Lymphocyte Subpopulations and the Expression of Intercellular Adhesion Molecules in Chronic Periodontitis

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ABSTRACT

Immunological responses to invading bacteria play a major role in the course of inflammatory periodontal diseases, such as CP. It was suggested that one of the major elements in determining the course of the disease is the expression of cellular adhesion molecules. We therefore investigated the expression of cellular adhesion molecules, ICAM-1 and beta-1 integrins, capillary density and lymphocyte subpopulations in gingival biopsies obtained from 20 patients with CP who responded and 21 patient who failed to respond to initial treatment using immunohistochemical methods. We found no differences between the two groups in capillary density, ICAM-1 and beta-1 integrin expression. Patients who responded to treatment had a lymphocytic inflammatory infiltrate consisting predominantly of T cells, while those who failed to respond had an approximately equal number of T and B cells. Our findings support the role of host immunological mechanisms in determining the outcome of CP and argue against a major role of differential cellular adhesion molecule expression.

Key words: chronic periodontitis, B and T lymphocytes, ICAM-1 expression, immunohistochemistry

Introduction

Chronic periodontitis (CP) is a chronic inflammatory periodontal disease characterised by gingival inflammation, formation of periodontal pockets and alveolar bone resorption. It is continuously progressive with brief episodes of localized exacerbations and occasional remissions. Dental plaque bacteria trigger the inflammatory response of host defence mechanisms that ultimately lead to tissue...
destruction\textsuperscript{1}. Treatment of CP includes measures designed to eliminate or control plaque infection in combination with removal of plaque retention factors.

Components of microbial plaque have the capacity to induce the initial infiltration of inflammatory cells including lymphocytes, macrophages and polymorphonuclear granulocytes (PMNs) in CP\textsuperscript{2,3}. Homing and retention of lymphocytes is dependent upon the expression of adhesion molecules\textsuperscript{4,5}. Intercellular adhesion molecule-1 (ICAM-1) is involved in the accumulation and activation of leukocytes in inflammatory sites through binding to beta-1 integrins expressed on leukocytes\textsuperscript{6,7}. Beta-1 integrins are adhesion molecules that appear on lymphocytes after activation and bind to the extracellular matrix, specifically collagen, laminin and fibronectin. PMN accumulation and activity in the gingival crevice results in release of enzymes that have detrimental effects on bacteria as well as on host tissue\textsuperscript{8,9}. Many cells within the inflammatory cell infiltrate produce matrix degrading enzymes and cytokines that directly and indirectly further degrade the connective tissue and bone.

Some\textsuperscript{10–13}, but not all authors\textsuperscript{14–16} have found an increase in ICAM-1 expression in gingiva of patients with more severe inflammatory periodontal diseases. One group has found an increase in beta-1 integrin (also called VLA) expression in the gingival tissue of patients with periodontitis\textsuperscript{17,18}. Also, differences were found in lymphocyte subpopulations infiltrating gingival tissues in patients with severe and mild types of inflammatory periodontal diseases.

However, it is unknown whether patients who respond differently to initial therapy show differences in characteristics of gingival inflammatory cell infiltrates, specifically lymphocytes and/or differences in expression of ICAM-1 and beta-1 integrins on gingival tissue and infiltrating inflammatory cells. To answer this question we have studied the expression of ICAM-1 and beta-1 integrins, capillary density and T and B lymphocyte infiltration in gingival biopsies obtained from patients with CP who responded to or failed to respond to initial periodontal therapy.

**Subjects and methods**

The study was performed in a group of 41 patients with CP at the time of reevaluation after initial therapy, i.e. three months after the start of treatment. Twenty patients responded favorably while 21 failed to respond to initial treatment. There were 31 males and 10 females, 30–60 years of age. Inclusion criteria were at least 20 permanent teeth remaining, adequately restored and without signs of active caries as determined by radiographs and clinical inspection, probing depths more than 5 mm on at least 6 teeth in three different quadrants and radiographically visible alveolar bone loss. Excluded from the study were patients with inadequate oral hygiene despite given repeated instructions in oral hygiene, with serious systemic diseases such as diabetes and cancer, or which have taken antibiotics 6 months prior to start of the study. All patients participating gave written informed consent for this study.

Standard initial periodontal examination was performed in all patients prior to the initial therapy including determination of bleeding on probing, probing depth measured on 6 sites per tooth, furcation involvements, tooth mobility and radiography. Initial treatment consisted of instructions in oral hygiene, removal of local irritations and subgingival root debridement. Scaling and root planing was performed quadrantwise under local anesthesia using Gracey curettes and sonic scalers by one trained periodontist. The periodontal examination was repeated af-
ter three months. Patients were considered to have favorably responded if they had less than 15% sites positive for bleeding on probing and their mean pocket depth was reduced by 1.5 mm or more.

Biopsies of gingival tissue, approximately 3 mm wide, were obtained during therapeutic periodontal surgery procedures under local anesthesia, that were undertaken as a part of the corrective phase of the periodontal treatment. Biopsies were taken from interproximal sites of teeth with deepest probing depth values in the surgical area. The tissue samples were instantly frozen in liquid nitrogen and stored at −20 °C. All samples were analyzed using standard histological hemalaun-eosin (HE) staining and immunohistochemical techniques. The latter were performed using murine monoclonal antibodies directed against the T cell marker CD3, B cell marker CD20 (Boehringer, Ingelheim, Germany), endothelial cell marker von Willebrand factor, ICAM-1 (DAKO Cytomation, Glostrup, Denmark) and beta-1 integrin (Mario Negri, Milano, Italy). Primary antibodies were detected using biotynilated anti-murine secondary antibodies (DAKO) and the avidin-biotin peroxidase (Vector, Burlingame, Ca, USA).

Lymphocytic infiltrates were semiquantitatively graded and divided into three groups:
1) predominantly T cells
2) similar numbers of B and T cells
3) predominantly B cells.

Capillary density was determined as follows. Tissue sections were examined under low magnification (40× and 100×). Three hot-spots, with a large number of vessels were identified. These hot-spots were analyzed using higher magnification (200×). In each of them vessels were counted in an area of 0.785 mm². Results were expressed as the highest count (maximal vessel density) and the average of the three counts (average vessel density).

Expression of ICAM-1 and beta-1 integrins was determined in gingival epithelium. In case of beta-1 integrins, a distinction was made between basal and subbasal expression. Expression was graded qualitatively as present or absent.

Statistical analysis was performed using »Statistica for Windows 4.0« software, and the Chi-square test was used to describe differences between groups.

Results

In the group of patients who favourably responded, the mean probing depth before the therapy was 3.8±0.9 mm, and after the therapy 2.2±0.6 mm. In the group of patients who did not respond to therapy, initial mean probing depth was 3.9±1.0 mm, and 3.3±0.7 mm after the reevaluation. Inflammation was present in all gingival samples analyzed, as determined by the presence of inflammatory mononuclear cell infiltrate.

The ratio of T to B lymphocytes was higher in gingival inflammatory patients who responded to treatment than in those who did not (Fig. 1). In the former group 11 patients had predominantly T cells, 4 similar number of T and B cells and 4 predominantly B cells in their gingival biopsies (Fig. 1). In one patient the quality of the bioptic material was insufficient for the analysis. In the group of patients who failed initial treatment 4 had predominantly T cells, 11 similar numbers of T and B cells (Fig. 2) and 6 predominately B cells in their gingival biopsies. This difference is statistically significant \( p=0.033, \chi^2=6.850 \).

Results of other immunohistochemical analyses are presented in Table 1. Patients who did and did not respond to initial therapy had similar capillary density, ICAM-1 expression (Figures 3a, 3b) and basal and subbasal beta-1 integrin expression (Figures 4a, 4b).
Discussion

Treatment of inflammatory periodontal disorders is frequently unsatisfactory and ultimately results in tooth loss. Modern periodontological research therefore focuses on investigations of molecular and cellular mechanisms in the hope that this will lead to changes in diagnostic approaches or treatment by blocking or modulating these mechanisms. Some authors\textsuperscript{19,20} actually suggest that blocking ICAM-1 might be an effective treatment for otherwise refractory cases of periodontitis.

In some of the previous studies differences in ICAM-1 and beta-1 integrin expression were found to be related to the degree of gingival inflammation or type of periodontal disease (periodontitis vs. gingivitis)\textsuperscript{11,13,17}. We therefore speculated that the same might be true for patients with CP who respond or fail to respond to initial treatment. However, in this study we found no indications that the differences in response to initial treatment in CP are related to differences in expression of intercellular adhesion molecules on gingival tissue or infiltrating inflammatory cells. Our results therefore seem to be in accordance with earlier studies that found no differences in ICAM-1 expression in gingival tissue of periodontally healthy persons in comparison to patients with inflammatory periodontal disease and contradict those who found a difference\textsuperscript{15,21}. However, this might not be completely true since inflammation in our study was present in all analyzed samples and the degree of inflammation did not correlate with the clinical response. The reason for this discrepancy lies probably in the fact that in periodontal disorders the important pathologic processes take place in deep supportive tissues and not on the surface. Therefore changes seen in the biopsies of epithelial and superficial supportive tissues probably do not correlate very well with the disease course. Still, our data suggest that the expression of cellular adhesion molecules in the gingiva of patients with CP does not affect the disease course.

The clinical response to initial treatment correlated with a lymphocytic inflammatory infiltrate that consisted mainly of T cells. T cells have various functions, they can act as cytotoxic killer cells or cells that regulate the inflammatory response. Specific subsets of T cells can stimulate the inflammatory response but also suppress it. Th1 T lymphocytes stimulate cellular immunity, while Th2 T lymphocytes stimulate humoral immunity and counteract some of the Th1 mediated effects\textsuperscript{22,23}. On the other hand, B cells secrete immunoglobulins and act as antigen presenting cells. Secretion of immunoglobulins in the gingiva would cause an in-
Fig. 2. Gingival biopsy from a non-responding patient. The gingival surface is covered with hyperplastic epithelium. An inflammatory infiltrate consisting of similar numbers of T- (CD3 positive cells - Fig. 1a) and somewhat more superficially located B-lymphocytes (CD20 positive cells - Fig. 1b) can be seen. Magnification ×40.

**TABLE 1**
RESULTS OF IMMUNOHISTOCHEMICAL ANALYSES

<table>
<thead>
<tr>
<th>Subject</th>
<th>Responders</th>
<th>Non-responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary density (medium/minimal-maximal)</td>
<td>23/9-45</td>
<td>29/11-25</td>
</tr>
<tr>
<td>ICAM-1 expression positive expression (N/%)</td>
<td>8/40%</td>
<td>9/43%</td>
</tr>
<tr>
<td>Subbasal beta-1 integrin expression positive expression (N/%)</td>
<td>9/45%</td>
<td>11/52%</td>
</tr>
<tr>
<td>Basal beta-1 integrin expression positive expression (N/%)</td>
<td>20/100%</td>
<td>20/95%</td>
</tr>
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increase in migration of PMNs and other phagocytes, thereby increasing local inflammatory activity\textsuperscript{24,25}. The antigen presenting activity of B cells would also in the end augment the inflammatory response. Since tissue destruction in CP, as in other, more severe types of periodontitis, is ultimately caused by host defence mechanisms and not by microbes, it is conceivable that patients who fail initial periodontal therapy have an inappropriately increased local inflammatory response. Alternatively, an effective cellular immune response, mediated by Th1 T lymphocytes could lead to a fast sanation of the primary pathogenic process, while a response involving Th2 T lymphocytes that stimulate the accumulation of B cells and immunoglobulin secretion might be less effective in dealing with the factors triggering CP. To distinguish between these possibilities it would be necessary to further characterize T cell infiltrates in both groups of patients.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3.png}
\caption{Two gingival biopsy specimens from responding patients. Fig. 3a shows strong positivity for ICAM-1. Fig. 3b shows negative staining for ICAM-1. Magnification $\times$100.}
\end{figure}
Conclusions

The expression of adhesion molecules ICAM-1 and beta-1 integrins in gingival biopsies of patients with CP who respond or fail to respond to initial periodontal treatment is similar. This argues against the importance of these molecules in the modulation of the disease course.

Patients with CP who respond to initial treatment have a lymphocytic inflammatory infiltrate in the gingiva consisting predominantly of T cells, while in those who fail treatment approximately equal numbers of T and B cells are found. This finding supports the thesis that the differences in immunological response of the host are most important in determining the disease course.

Fig. 4. Two gingival biopsy specimens from non-responding patients stained for beta-1 integrin with positive expression. In Fig. 4a positive cells are located in the basal, and in Fig. 4b in the suprabasal layer.
REFERENCES


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SUBPOPULACIJA LIMFOCITA I EKSPRESIJA INTERCELULARNIH ADHEZIJSKIH MOLEKULA U KRONIČNOM PERIODONTITISU

SAŽETAK

Imunološki odgovor na invazivne bakterije ima glavnu ulogu u upalnim bolestima periodontita, kao što je kronični periodontitis (CP). Predloženo je da je jedan od glavnih elemenata te bolesti ekspresija adhezivnih molekula. Korištenjem imunohistokemijskih metoda istražili smo ekspresiju adhezivnih molekula ICAM-1 i β1-integrina, te kapilarnu gustoću i subpopulacije limfocita iz gingivalnih biopsija dobivenih od 20 pacijenata s CP koji su reagirali na terapiju i 21 pacijenta koji nisu reagirali na terapiju. Između te dvije grupe nismo pronašli nikakvu razliku u ekspresiji ICAM-1 i β1-integrina. Pacijenti koji su reagirali na terapiju pokazivali su uglavnom T limfocitni infiltrat, dok su oni koji nisu reagirali na terapiju pokazivali jednak broj T i B limfocita. Ovi nalazi podupiru ulogu imunoloških mehanizama koji određuju CP i govore protiv centralne uloge adhezivnih molekula.