Immunohistological findings in lip biopsy specimens from patients with Crohn’s disease and healthy subjects

GRACIELA CRAMA-BOHBOUTH, F T BOSMAN, B J VERMEER, A M VAN DER WAL, I BIEMOND, I T WETERMAN, AND A S PEÑA

From the Departments of Gastroenterology, Pathology, and Dermatology, University Medical Centre, Leiden, The Netherlands

SUMMARY Biopsies of apparently normal buccal mucosa were taken from 14 patients with Crohn’s disease and 13 healthy controls who were matched for dental status. Most patients had an increased number of lymphocytes around vessels in the subepithelial tissue and two showed fibrosis with moderate atrophy of minor glands. Plasma cells which contained immunoglobulin, predominantly IgA, were only found around minor salivary glands in both patients and controls. Quantitative studies showed a significant increase in the number of cells containing IgA in patients compared with controls. No correlation was found between immunoglobulin pattern and disease activity, age, sex, or duration of Crohn’s disease. A significant correlation was found between the activity of the disease, as defined by the Crohn’s Disease Activity Index, and the number of plasma cells containing IgM.

Oral lesions in Crohn’s disease which were first described in 19691 have been reported by various groups since then.1-12 Basu et al13 found that 9% of patients with Crohn’s disease had macroscopic abnormalities in the buccal mucosa while others have shown that buccal mucosa which is apparently normal on clinical inspection contains aggregations of lymphocytes in the connective tissue of minor salivary glands, compatible with chronic inflammation.14-18

In the present study we investigated the histology and certain immunological features of uninvolved oral mucosa of Crohn’s disease patients during different phases of the disease, and compared the findings with those obtained in healthy controls.

Methods

PATIENTS

Fourteen patients with Crohn’s disease, nine women and five men (Table) with a mean age of 34-5 years (range 21-71 years), were included in the study. In all cases the diagnosis had been confirmed histologically. The total duration of the disease ranged between four months and 29 years. Disease activity was recorded as a Crohn’s Disease Activity Index with criteria used by the National Cooperative Crohn’s Disease Study.19 Treatment at the time of the lip biopsy was noted, with the site, and extent of disease, any extra-intestinal manifestations, and the dental status. None of the patients had a deficiency of folic acid, iron, or vitamin B12.

CONTROLS

Thirteen healthy volunteers, eight women and five men with a mean age of 28 years (range 24-40 years) and a dental status matching those of the patients, formed the control group.

ETHICAL ASPECTS

This study was approved by the Ethics Committee of our hospital, and all persons who took part were thoroughly informed about the purpose of the study and gave their consent.

PROCEDURES

Two biopsy specimens, each 4 mm in diameter, were taken under local anaesthesia with a biopsy punch from the inside part of the lower lip, one to the right of the midline of the lip, the other to the left of it. One of the biopsy specimens was snap-frozen and used for detection, by an indirect immunofluorescence method,20 of deposits of IgA, IgM, IgG, and complement along the basement membrane of the epithelium and around vessels and salivary glands.
Table  Clinical data of patients and healthy controls

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Duration of disease (yr)</th>
<th>Localisation</th>
<th>Resection</th>
<th>CDAI</th>
<th>Therapy</th>
<th>No.</th>
<th>Sex</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>44</td>
<td>20</td>
<td>Distal small bowel–colon–perianal</td>
<td>+</td>
<td>245</td>
<td>C + A</td>
<td>1</td>
<td>F</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>33</td>
<td>16</td>
<td>Jejunum–ileum</td>
<td>+</td>
<td>127</td>
<td>None</td>
<td>2</td>
<td>F</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>23</td>
<td>13</td>
<td>Ileocecum</td>
<td>+</td>
<td>287</td>
<td>None</td>
<td>3</td>
<td>F</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>21</td>
<td>13</td>
<td>Ileum–colon</td>
<td>–</td>
<td>50</td>
<td>C + A + S</td>
<td>4</td>
<td>F</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>22</td>
<td>4</td>
<td>Distal ileum, colon–perianal</td>
<td>–</td>
<td>270</td>
<td>TPN</td>
<td>5</td>
<td>M</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>35</td>
<td>7</td>
<td>Colon–rectum</td>
<td>–</td>
<td>25</td>
<td>C + A</td>
<td>6</td>
<td>M</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>32</td>
<td>6</td>
<td>Ileocecum</td>
<td>+</td>
<td>23</td>
<td>S</td>
<td>7</td>
<td>F</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>34</td>
<td>20</td>
<td>Distal ileum, colon</td>
<td>+</td>
<td>154</td>
<td>None</td>
<td>9</td>
<td>F</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>43</td>
<td>15</td>
<td>Ileocecum</td>
<td>+</td>
<td>175</td>
<td>S</td>
<td>10</td>
<td>M</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>28</td>
<td>8</td>
<td>Stomach–duodenum, ileum–colon</td>
<td>+</td>
<td>167</td>
<td>C + A + S</td>
<td>11</td>
<td>F</td>
<td>27</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>71</td>
<td>29</td>
<td>Ileum–colon, stomach</td>
<td>+</td>
<td>201</td>
<td>S</td>
<td>12</td>
<td>M</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>21</td>
<td>3</td>
<td>Stomach–ileum–colon</td>
<td>–</td>
<td>339</td>
<td>C + S</td>
<td>13</td>
<td>M</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>54</td>
<td>8</td>
<td>Colon</td>
<td>–</td>
<td>281</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Crohn’s Disease Activity Index was determined according to Best et al at the time of the lip biopsy. C = corticosteroids, S = sulphasalazine, A = azathioprine, TPN = total parenteral nutrition.

**Immunohistological studies**

Serial cryostat sections, 2–4 μ thick, were dried, incubated with fluorescein-conjugated antisera for 30 minutes at room temperature, washed in 0.01M phosphate-buffered saline (pH 7.2), mounted in Tris-buffered glycerol (0:1 v/v, pH 7-8), and examined under a Leitz fluorescence microscope. Monospecific FITC-conjugated rabbit antisera against the heavy chain of human IgM, IgA, and IgG were obtained from NORDIC (Tilburg, Holland). Specific antisera against total complement was produced in rabbits and conjugated with FITC. The specificity of the antisera was confirmed by both immunoelectrophoresis and immunodiffusion and also by staining of bone marrow plasma cells producing monoclonal IgG, IgA, or IgM.

The other biopsy specimen was fixed in a sublimate-formaldehyde mixture for three hours and embedded in Paraplast®. Tissue sections were cut (4 μ thick) perpendicular to the mucosal surface and mounted on glass slides. Sections were stained with haematoxylin and eosin, and specifically for IgA, IgM, IgG by an indirect immunoperoxidase method described elsewhere. Plasma cells containing immunoglobulins, which were found only around minor salivary glands, were counted and the results expressed per mm². Student’s t test was used for statistical analysis of the results.

**Results**

The biopsy specimens of almost all patients showed an increase of submucosal lymphocyte infiltrate. In two patients the biopsy showed fibrosis and atrophy of the minor salivary glands, and in one case there was an abnormal aggregation of lymphocytes in the connective tissue around minor glands. There was no correlation between the presence or degree of histological abnormality and the activity, site, extent or duration of disease: nor was there a relationship with the patients' age and treatment (Table).

The immunofluorescence studies showed no clear deposits of IgA, IgG, IgM, or complement in either patients or controls. With the immunoperoxidase technique we found Ig-containing plasma cells around salivary glands in both patients and controls, and IgA was the predominant immunoglobulin (77%). The number of plasma cells containing IgA, IgG, and IgM around minor salivary glands was higher in Crohn's disease patients than in controls. IgA immunocytes per mm² (mean ± SD) for controls 105±68; for patients 205±104. IgM immunocytes per mm² for controls 11±14; for patients 24±24. IgG immunocytes per mm² for controls 16±15; for patients 29±24.

The difference was only statistically different for IgA (p<0.01). A significant correlation was found between the activity of the disease, as defined by the Crohn's Disease Activity Index, and the number of IgM-containing plasma cells (r=0.67, p<0.02) (see Figure).

**Discussion**

In agreement with previous reports, we found that the buccal mucosa from patients with Crohn's
patients. but these authors did not investigate the correlation between their observations and the activity of the disease.

The present study documents yet another immunological abnormality in Crohn's disease. Further studies are needed to discover whether these abnormalities are the cause of, or are caused by, the underlying disease process.

We are indebted to Professor W van Vloten for instructing us in the lip-biopsy procedure, to Mr Wendel Hillebrands, Department of Dermatology, for technical help, to Mrs I Seeger for reading the English text, and to Gabrielle W M Verhoef for typing the manuscript.

References

Immunohistological findings in lip biopsy specimens

Immunohistological findings in lip biopsy specimens from patients with Crohn's disease and healthy subjects.

G Crama-Bohbouth, F T Bosman, B J Vermeer, A M van der Wal, I Biemond, I T Weterman and A S Pena

*Gut* 1983 24: 202-205
doi: 10.1136/gut.24.3.202

Updated information and services can be found at:
http://gut.bmj.com/content/24/3/202

These include:

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections
Crohn's disease (932)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/