Biclonal Lymphoplasmacytic Immunocytoma Associated with Crohn’s Disease

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A 33-year-old man with a 4-year history of Crohn’s disease presented with marked ascites and an abdominal tumor. Two M-protein peaks, immunoglobulin (Ig) G-κ and IgA-κ, were detected in the serum. Neoplastic lymphoplasmacytic cells were infiltrated in the bone marrow and ascites. Histological examination of the abdominal tumor showed marked proliferation of lymphoplasmacytic cells that were positive for either IgG or IgA. Moreover, DNA sequences of the expressed IgG and IgA genes were different in the complementarity-determining region 3. These results suggest that chronic inflammation in Crohn’s disease contributes to the simultaneous development of biclonal lymphoplasmacytic immunocytoma of the small intestine.

Key words: malignant lymphoma, biclonal gammopathy, immunoglobulin gene

Introduction

Inflammatory bowel diseases (IBD) such as Crohn’s disease and ulcerative colitis are chronic systemic disorders and are risk factors for gastrointestinal malignant tumors. The association between ulcerative colitis and adenocarcinoma of the affected gastrointestinal tract is well established, suggesting that chronic inflammation and stimulation provoke malignant transformation of the damaged tissue (1). Several authors have recently reported the development of malignant lymphoma in patients with Crohn’s disease (2, 3), however, the risk of lymphoma in Crohn’s disease is not well defined (4–6).

Lymphoplasmacytic immunocytoma is a B-cell neoplasm characterized by different stages of tumor-cell maturation, such as small lymphocytes, lymphoplasmacytic cells, and plasma cells (7). The incidence of biclonal gammopathy in this type of lymphoma is extremely rare (8, 9). Biclonal gammopathy may result from the proliferation of two unrelated B-cell clones, or from a transformation event in a malignant B-cell undergoing an isotype switch. Clonal analysis of neoplastic B-cell is, therefore, important for understanding the pathogenesis of biclonal gammopathy, especially when tumors are associated with chronic inflammatory conditions.

Here, we report a case of Crohn’s disease which developed lymphoplasmacytic immunocytoma with biclonal gammopathy. DNA sequence analysis of the immunoglobulin genes provided evidence of two different clones in the tumor, indicating that independent clonal evolution occurred during the chronic inflammation of Crohn’s disease.

Case Report

A 33-year-old man was admitted to our hospital in April 1997 for the investigation of a lower abdominal mass. Four years earlier, he presented with diarrhea and colonoscopic examination showed cobblestone appearance with longitudinal ulcers of the descending colon (Fig. 1) and ulceration areas of the sigmoid colon. Pathological examination of biopsied specimens from the colon was consistent with Crohn’s disease, showing segmental edema, fibrosis, and chronic inflammatory infiltration. Small-bowel enema showed no abnormal findings. M-protein was not found in the serum. Treatment was started with corticosteroids and salazosulfapyridine in April 1996. The ulcer lesions of the colon were improved, however, hypoalbuminemia and the accelerated erythrocyte sedimentation rate were still observed. On admission, the patient was cachectic with marked ascites and a 5 × 7 cm mass in the left iliac fossa. There was no superficial lymphadenopathy. Laboratory tests showed a microcytic anemia (hemoglobin, 10.7 g/dl) and mild leukocytosis (white blood cell, 11,400/μl) with 1% atypical plasma cells. Bone marrow aspiration showed the infiltration of lymphoplasmacytic cells (11.4%). Two M-protein peaks were
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Figure 1. Endoscopic view of the descending colon showing cobblestone appearance (A) and longitudinal ulcers (B).

Figure 2. Neoplastic lymphoplasmacytic cells in ascites (Wright-Giemsa stain, ×330).

detected in the serum. Serum immunoglobulin levels were as follows; immunoglobulin (Ig) G, 1,330 mg/dl; IgA, 575 mg/dl; and IgM, 29 mg/dl. Immunoelectrophoresis revealed biclonal gammopathy for IgG-κ and IgA-κ in the serum and Bence Jones protein of κ-type in the urine. A computed tomography (CT) scan of the abdomen and pelvis showed a mesenteric mass in the left iliac fossa with a maximum diameter of 7 cm. An abnormal loop of the small intestine passing through a large mass was also found. While enlarged lymph nodes were observed in the paraaortic regions, no hepatosplenomegaly was noted. Cytological examination of ascites showed infiltration of neoplastic lymphoplasmacytic cells (Fig. 2). These cells were positive for CD19, CD20, CD21, CD22, CD23, and human leukocyte antigen (HLA)-DR. In addition, 80% of these cells were positive for surface IgG-κ and 5% for IgA-κ. The cytogenetic analysis of the ascites cells showed an abnormal karyotype; 48, XY, t(2;8) (p11;q24), del(3) (q13q21), add(4) (q31), add(7) (q36), −18, +21, +2mar, in all 20 metaphases examined, providing evidence of clonality. He was treated with combination chemotherapy (vincristine, doxorubicin, dexamethasone), but the tumor rapidly progressed to involve the pleura and he died of the disease.

At autopsy, a large tumor (20 × 20 cm) was found at the ileum with abnormal lymph nodes in the mesentery. Histological examination of the tumor showed extensive infiltration of the intestinal wall by small lymphocytes, lymphoplasmacytic cells, and plasma cells (Fig. 3A). Immunohistochemistry revealed that tumor cells were positive for either IgG or IgA (Fig. 3B, C). These findings were compatible with the features of lymphoplasmacytic immunocytoma which was composed of two subpopulations of B cells. There was no histological finding of Crohn’s disease in the esophagus, stomach, small intestine, or colon. However, tumor invasion was also observed in the stomach, colon, bladder, pancreas, peritoneum, lungs, and pleura.

To investigate whether these tumor cells originated from the same clone, we amplified and sequenced the expressed
immunoglobulin genes from the tumor. Total RNA was extracted from the tumor and cDNA was synthesized by reverse transcriptase. DNA of immunoglobulin variable region was amplified by polymerase chain reaction using 5' V\textsubscript{H} family-specific leader (V\textsubscript{H}1, V\textsubscript{H}2+4, V\textsubscript{H}3, V\textsubscript{H}5 or V\textsubscript{H}6) and 3' C\textgamma-or C\alpha-specific primers. DNA sequencing was performed by direct sequencing method using an autosequencer. Each sequence was searched for homology with GENBANK DNA database. A major amplification product was obtained with the V\textsubscript{H}4 family-specific primers in both IgG and IgA genes. However, as shown in Fig. 4, DNA sequence analysis demonstrated that V\textsubscript{H} region genes of IgG and IgA were different with somatic mutations in the complementarity-determining region 3 (CDR3).

**Figure 3.** Histological features of the small intestine. Neoplastic lymphoplasmacytic cells infiltrated the intestinal wall (A) (HE stain, ×132). By immunohistochemical studies of serial sections, the tumor cells were positive for either IgG (B) or IgA (C) (immunohistochemical stain, ×132).

**Figure 4.** DNA sequences of the CDR3 from the tumor clones. Germline D and JH genes with maximum homology to the segments are shown under each sequence. #: replacement mutation, *: silent mutation.

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Discussion

We reported a case of Crohn’s disease which subsequently developed lymphoplasmacytic immunocytoma with IgG-κ and IgA-κ biclonal gammapathy. DNA analysis of the tumor showed that the sequences of CDR3 of IgG and IgA were different, confirming the presence of two independent proliferating clones of B cells. Moreover, we found frequent somatic mutations and no intraclonal variation of these IgG and IgA genes, which indicate that the tumor cells of the patient originate from a B lineage cell that had already undergone repeated antigenic stimulation and selection in the germinal center of lymphoid tissue and had acquired the ability to produce the high-affinity immunoglobulin (10). Therefore, these findings suggest that the antigen stimulation played a role in the evolution of two independent B-cell clones.

Previous clinical data support an association between gastrointestinal malignant tumors and IBD (1). Although the pathogenesis of malignant lymphoma complicating Crohn’s disease is obscure (4–6), several factors such as primary immunologic defects, immunosuppressive therapy, and chronic inflammation and stimulation have been postulated (2). To date, 22 cases of gastrointestinal lymphoma have been reported in association with Crohn’s disease (3). The observations that all of these tumors have arisen at the sites of active IBD suggest a correlation between Crohn’s disease and gastrointestinal lymphoma. In addition, our data are consistent with the possibility that such evolution occurs in several stimulated lymphoid populations of active IBD, and that clonal B cells eventually give rise to lymphomas. Similarly, the development of biclonal or oligoclonal lymphomas has also been documented in chronic inflammatory conditions such as Helicobacter pylori infection (11). Although there is no other case report of biclonal lymphoma associated with Crohn’s disease, it might be important to evaluate the clonality of previous cases of IBD-associated lymphoma by molecular studies.

It has previously been shown that chromosomal translocations which affect c-myc transcription are associated with development of plasmacytoma that occurs on a background of chronic inflammation (12). Chromosome abnormality of t(2;8) was found in this case, suggesting that c-myc gene may participate in the evolution of lymphoma cells. However, we found only one pattern of chromosome abnormality in ascites cells despite the presence of two B-cell clones. One possibility is that this abnormality resulted from IgG-producing cells which proliferated dominantly in ascites. Alternatively, IgG- and IgA-producing clones might have arisen from B cells which already had this chromosomal change. Although we were unable to demonstrate different breakpoints within c-myc gene between IgG- and IgA-producing tumors, this analysis may provide further evidence for their clonal distinctiveness and elucidate its possible role in the tumorigenesis of IBD-associated lymphoma. Further investigation is still required to establish a definite relationship between IBD and lymphoma of affected gastrointestinal tract.

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References