Immunohistochemical Evaluation of CD86 Expression in Erosive Oral Lichen Planus

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Abstract

Background and Aim: Lichen planus is an inflammatory autoimmune mucocutaneous disease. Stimulation of T cells and antigen-presenting cells (APCs), such as dendritic cells, mediates apoptosis of keratinocytes and basement membrane destruction. The aim of this study was to investigate the expression of one of the immune cell markers (CD86) in erosive oral lichen planus.

Materials and Methods: In this paired analytical cross-sectional study, 22 patients with erosive oral lichen planus were included. Biopsies from oral lesions of these patients were obtained. The surrounding normal marginal mucosa around these lesions served as controls. To visualize CD86-expressing cells, immunohistochemistry (IHC) processes were used. Data were transferred to SPSS 11.0 software and were analyzed using marginal homogeneity test.

Results: This study showed an increased number of CD86-expressing cells in erosive lichen planus lesions, while in normal mucosa, no cells were found to express CD86. Marginal homogeneity test revealed that the difference in positivity and negativity between the groups was significant (P<0.001).

Conclusion: A high expression of CD86 in erosive lichen planus lesions shows an increased activation of APCs, while these cells are inactive in normal mucosa.

Key Words: CD86, Dendritic Cells, Lichen Planus

Introduction

Lichen planus is a relatively common chronic mucocutaneous disease with a prevalence rate of approximately 1-2% [1-5]. Different clinical types of the disease can be seen, including reticular, papular, plaque-like, bullous, erythematous, and erosive subtypes [1-5]. Due to the presence of symptoms in the erosive form and the precancerous nature of this lesion, patients must undergo periodic follow-ups [6]. The cause of this disease is unknown but one theory refers to immune dysfunction and the role of cytotoxic cells and monocytes in apoptosis of keratinocytes by killer T cells.
(CD8+) [4,7,8]. These cells are activated by cytokines secreted by T helper cells, such as tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2), and recognition of antigens, such as major histocompatibility complex class II (MHC CL II), on basal keratinocytes and their destruction. [5] T cells require an intermembrane signal to act as effective cells [8]. The CD86 molecule is one of the important mediators of T-cell activation in immune responses [9]. Also, it is one of the stimulatory molecules that is expressed on antigen-presenting cells (APCs), such as dendritic cells and macrophages, binding its receptor to T-lymphocyte-associated molecule-4 (CTLA-4) on T cells, causing T-cell proliferation, clonal expansion, and IL-12 production by keratinocytes, which lead to apoptosis [8,10-12].

Active human dendritic cells exhibit direct anti-tumor killing activity in response to some pathogens and cytokines. When dendritic cells mature in the presence of different stressors, such as mechanical trauma and some systemic drugs, they present more costimulatory molecules, such as CD86, compared to immature dendritic cells [13,14].

Some studies on various clusters of differentiation (CD) molecules and dendritic cells in inflammatory diseases, such as lichen planus and rheumatoid arthritis, have shown that activated Langerhans cells have special roles in the progression of oral lichen planus lesions [15].

In another study by Gustafson et al [16], the presence of dendritic cells (CD86+) was observed with lymphocyte aggregation in the connective tissue of oral lichen planus erosions. T helper cells activated by MHC II antigen and stimulatory molecules, such as CD80 and CD86, on APCs are involved in the pathogenesis of rheumatoid arthritis [17,18].

In the present study, we investigated the presence of CD86 in erosive lichen planus in comparison with normal tissue to define the role of the mentioned molecule in this disease.

Materials and Methods

The subjects in this paired analytical cross-sectional study were patients with erosive oral lichen planus, who were referred to the Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran. The diagnosis of erosive lichen planus was based on clinical and pathological features of the disease according to the modified World Health Organization (WHO) criteria. Biopsied specimens from erosive areas were assigned to the case group, and the surrounding normal tissue was used as the control group. This study was approved by the Ethical Committee of Tabriz University of Medical Sciences (IR.Tbzm.REC.1387.35). Because erosive lichen planus is not prevalent, we included every patient with erosive lichen planus, who were referred to the Department of Oral Medicine in the period of research. The optimal sample size was determined to be 22 samples in each group.

The inclusion criteria consisted of patients with erosive oral lichen planus, and the diagnosis of lichen planus was confirmed based on the histopathological evaluation. In addition, the patients who were enrolled in the study were in the age range of 18-60 years and completed an informed consent form.

The exclusion criteria consisted of the followings [9]:

1. Consumption of any drugs for treatment of lichen planus in the past three months.
2. Consumption of medications associated with lichenoid reactions, including penicillin, nonsteroidal anti-inflammatory drugs (NSAIDs), methyl dopa, propranolol, metformin, and sulfonamide.
3. The presence of acquired and congenital immunodeficiency disorders, such as acquired immune deficiency syndrome (AIDS) and other immunologic diseases, chemotherapy, addiction to injectable opioids, hemophilia, and hemodialysis. The reason for excluding these patients from our study was the difficulty of biopsy procedures, difficulty of infection control, the possible interaction with the clinical findings of lichen planus, and the patients' doubtful cooperation.
4. The presence of any contraindication for biopsy.
5. Patients with no interest in being involved in the study.
6. Patients with a history of steroid and immunomodulator consumption in the past three months.
7. Patients with diabetes or other medical conditions.

The patients’ records were completed, and the necessary examinations were performed. Biopsies were then taken from all the patients. The biopsies were immersed in 10% formalin and were sent to Imam Khomeini Hospital. After the diagnosis of lichen planus, the prepared tissue sections from these blocks were attached to slides, left overnight at 37°C and then stained for immunohistochemistry (IHC). Also, the surrounding normal tissue from the area near the lesion was immersed in formalin solution and was sent to Imam Khomeini Hospital, and after preparing paraffin blocks, the sections were attached to slides and were stained. The manufacturer’s guidelines for detecting CD86 were as follows:

CD86 was detected in immersion-fixed paraffin-embedded sections of human mucosa using Human CD86/B7-2 Antigen Affinity-purified Polyclonal Antibody (Novus Biologicals, City of Centennial, USA; Catalog #AF-141-NA) at 15 µg/ml concentration overnight at 4°C. The tissues were stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; R&D systems, Minneapolis, USA; Catalog #CTS008) and were counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasm of lymphocytes. The sections (erosions and normal tissues) were evaluated in three fields at ×0.1, ×10, and ×40 magnifications under a light microscope. All the specimens were evaluated by one experienced observer.

The staining intensity of cells and the number of cells with a staining intensity of different colors (light yellow, light brown, and deep brown) were determined. Stain intensity revealed CD86 density; the deeper the stain, the greater the prevalence [15]. Light yellow suggests mild, light brown suggests moderate, and deep brown suggests severe expression (Figure 1). For evaluation of the accuracy of the results, marginal homogeneity test was used. Data were analyzed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).

Results
For the evaluation of the slides stained with IHC, the stained cells were defined as positive, and the unstained cells were defined as negative. The evaluation with marginal homogeneity test revealed that the difference in positivity and negativity between the groups was significant (P<0.001). Evaluation of the slides of erosive lesions was positive, and the connective tissue and epithelial cells were stained but two of the specimens were not stained and were categorized as negative. In staining, positivity was based on the color intensity; nine specimens stained light yellow (mild), nine stained light brown (moderate), and two stained deep brown (severe). There was no expression of CD86 in normal tissues (Table 1).

Discussion
This study evaluated the rate of CD86 expression in erosive lichen planus, compared to the surrounding normal tissue, indicating that CD86 was expressed in erosive lichen planus at high levels in epithelial and connective tissue cells but was not expressed in normal tissues. The results of this study showed increased activity of APCs, such as Langerhans cells, in lichen planus; these cells have a significant role in activating and increasing T lymphocytes although they are active in normal tissues. Confirmation of these findings requires further studies.

There is no study that compares CD86 expression in lichen planus lesion and the surrounding normal tissue but there are studies focusing on other CDs and similar diseases. In this study, we studied another kind of CD in these lesions, which its presence confirms the immunologic etiology of lichen planus. The results of this study are comparable to that of a study by Gustafson et al in 2007 [16].
Gustafson et al [16] studied the expression of CD83, Langrin, and CD1a on Langerhans cells of oral lichen planus lesions and showed that the cells expressing CD1a, CD83, and Langrin increased significantly in counts in lichen planus lesions compared to normal tissues. The results of the cited study are similar to that of our study.

In a study by Hasséus et al [19] in 2001, graft versus host disease (GVHD) and oral lichen planus were compared based on the expression of CD1a, CD86, and CD80. Although the pathogenesis of these two diseases is different, there is an irregularity of the immune system in both of them and they have similar clinical presentations. The results of the mentioned study showed that cells with CD1a+, CD80+, and CD86+ in the epithelium of GVHD and lichen planus lesions had the dendritic form of Langerhans cells, and lichen planus lesions exhibited higher expression of CD1a and CD86 than GVHD [19]. The high expression of CD86 in oral lichen planus was shown in their study and ours. However, they compared oral lichen planus with GVHD, but in our study, oral lichen planus was compared with the surrounding normal tissue. In comparison, they evaluated three cell markers but we assessed only CD86. In addition, their sample size was smaller than ours. The similarity of the cited study with our study was the finding of increased expression of CD86 in oral lichen planus lesions.

In another study by Katou et al [20] in 2000, Langerhans cell specifications were studied in dermal and epithelial tissues in relation to lymphocytic secretions. They used thermal flaps for repair of defects after facial cancer and oral cavity surgery and compared them with 15 flaps

Table 1. CD86 expression using color intensity in the study groups

<table>
<thead>
<tr>
<th>Color Groups</th>
<th>Color intensity (CD86 expression)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deep brown (severe) Number (%)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>2(9.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light brown (moderate) Number (%)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>9(40.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Light yellow (mild) Number (%)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>9(40.9)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative Number (%)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>2(9.1)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Number (%)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>22(100)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22(100)</td>
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that were contaminated with Candida albicans, which were severely infected. IHC staining showed that Langerhans cells expressed CD86 and CD80 in significant amounts but no cell in normal skin expressed CD80 or CD86. These findings revealed no activated Langerhans cells in normal tissues [20]. In 2017, Marshall et al [21] stated that CD40 and CD86 expressions in H357 cells are enhanced by interferon gamma (IFN-γ) stimulation. Their results suggest that CD40 and CD86 play an important role in the pathophysiology of oral immunologic diseases such as oral lichen planus [21]. The mentioned study was consistent with our study in showing the increased expression of CD86 following the activation of the immune system by Langerhans cells in contrast to a lack of expression in the surrounding normal tissue. The results of our study are similar to those of another study performed by Farthing et al in 1990 [15]. They evaluated the number of CD1a⁺, HLA DR⁺, and HLA DP⁺/HLA DQ⁺ cells in the epithelium of oral lichen planus lesions and normal oral mucosa. In lichen planus lesions, there were more dendritic cells but there was no difference in total Langerhans cell counts in these lesions compared to normal tissues. However, the level of MHC Cl II (HLA DQ/HLA DP) increased, suggesting an increase in the activity of Langerhans cells with important roles in the progression of the lesions [15]. It may seem that the cited study is in contrast to our study as the total number of Langerhans cells did not increase in lichen planus but the activity and expression of CD86 antigen increased; however, these findings are similar. Based on our data, there is no research available with a similar methodology.

Conclusion
Based on the results of this study, CD86 is expressed in significant amounts in erosive lichen planus but not in the surrounding normal tissue. High levels of expression of CD86 molecule in erosive lichen planus lesions may indicate the high activity of Langerhans cells; nevertheless, more studies are needed to prove the exact role of these cells in the pathogenesis of lichen planus.

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