Abstract. Background: The diagnosis of mucosa-associated lymphoid tissue (MALT) lymphoma in the intestine is occasionally difficult from histological examination on small biopsy specimens obtained by endoscopy. This study focused on unusual cases of reactive lymphoproliferative disorders in the intestine in order to make a differential diagnosis of MALT lymphoma. Materials and Methods: Five patients were examined with regards to clinical symptoms, endoscopic findings and multiparameter analysis (the morphological examination using routine hematoxylin and eosin staining by light microscopy, immunophenotyping by flow cytometry (FCM), immunohistochemistry and genotyping of extracted DNA). Results: All cases showed an aggregation of lymphocytes and one case showed similar features to lymphoepithelial lesions. Analyses of FCM and genetic rearrangements denied the monoclonality in all cases. Consequently, we considered that all cases should be diagnosed as reactive lymphoid hyperplasia and inflammatory change. Conclusion: Multiparameter analysis is useful in making an exact diagnosis of MALT lymphoma and therefore contributes to prevent unnecessary overtreatment.

Mucosa-associated lymphoid tissue (MALT) lymphoma in the intestine is a rare subset of low-grade malignant neoplasms. It belongs to extranodal marginal zone lymphoma, characterized histopathologically by centrocyte like cells (CCLs), lymphoepithelial lesion (LEL) and follicular colonization etc.

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Key Words: Intestinal mucosa-associated lymphoid tissue (MALT) lymphoma, flow cytometry (FCM), monoclonal gene rearrangement.

Materials and Methods

Subjects. Five patients were enrolled in this study. All of them were referred to our hospital because of diagnosis or suspicion of intestinal MALT lymphoma from physical, endoscopic and histopathological findings from forceps biopsy specimens. All patients underwent reexamination of the clinical symptoms, laboratory data and endoscopic findings and then multiparameter analysis was performed. The details of the multiparameter analysis were described elsewhere (7, 8).

Micromorphological examination. One-third of the respective specimens obtained from multiple biopsies or EMR of the lesions was fixed in 20% non-buffered formalin. Paraffin-embedded specimens were stained with routine hematoxylin-eosin for histological diagnosis.
Two-color flow cytometry. The monoclonal antibodies (CD5, CD10, CD19, CD20, CD21, CD23, CD35, immunoglobulins etc.) were used in two-color flow cytometry (2-FCM). The cell suspension obtained from the unfixed material was stained with a set of two kinds of various monoclonal antibodies listed above. The immunostained cells were analyzed by a FACScan (Becton-Dickinson Immunocytometry Systems, Mountain View, CA, USA).

Immunohistochemistry. Small pieces of the specimens were fixed in PBS with 1% paraformaldehyde and preserved in PBS with 20% sucrose. Then the material was mounted in the OCT compound, quickly frozen and was sectioned by a cryostat. Frozen and paraffin sections were stained with the unlabeled monoclonal antibodies, followed by avidin-biotin peroxidase method.

Genotyping. DNA samples were extracted from the material and were digested with the restriction enzymes BamHI, EcoRI and HindIII endonucleases. Gene rearrangements were examined using ³²P-probes for JH gene of the immunoglobulin heavy chain (IgH), human T cell receptor β, γ and δ chain genes and BCL-2 (Biomedical Laboratories, Kawagoe, Japan).

Results

Table I shows the clinical and endoscopic features of the 5 patients. All the patients had some symptoms. They had no ulcer, cancer, malignant lymphoma or H. pylori infection in the stomach. A high level of soluble interleukin-2 receptor (sIL-2R) was observed in case 2. Concerning the intestinal endoscopic findings, three cases had multiple protrusions and erosions in the large intestine or terminal ileum. In the other two cases, one had a solitary lesion of polypoid appearance in

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Clinical symptoms</th>
<th>CRP</th>
<th>CEA</th>
<th>sIL-2R</th>
<th>Endoscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>f</td>
<td>Right lower abdominal pain</td>
<td>-*</td>
<td>-</td>
<td>-</td>
<td>Multiple protrusions and erosions in the terminal ileum and total colon</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>m</td>
<td>Upper abdominal pain, diarrhea</td>
<td>1.0 mg/dl</td>
<td>-</td>
<td>1279 U/ml</td>
<td>Multiple protrusions in the ileum</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>f</td>
<td>Anal bleeding</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Multiple protrusions and erosions in the rectum</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>f</td>
<td>Lower abdominal pain, diarrhea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Erosions and redness in the rectum</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>m</td>
<td>Diarrhea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Solitary polypoid lesion in the rectum</td>
</tr>
</tbody>
</table>

f: female; m: male; CRP: C reactive protein; CEA: carcinoembryonic antigens; sIL-2R: soluble interleukin-2 receptor

*- within normal limit
the rectum, while the other had diffuse erosions and redness in the rectum. The endoscopic finding of case 1 is shown in Figures 1 and 2. The results of the multiparameter analysis are summarized in Table II. In multiple biopsied or EMR specimens of the intestine, all cases showed an aggregation of lymphocytes in the mucosal and submucosal layer and only case 1 showed wide marginal zone and LEL like lesion (Figures 3, 4). The distinct CCLs and LEL were not observed in the other four cases. As a result of immunophenotyping by FCM, a mixed pattern of B and T lymphocytes, that is, a reactive pattern was observed in all cases. Figure 5 shows the FCM of case 1. Immunohistochemical examination showed that many of the lymphocytes were positive for CD20 and negative for both CD5 and cyclinD1 in case 1. Analysis of IgH, BCL-2 or human T cell receptor β, γ and δ chain genes rearrangements showed negative results for any monoclonality in all cases (Figure 6). Finally, four cases were diagnosed as reactive lymphoid hyperplasia and one as inflammation change. The symptoms of all cases subsided spontaneously and they have been followed-up periodically. The intestinal lesions disappeared within six months in three patients, but they remained without apparent changes in two patients.

Discussion

Lymphoproliferative disorders in the intestine are relatively rarely observed. Intestinal disorders involving lymphocytes are divided into two major groups, that is,
malignant lymphoma including MALT lymphoma, which represents monoclonal neoplastic proliferation of lymphocytes and reactive lymphoid hyperplasia, which shows hyperplastic and polyclonal aggregation of lymphocytes. Although MALT lymphoma and lymphoid hyperplasia are distinct clinicopathological entities, they can frequently cause serious problems in routine histological differential diagnosis.

The cases we described in the current report closely resembled MALT lymphoma when examined on the endoscopic findings and histological features. Specifically, the infiltrating cells of the lymphoid hyperplasia of one case were similar in size and morphology to CCLs of MALT lymphoma. The distribution of proliferating lymphocytes may also make the diagnosis difficult. There was focal infiltration of crypt epithelium like LEL, which was difficult to distinguish from MALT lymphoma.

The most important procedures to distinguish between them are combined multiparameter analysis including immunophenotyping using FCM and/or immunohistochemistry and monoclonal gene rearrangement (7, 8). The diagnostic criteria of MALT lymphoma has been proposed to be the characteristic of tumor cells, positive for CD19, CD20, CD21 and CD35 and negative for CD5, CD10, CD23 and cyclinD1 (2, 9). In addition, it has been recently reported that monoclonal gene rearrangement, including IgH and t(11;18)(q21;q21) chromosomal translocation, are frequently observed in MALT lymphoma (6, 10). All current cases clearly exhibited the mixed pattern of B and T lymphocytes in FCM which are considered to be reactive changes. Genotyping to detect clonal immunoglobulin, T cell receptors and BCL-2 revealed no monoclonal proliferation. Therefore, we concluded that all current cases should be diagnosed as reactive and inflammatory lymphoid changes rather than MALT lymphoma. The symptoms of all cases have subsided spontaneously and none of them showed an aggravation. In addition, the level of sIL2-R in case 2 decreased gradually. Three patients presented disappearance of the lesions endoscopically. These findings also support that the disorders can be considered as reactive changes.

The etiology of lymphoid hyperplasia and inflammation changes are still not clear in detail. Immunological factors such as hypogammaglobulinemia or an isolated IgA deficiency, which are frequently observed in patients with diffuse lymphoid hyperplasia, may play some role in the etiology (11-13). Intestinal parasitosis or viral infection...
may also be implicated as a possible cause of lymphoid hyperplasia (14-17). However, stool culture tests of the current cases were negative for harmful bacteria.

There are several reports of “intestinal MALT lymphoma” that underwent operation and/or chemotherapy without multiparameter analysis (6, 18, 19). Here we described 5 cases of reactive lymphoid hyperplasia and inflammatory changes in the intestine which should be distinguished from MALT lymphoma. Multiparameter analysis including FCM and gene rearrangement was useful in making discrete differential diagnosis between these benign lesions and MALT lymphoma, suggesting that it might contribute in preventing overtreatments for those lymphoproliferative disorders.

References


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