CD27 and CD38 lymphocytes are detected in oral lichen planus lesions

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Objective. Oral lichen planus (OLP) is a chronic disease with bandlike lymphocyte infiltration.

Study design. To elucidate the immunologic phenotype of OLP, we analyzed the presence of CD5+/H11001, CD20+/H11001, CD27+/H11001 and/or CD38+/H11001 lymphocytes in a series of 70 atrophic OLP biopsy samples.

Results. CD27+/H11001 and CD38+/H11001 cells were present in 84% and 54% of the lesions, respectively. The lesions were graded as T-cell dominant, B-cell dominant, or a mixed lesion based on CD5+ and CD20+ cells in the inflammatory infiltration with the following results: 26%, 7%, and 67%, respectively. CD27 expression was found in 67% of the T-cell dominant, in 80% of the B-cell dominant, and in 91% of mixed lesions. The corresponding figures for CD38 were 72%, 80%, and 62%.

Conclusions. CD27+/H11001 and CD38+/H11001 lymphocytes represent abnormal mononuclear cell populations in atrophic OLP lesions indicating 2 forms of OLP might exist with different pathogenesis, despite similar histology and clinical behavior. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:211-217)

Inflammatory infiltrates in oral lichen planus (OLP) have been assessed to identify some clues to the etiopathogenesis of the lesions.1-6 Lichen planus is considered a chronic systemic disease of established immunemediated pathogenesis. The disease can affect oral and genital mucosa or skin.7-9 OLP is histologically characterized by hyperkeratosis, basal layer vacuolization with apoptotic keratinocytes, and a mononuclear cell infiltrate at the epithelium-connective tissue interface.1-6 Scattered lymphocytes also can be detected within the epithelium. The etiology and triggers of OLP are still unknown, although the presence of lymphocytic infiltration supports the view of cell-mediated immune reaction or autoimmune reaction targeting the basal keratinocytes.9,10

Previous studies have shown that CD4+ T cells and CD8+ T cells predominate in the lymphocytic infiltration of oral and cutaneous LP lesions.1,3,6 These T cells produce and respond to a range of cytokines, such as tumor necrosis factor-alpha (TNF-α), which have an important role in the initiation and progression of OLP11-13 and other inflammatory mediators. All of these might in turn affect epithelial cells and stimulate cell proliferation or cell death.13 However, activation of B cells may also be important in the pathogenesis of OLP, which has not been excessively studied by immunohistochemistry.

CD27 is a member of the tumor necrosis factor receptor superfamily and provides costimulatory signals for a secondary immune response. CD27 is expressed on discrete subpopulations on CD4+ and CD8+ effector T cells and memory B cells in vessels (both in blood and in the lymphatic) as well as in peripheral lymphoid organs but not in other peripheral tissues.14-19 Recently it was shown that CD20+ and CD27+ B cells...
predominate in the lymphocyte infiltrate of salivary glands in patients with primary Sjögren’s syndrome. The homing of CD20+, CD27+ B cells into the glandular tissues was regarded as a key event in the pathogenesis of this autoimmune disease. The homing of these cells is not fully understood. This observation leads us to an idea that CD27+ intravascular lymphocytes could also home into oral mucosa. Atrophic OLP was selected as a disease model to investigate the presence of CD27+ lymphocytes in oral mucosa. The presence of CD27+ cells would support the hypothesis that OLP or at least part of the lesions could be caused by autoimmunity as suggested earlier.

As a subject, we selected an additional molecule, CD38, which is a receptor that triggers transmembrane signaling on engagement with CD31 (platelet-endothelial cell adhesion molecule [PECAM-1]). The effects mediated include (1) production of proinflammatory and regulatory cytokines by monocytes, natural killer (NK) cells, and activated B and T cells; (2) proliferation of T cells; and (3) protection of mature B lymphocytes from apoptosis. CD38 may act as a surface molecule modulating adhesion and signaling between B and T lymphocytes. The differentiation of memory B cells into CD38-expressing cells is interleukin (IL)-10 dependent, and increases in frequency with cell division. Thus, when memory B cells are stimulated with T-cell help, they are capable of generating numerous populations of cells discernible by division history and the differential expression of CD27 and CD38. To best of our knowledge, the expression of these B-cell markers in association with T cells has not been previously studied in OLP lesions.

**MATERIAL AND METHODS**

The material of this study consisted of 70 biopsy specimens taken from 66 consecutive patients with atrophic OLP, as defined by World Health Organization (WHO) criteria, identified between 1991 and 2002, and retrieved from the files of the Department of Oral Pathology and Radiology, University of Turku. All patients born before 1928, those with any medication, or suffering from any systemic disease were excluded from this study. Of the 66 patients, 49 (74.2%) were women with the mean age of 53.9 years (range 35 to 73 years) and 17 (25.8%) were men with the mean age of 51.0 years (range 36 to 65 years). Thus, the patients represented a typical age and sex distribution of OLP patients. The mean follow-up time of the patients was 62.4 months (range from 7.2 to 117.4 months). At the first visit, all lesions were clinically typical atrophic OLP fulfilling the WHO criteria, such as presence of bilateral lesions on oral mucosa with atrophic or ulcerative outlook.

**Histologic examination**

The biopsies were fixed in formalin and processed to paraffin-embedded tissue blocks according to the routine practice as described earlier. Hematoxylin and eosin stained slides (6 µm) were cut for routine diagnostics. Re-examination of the slides confirmed the diagnosis of OLP in all cases. The WHO criteria for OLP were used when reexamining the histopathology of the sections. Candida positive biopsies were not used in this investigation.

**Immunohistochemistry**

For immunohistochemical staining of CD5, CD20, CD27, and CD38, 4-µm-thick consecutive sections from formalin-fixed, paraffin-embedded tissue blocks were cut. Briefly, the sections were de-paraffinized and boiled in a microwave 2 times for 5 minutes in 0.01 M citrate buffer, pH 6, then cooled at room temperature for 20 minutes. The monoclonal antibodies were used as follows: CD5 (T cell, clone 54/F6, Dako, A/S, Glostrup, Denmark), CD20cy (B cell, clone L26, Dako), CD27 (clone 137B4, NovoCastra, Newcastle upon Tyne, UK), and CD38 (clone SPC32, NovoCastra) at a 1:50, 1:400, 1:60, and 1:100 dilution, respectively. The staining was made by the Dako TechMate 500 plus staining system, following the manufacturer’s instructions. Sections from tonsil were used as a positive control for all the antigens used. Negative controls were processed in parallel with test samples, by replacing the primary antibody by buffer.

**Evaluation of immunostaining**

On light microscopy, each section was examined for the presence of CD5+, CD27+, CD20+, and/or CD38+ lymphocytes at ×200 magnification. For immunohistochemical staining, consecutive sections were made from the specimens. Thus, it was possible to correlate the location of CD5+, CD20+, CD27+, and CD38+ lymphocytes with each other.

The CD5 and CD20 expression was graded into (1) no expression, (2) fewer than 50% of the cells were positive, or (3) 50% or more of the lymphocytes were positive. Also, the presence of CD5+ lymphocytes in epithelia was graded as present or not. The location of the CD20 B+ lymphocytes among the inflammatory infiltrate was graded as (1) diffusely distributed, (2) bandlike zone under the epithelia, or (3) clusterlike distribution (Fig. 1, A and B).

The proportions of CD27+ or CD38+ cells were separately graded as (1) negative, (2) only a few scattered CD27+ or CD38+ lymphocytes, or (3) nearly whole inflammatory infiltration containing CD27+ or CD38+ cells. The number of individual cells with expression was not counted. The pattern of CD27+ or...
Fig. 1. Immunohistochemical staining of A, CD5 (>50% of the lymphocytes stained), B, CD20 (>50% of the lymphocytes stained), C, CD27 (nearly whole inflammatory infiltration consisted of CD27+ lymphocytes), and D, CD38 (only a few scattered lymphocytes were CD38+) in an atrophic OLP biopsy sample (original magnification ×50 and ×100).
CD38$^+$ lymphocytes among the inflammatory infiltrate was graded as (1) diffuse, (2) bandlike zone, or (3) clusters (Fig. 1, C and D).

All lesions were graded either as T-cell dominant, B-cell dominant, or mixed subtypes. As all the lesions were stained with CD5, the division was defined through the CD20 staining compared with the CD5 expression. In the T-cell-dominant lesion, CD5 expression was 50% or more of the lymphocytes and CD20 expression was negative. In the mixed group, CD5 expression was 50% or more of the lymphocytes, and CD20 was 10% to fewer than 50%, or 50% or more of the lymphocytes were positive. In the B-cell-dominant lesions, CD5 expression was found in 10% to fewer than 50%, and CD20 expression in 50% or more of the lymphocytes.

**Statistical analysis**

SPSS for Windows, version 16.0.2 (Chicago, IL) was used for statistical analysis. Frequency tables were analyzed using the $\chi^2$ test, with Pearson’s R or likelihood ratio being used to assess the significance of the correlation between the categorical variables. Differences in the means of continuous variables between the groups were analyzed using analysis of variance (ANOVA) (when appropriate) or nonparametric tests (Mann-Whitney, Kruskal-Wallis). In all analyses, the probability values less than .05 were regarded as significant.

**RESULTS**

Table I summarizes the proportion of CD5$^+$ and CD20$^+$ lymphocytes among the inflammatory infiltrate in atrophic OLP. CD5$^+$ cells were present in all samples, whereas 25.7% (18/70) of the samples were totally lacking CD20$^+$ cells. More than 50% of the lymphocytes in the infiltrate were CD5$^+$ or CD20$^+$ in 68.6% and 32.9% of OLP samples, respectively. In 85.7% (60/70) of the lesions CD5$^+$ cells had infiltrated into the epithelium. Of the OLP lesions only 25.7% (18/70) were T-cell-dominant, 7.1% (5/70) were B-cell-dominant, and 67.1% of the lesions showed an infiltration with mixed T and B cells. In the T-cell-dominant group, epithelial infiltration of CD5$^+$ cells were detected in 78% (14/18) of the specimens (Fig. 1, A and B).

The distribution of CD27$^+$ and CD38$^+$ lymphocytes in atrophic OLP biopsy samples is summarized in Table II. CD27$^+$ and CD38$^+$ cells were found in 84.3% and 54.3% of the lesions, respectively. CD27$^+$ cells were scattered among the inflammatory infiltration in 44.3% (31/70) of the biopsies, whereas a clustered pattern was found in 40% (28/70). The corresponding figures for CD38$^+$ cells were 32.9% (23/70) and 24.7% (15/70), respectively (Fig. 1, C and D).

In the T-cell-dominant group, CD27$^+$ lymphocytes were found in 67% (12/18) of the lesions. In the B-cell-dominant group, 80% (4/5) of the biopsy samples were CD27$^+$. CD27$^+$ lymphocytes were the predominant cells in the mixed T- and B-cell lesions (91%, 43/47) (Fig. 2).

In the T-cell-dominant group, 72% (13/18) of the specimens had no CD38$^+$ cells. In the B-cell-dominant group, only 1 of 5 samples had no CD38$^+$ cells. CD38$^+$ cells were present in 62% of the lesions, mixed both with B and T cells (29/47). CD38$^+$ but not CD27$^+$ lymphocytes were statistically significantly related to the inflammatory subgroups ($P = .008$; Fisher’s exact test) (Fig. 2, Table III). Both CD27$^-$ and CD38$^-$ lesions were found in one third of the T-cell-dominant group.

**DISCUSSION**

Traditionally, OLP has been regarded as a chronic inflammatory T-cell lesion, and the presence of B cells within the inflammatory cell infiltration has been associated with reactive lichenoid lesions. During the past several years, an attempt has been made to differentiate oral lichenoid reactions from OLPs. The presence of a substantial number of plasma cells in the lymphocytic infiltrate has been regarded as one of the definitions of a lichenoid reaction. It was recently agreed, however, that there is no way to differentiate lichenoid reactions from OLP lesions either clinically or histologically.

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**Table I.** Proportion of CD5$^+$ and CD20$^+$ lymphocytes in OLP lesions

<table>
<thead>
<tr>
<th>Staining pattern</th>
<th>No. of specimen</th>
<th>%</th>
<th>No. of specimen</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>25.7</td>
</tr>
<tr>
<td>10% ≤50% of the</td>
<td>22</td>
<td>31.4</td>
<td>29</td>
<td>41.4</td>
</tr>
<tr>
<td>lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50% of the</td>
<td>48</td>
<td>68.6</td>
<td>23</td>
<td>32.9</td>
</tr>
</tbody>
</table>

**Table II.** The distribution of CD27$^+$ and CD38$^+$ lymphocytes in biopsy samples of atrophic OLP lesions

<table>
<thead>
<tr>
<th>Staining pattern in the lymphocytic infiltrate</th>
<th>No. of specimen</th>
<th>%</th>
<th>No. of specimen</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>11</td>
<td>15.7</td>
<td>32</td>
<td>45.7</td>
</tr>
<tr>
<td>Scattered lymphocytes were positive</td>
<td>31</td>
<td>44.3</td>
<td>23</td>
<td>32.9</td>
</tr>
<tr>
<td>Positive lymphocytes predominated in several different areas</td>
<td>28</td>
<td>40.0</td>
<td>15</td>
<td>21.4</td>
</tr>
</tbody>
</table>
In the present study, we used strict criteria in selecting patients. Also, none of the patients were taking any medications known to cause lichenoid lesions in oral mucosa as a side effect. Our main aim was to find, first, B cells in atrophic OLP and, second, the presence of CD27\(^+\)/H11001 cells in these lesions. This should lend some support to the concept that at least part of the OLP lesions could be related to autoimmunity, as previously suggested. Accordingly, Sugerman et al.\(^{21,34}\) described evidence for autoimmunity in OLP, one of which is coexistent with a known autoimmune disease and OLP in the same patient. Another possible contribution of autoreactivity in the pathogenesis of OLP has been provided by studies on a CD4\(^+\) helper-induced T-cell subset in the peripheral blood and in lesions of OLP patients.\(^{13}\)

To support our hypothesis, we found that CD20\(^+\) expressing B cells were detected in 74.3% of the OLP lesions. CD5 expression is accepted as a sign of T cells in OLP lesions, even if the phenotype of the inflammatory infiltrate in OLP results in a highly controversial picture.\(^{1-6}\) The CD5 molecule has been implicated in the proliferate response of activated T cells and in T-cell helper function. We could clearly confirm the expression of CD5 on lymphocytes in OLP lesions. However, B-cell expression was remarkably common, detectable in close to 75% of all lesions. This suggests that B cells might play an important role in OLP lesions, and widens the repertoire of different subtypes of lymphocytes in OLP lesions.

CD20 expression is detected in different stages of B-cell maturation, including pre-B cells and immature and mature cells as well as activated B cells, but not plasma cells.\(^{35}\) Functional studies using monoclonal antibodies have shown that antibody binding to CD20 inhibits B-cell proliferation caused by mitogens and inhibits B-cell differentiation. However, although the exact function of the CD20 gene is not clear, it has been

Table III. Distribution of CD38\(^+\) lymphocytes according to the predominance of T cells, B cells, or both in the infiltrate in the biopsy samples of OLP lesions

<table>
<thead>
<tr>
<th>CD 38–</th>
<th>Scattered lymphocytes CD 38+</th>
<th>CD 38+ cells found in several different areas</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell predominance</td>
<td>13</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>72.2%</td>
<td>27.8%</td>
<td>0%</td>
</tr>
<tr>
<td>Mixed</td>
<td>18</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>38.3%</td>
<td>36.2%</td>
<td>25.5%</td>
</tr>
<tr>
<td>B cell predominance</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>20.0%</td>
<td>20.0%</td>
<td>60.0%</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>45.7%</td>
<td>32.9%</td>
<td>21.4%</td>
</tr>
</tbody>
</table>

\(P = .008,\) Fisher’s exact test.

Fig. 2. The number of OLP samples according to the predominance of T cells, B cells, or both in the infiltrate of the lesions. *Fisher’s exact test \((P = .008)\).
the target gene in therapy of B-cell lymphomas and some autoimmune disorders. On the basis of immunohistochemical staining, one cannot assess the stage of B cells, whether activated or immature. BAFF (B-cell-activating factor belonging to the TNF family) is critical for B-cell survival and maturation but it does not affect proliferation. In BAFF-transgenic mice, it has been shown that CD27+ B cells migrate from serum to cutaneous tissue and exocrine glands, causing similar changes as found in certain autoimmune diseases, e.g., systemic lupus erythematosus (SLE) and Sjögren’s syndrome. So far, the expression of BAFF in relation to OLP has not been studied.

Here we report that CD27+ cells were detected in nearly 85% of the OLP lesions and were the predominating cells in 40% of the OLP lesions. The staining pattern in the present study suggests that the target molecule of CD27 cells is not in the epithelium of OLP, because the CD27+ lymphocytes are located deep in the lamina propria rather than the subepithelial zone. These results support the hypothesis that in at least part of the OLPs, autoimmune mechanisms might be involved. However, we cannot exclude the possibility that CD27+ cells might have interactions with CD8+ T cells as well.

Similarly, CD38 expression in B cells has been connected with autoimmune diseases, such as type II diabetes mellitus and SLE. We found CD38+ cells in 55% of the OLP lesions. The targets of CD27 or CD38 in oral mucosa are unknown and further studies are warranted. It is known, however, that the target of CD38 in intestinal mucosa is the mucosal addressing cell adhesion molecule-1 (MADCAM-1). Oral mucosa and tonsils lack this expression. Other ligands have not been identified yet. As discussed previously, CD27 BAFF plays a central role in B-lineage cell biology; however, the regulation of BAFF-binding receptor expression during B-cell activation and differentiation is not completely understood.

We could identify a subgroup of the OLPs where no CD27+ or CD38+ cells were found, indicating that these lesions could be the real T-cell lesions and activated B cells have no role in their pathogenesis. This was supported by the predominance of CD5+ T cells in 6 of 18 CD27- or CD38-negative samples.

To conclude, the present results suggest that at there are at least 2 pathogenetically different forms of OLP according to the presence or absence of CD27+ and CD38+ lymphocytes. OLPs with CD27+ lymphocyte predominance might be an autoimmune-related disease, but this needs further confirmatory studies.

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REFERENCES


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