there is a deficiency of proliferating active cells for B-cell response to mitogenic stimulation in RTTH. In other words, new follicles might have formed with B cells supplied from other sites because of the deficiency of proliferating active cells, thus causing follicular hyperplasia.

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