CASE REPORT

Immunopathological Analysis of Erdheim-Chester Disease with Massive Ascites

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Abstract

We treated a 77-year-old woman with pleural and pericardial effusion and ascites. Initially, collagen vascular disease was suspected due to the presence of anti-centromere antibodies and suspected complication of pulmonary arterial hypertension. However, soft-tissue abnormalities surrounding the bilateral kidneys detected on computed tomography (CT) and symmetrical lesions of the long bones detected on bone scintigraphy made us consider a diagnosis of Erdheim-Chester disease (ECD), which is a rare form of histiocytosis. We immunochemically analyzed the cells derived from the ascites in detail and confirmed the diagnosis. Immunocytochemical analyses may therefore help to achieve a better understanding of the pathogenesis of this rare disease.

Key words: Erdheim-Chester disease, histiocytosis, flow cytometry, interferon

(DOI: 10.2169/internalmedicine.51.8233)

Introduction

Erdheim-Chester disease (ECD) is a rare form of histiocytosis of unknown origin (1). It is distinguished from Langerhans histiocytosis (LCH) in that the abnormal histiocytes of ECD are positive for CD68 and negative for CD1a and S100 proteins. ECD is a multisystem disease that primarily affects the skeletal system but also involves the central nervous system, cardiovascular system, hypothalamic-pituitary system, lungs, kidneys, retroperitoneum and orbits. It remains unclear whether ECD is a malignant or reactive inflammatory disease. Since the first report in 1930, less than 200 cases of ECD had been reported as of the end of 2007 (2). Since then, however, more than 200 cases have been reported in less than four years (1). The increased number of reported cases is probably due to the fact that awareness of the disease has become more widespread in recent years. Hopefully, accumulating data related to ECD will increase our understanding of the pathogenesis of this disease and also help to establish better treatment strategies.

Case Report

A 77-year-old woman was admitted to this hospital with dyspnea, palpitations and ascites. One year before admission, she developed dyspnea on exertion. Pericardial effusion was detected at a nearby hospital and a diuretic was prescribed. The dyspnea persisted and peripheral edema newly developed. She was admitted to another hospital, where pericardiocentesis was performed. The pericardial effusion was reported to be bloody and exudative. The cytological grade was Class I, and no bacteria were detected, including Mycobacterium tuberculosis.

The amount of pericardial effusion decreased spontaneously and she was discharged on the 16th day of hospitalization. Within one month, however, abdominal bloating developed and she was referred to our hospital. As the antinuclear antibody test was positive at 1:640, she was referred to this department.

On examination, the patient’s temperature was 37.0°C, pulse was 78 beats per minute and blood pressure was 170/80 mmHg. Her weight was 54.0 kg and height was 143.4 cm.
Platelet count was 176×10^3/μL (72.3% neutrophils, 19.1% lymphocytes), the hemoglobin level was 10.1 g/dL and the platelet count was 176,000/μL. The C-reactive protein (CRP) level was 0.18 mg/dL. The patient’s liver function was normal; however, the creatinine level was 1.32 mg/dL, the blood urea nitrogen level was 23 mg/dL and the potassium level was 5.4 mEq/L, suggesting the presence of chronic kidney disease. The free T3 and free T4 levels were low (1.53 pg/mL and 0.83 pg/mL, respectively), while the thyroid stimulating hormone (TSH) level was high (6.64 μIU/mL), indicating hypothyroidism. The anti-nuclear antibodies were of the discrete speckled type. As expected, the patient was positive for anti-centromere antibodies (INDEX: 128; reference range: 0-9.99); however, no other disease-specific antibodies were detected, including antibodies to double-stranded deoxyribonucleic acid (DNA), Sm, ribonucleoprotein (RNP) or Scl-70. A urinalysis revealed the presence of slight proteinuria (45 mg/dL), scant red blood cells (1-4/high power field (HPF)), hyaline casts (50-99/whole field (WF)) and granular casts (5-9/WF).

Chest X-ray revealed cardiac enlargement and computed tomography (CT) revealed bilateral pleural effusions, pericardial effusion and ascites (Fig. 1). Characteristically, soft-tissue abnormalities surrounding the circumference of the bilateral kidneys were observed (Fig. 1B). The right ventricular systolic pressure (RVSP) was estimated to be 60.4 mmHg using echocardiography, which suggested the presence of pulmonary arterial hypertension (PAH).

A diagnosis of systemic sclerosis was initially suspected; however, no dermal sclerosis was apparent. Diagnostic thoracentesis was performed. The levels of protein and lactate dehydrogenase (LDH) in the pleural effusion were 3.6 g/dL and 53 IU/L and those in the plasma were 6.2 g/dL and 84 IU/L, respectively. In addition, the specific gravity and white blood cell concentration were both relatively high (1.027 and 978/μL, respectively). Therefore, the effusion was considered to consist of exudates. Although a diagnosis of systemic sclerosis was not confirmed, the presence of inflammation and the negative test results for infection led us to decide to treat the patient with 30 mg of prednisolone per day. The patient’s hypothyroidism was treated with synthetic

**Figure 1.** Chest and abdominal CT scans. (A) Pleural effusion and pericardial effusion were evident. (B) Bilateral and symmetrical perirenal soft tissue lesions were detected (“hairy kidney” appearance). A small amount of ascites was also present.

**Figure 2.** Skeletal imaging of the legs. (A) X-ray showed the presence of osteosclerotic lesions in the tibia. (B) Symmetrical increased osteoblastic activity primarily in the lower limbs was detected on bone scintigraphy. The diaphyses and metaphyses of the femurs and the tibiae were primarily affected, whereas the mid-diaphyses and epiphyses were spared. (C) MR imaging revealed bone lesions at the same positions as those shown in (A).
These findings, along with the peculiar soft tissue abnormalities and a small number of activated mesothelial cells, always revealed Class II and the presence of histiocytes, excluding the diagnosis of IgG4 syndrome. Unexpectedly, four months after the initial assessment, the RVSP estimated with echocardiography was 38 mmHg, although we did not use any medications for PAH, casting doubt on the presence of PAH. To relieve the symptoms of abdominal distension and edema of the lower extremities again worsened. She was therefore readmitted to this department for further evaluation. Taking into account the possibility that the abnormal soft tissue around the kidneys represented retroperitoneal fibrosis, we performed a peritoneal biopsy. Only non-specific findings were observed such as mild fibrosis and congestion (data not shown). Immunostaining revealed no signs of IgG4 deposition or IgG4-producing plasma cells, excluding the diagnosis of IgG4 syndrome.

In a flow cytometric analysis (Fig. 3A), two distinct populations (A and B) were gated using forward scatter (FSC) and side scatter (SSC). The cells in gate A were found to be double-positive for CD11c and dendritic and epithelial cells, 205 kDa (DEC-205) antigens, while those in gate B were negative for the antigens. Cells cultivated in vitro for seven days were used for the analysis. Dead cells were excluded with 7-aminoactinomycin D staining. (B) In vitro cultured cells were analyzed 15 hours after treatment with 10 ng/mL IFN-α. Note that the number of cells in gate A decreased significantly. Moreover, most of the cells became negative for CD11c and DEC-205 antigens.

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Figure 3. Flow cytometric analysis of the cells in the ascites. (A) More than one-half of the cells in gate A were positive for both CD11c and DEC-205 antigens, whereas those in gate B were double-negative for the antigens. Cells cultivated in vitro for seven days were used for the analysis. Dead cells were excluded with 7-aminoactinomycin D staining. (B) In vitro cultured cells were analyzed 15 hours after treatment with 10 ng/mL IFN-α. Note that the number of cells in gate A decreased significantly. Moreover, most of the cells became negative for CD11c and DEC-205 antigens.

thyroid hormone (125 μg/day). The abdominal distension improved after treatment with the corticosteroid was initiated, and the patient was discharged approximately two months later. She received follow-up care as an outpatient of this hospital for one month; however, during the course of tapering the dose of prednisolone, dyspnea on exertion, abdominal distension and edema of the lower extremities again worsened. She was therefore readmitted to this department for further evaluation. Taking into account the possibility that the abnormal soft tissue around the kidneys represented retroperitoneal fibrosis, we performed a peritoneal biopsy. Only non-specific findings were observed such as mild fibrosis and congestion (data not shown). Immunostaining revealed no signs of IgG4 deposition or IgG4-producing plasma cells, excluding the diagnosis of IgG4 syndrome. Unexpectedly, four months after the initial assessment, the RVSP estimated with echocardiography was 38 mmHg, although we did not use any medications for PAH, casting doubt on the presence of PAH. To relieve the symptoms of abdominal fullness, it was necessary to perform abdominal paracentesis one to two times per week. Repeated cytological examinations always revealed Class II and the presence ofhistiocytes and a small number of activated mesothelial cells. These findings, along with the peculiar soft tissue abnormality around the kidneys, led us to suspect a diagnosis of ECD. Bone scintigraphy demonstrated a symmetrically increased osteoblastic activity in the lower limbs primarily affecting the diaphyses and metaphyses of the femurs and the tibiae but sparing the mid-diaphyses and epiphyses. These are the almost pathognomonic findings of ECD (2) (Fig. 2B). X-rays of the knees revealed sclerotic bone lesions and magnetic resonance (MR) imaging revealed multiple bone lesions with irregular boundaries (Fig. 2A, C). Although ECD was strongly suspected, the histological evidence was lacking. The patient refused to undergo a biopsy of the bone lesions. As a second-best option, we immunologically analyzed the cells derived from the ascites.

In a flow cytometric analysis (Fig. 3A), two distinct populations (A and B) were gated using forward scatter (FSC) and side scatter (SSC). The cells in gate A were found to be double-positive for CD11c and dendritic and epithelial cells, 205 kDa (DEC-205) antigens, while those in gate B were found to be negative for both antigens. We next performed immunocytochemistry of the pellet of the ascites cells (Fig. 4 and Table). More than 50% of the cells were positive for CD68 and none of the cells were positive for S100 proteins. The cells were also diffusely positive for DEC-205 and partially positive for dendritic cell-specific in-
tercellular adhesion molecule-3-grabbing non-integrin (DCSIGN). Interestingly, double staining for CD163 and Ki-67 revealed almost all of the cells to be positive for CD163 and 5-10% of them were also positive for Ki-67, thus indicating that the cells were still proliferating.

Two courses of steroid pulse (methylprednisolone (mPSL) 500 mg ×3) were administered over an interval of one month, and, during this period, oral mPSL was reduced from 32 mg/day to 18 mg/day. Thereafter, the amount of ascites decreased somewhat and it was no longer necessary to perform abdominocentesis. Although there is no standard treatment for ECD, several reports have indicated the efficacy of interferon (IFN)-α (3-5). We therefore attempted to treat the patient with IFN-α; however, the treatment had to be discontinued due to fever, appetite loss and deterioration of renal function.

The patient and her family did not wish for any further vigorous therapy to be administered; therefore, she was transferred to a nearby hospital and treated with oral mPSL.

**Discussion**

We encountered a case of ECD with ascites. The “hairy kidney” appearance of the kidneys caused by bilateral infiltration of the perirenal and posterior pararenal spaces was highly indicative of the disease (6), while bone scintigraphy showed the pathognomonic appearance of ECD skeletal involvement (2). Osteosclerotic lesions detected on X-rays and bone cyst-like lesions detected on MR imaging were also compatible with a diagnosis of ECD (7). A diagnosis of ECD is usually confirmed with typical pathological findings: xanthogranulomas infiltrated by foamy histiocytes that are positive for CD68 and negative for CD1a (8). As we did not obtain the consent of the patient to perform a bone biopsy, we confirmed the diagnosis based on an immunocytological analysis of the cells derived from the ascites. It was obvious from the analysis that the abnormal histiocytes were of monocyte/macrophage origin; however, negative staining for CD15 suggests that the cells had lost the characteristics of monocytes/macrophages. Moreover, the findings of flow cytometry that the cells were positive for DEC-205, DC-SIGN and CD11c suggest that the cells had developed into interstitial dendritic cells. Interestingly, some of the CD68-positive cells were also positive for Ki-67, thus indicating that the cells were still proliferating, which is unusual for differentiated cells.

Initially, we suspected a diagnosis of systemic sclerosis, even though the patient had no signs of dermal sclerosis, because the anti-centromere antibody test result was positive and the RVSP estimated on echocardiography was high. However, repeated echocardiography showed decreases in estimated RVSP to the level of 38 mmHg over four months.

![Figure 4. Immunostaining of cell pellets derived from the ascites. (A) Most of the cells were S-100-protein-negative. Counterstained with Hematoxylin and Eosin staining, ×40. (B) Cells were diffusely positive for DEC-205. Counterstained with Hematoxylin and Eosin staining, ×400. (C) Double staining for CD163 (brown) and Ki-67 (red). Some of the CD163-positive cells were also Ki-67-positive (black arrows), indicating that the cells were proliferating. A white arrowhead indicates a cell that was positive for Ki-67 and negative for CD163. Uncounterstained with Hematoxylin and Eosin staining, ×400.](image-url)
Table. Results of the Immunocytochemical Analysis of Cell Pellets Derived from Ascites

<table>
<thead>
<tr>
<th>Antibody (clone)</th>
<th>Ig subclass</th>
<th>Source</th>
<th>Cells</th>
<th>Results in this case</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD163 (10D6)</td>
<td>Mouse IgG1</td>
<td>Novocastra, Newcastle upon Tyne, UK</td>
<td>Monocytes/macrophages</td>
<td>Diffusely positive</td>
</tr>
<tr>
<td>CD68 (KP-1)</td>
<td>Mouse IgG1</td>
<td>DAKO, Carpinteria, CA</td>
<td>Monocytes/macrophages</td>
<td>Positive (&gt;50%)</td>
</tr>
<tr>
<td>Anti-macrophage (LN5)</td>
<td>Mouse IgM, κ</td>
<td>Invitrogen, Camarillo, CA</td>
<td>Monocytes/macrophages</td>
<td>Positive (&gt;50%)</td>
</tr>
<tr>
<td>Vimentin (V9)</td>
<td>Mouse IgG1</td>
<td>Santa Cruz, Delaware Avenue, CA</td>
<td>Stromal cells</td>
<td>Diffusely positive</td>
</tr>
<tr>
<td>DEC-205 (CD205) (11A10)</td>
<td>Mouse IgG1</td>
<td>Novocastra</td>
<td>Dendritic cells (macrophages)</td>
<td>Diffusely positive</td>
</tr>
<tr>
<td>DC-SIGN (CD209)</td>
<td>Rabbit IgG</td>
<td>Santa Cruz</td>
<td>Dendritic cells (macrophages)</td>
<td>Partially positive</td>
</tr>
<tr>
<td>CD15 (Leu M1)</td>
<td>Mouse IgG1, κ</td>
<td>Abcam, Cambridge, UK</td>
<td>Myelocytes/monocytes</td>
<td>Negative</td>
</tr>
<tr>
<td>EMA (E29)</td>
<td>Mouse IgG2a, κ</td>
<td>DAKO</td>
<td>Epithelial cells</td>
<td>Negative</td>
</tr>
<tr>
<td>Calretinin (DAK-Calret 1)</td>
<td>Mouse IgG1, κ</td>
<td>DAKO</td>
<td>Mesothelial cells</td>
<td>Negative (the few positive cells were mesothelial cells)</td>
</tr>
<tr>
<td>CD1a (O10)</td>
<td>Mouse IgG1, κ</td>
<td>Immunotech, Marseille, France</td>
<td>Langerhans cells</td>
<td>Negative</td>
</tr>
<tr>
<td>S-100 protein</td>
<td>Rabbit, heterologous</td>
<td>Nichirei, Tokyo, Japan</td>
<td>Langerhans cells</td>
<td>Negative</td>
</tr>
<tr>
<td>Ki-67 (MIB-1)</td>
<td>Mouse IgG1</td>
<td>Immunotech</td>
<td>Proliferating cells</td>
<td>5-10% positive</td>
</tr>
</tbody>
</table>

and to 27.4 mmHg over nine months despite the fact that the patient did not receive any medications for PAH. Therefore, we doubted the presence of PAH. Performing right heart catheterization would have been necessary to confirm the diagnosis (9); however, it was not performed. Considering that the patient did not show any signs of visceral involvement, such as esophageal hypomotility, small bowel hypomotility or pulmonary interstitial fibrosis and did not have a history of Raynaud’s phenomenon, we now believe that the coexistence of “systemic sclerosis sine scleroderma” (10) was unlikely. The positive result for the anti-centromere antibody test may thus have been non-specific.

Regarding the treatment of ECD, corticosteroids are known to have a limited effect. We did observe, however, that oral prednisolone (30 mg/day) decreased the amount of ascites somewhat for the time being. Moreover, two courses of steroid pulse therapy seemed to inhibit increases in the amount of ascites, rendering abdominocentesis unnecessary. The estimated systolic right ventricular pressure also normalized after treatment, although the causal linkage was not clear. On the other hand, a second abdominal CT scan repeated after approximately six months revealed almost no changes in the soft tissue abnormality around the kidneys.

Recently, treatment with IFN-α has been shown to improve the survival of ECD patients (3). Unfortunately, high fever, appetite loss and renal function deterioration, most likely due to dehydration, did not allow us to continue to administer IFN-α to our patient. The mechanisms by which IFN-α exerts therapeutic effects in this disease have not yet been clarified; however, i) maturation of abnormal cells, ii) immune-mediated destruction of cells and iii) anti-proliferative effects of IFN-α have been suggested. We added recombinant IFN-α (10 ng/mL) to the cells derived from the ascites in vitro and attempted to analyze the cells with flow cytometry 15 hours later. To our surprise, the cells in gate A (Fig. 3) significantly decreased in number and most of the remaining cells were found to be double-negative for CD11c and DEC-205. This may indicate that IFN-α specifically induces death in abnormal cells. We were unable to confirm whether the observed cell death was due to apoptosis because we were unable to obtain fresh ascites after two courses of steroid pulse therapy.

To the best of our knowledge, this is the first report of ECD in which abnormal cells were analyzed using both flow cytometry and detailed immunocytochemistry. The cells derived from the ascites were safely obtained and proved to be quite useful for confirming the diagnosis. Moreover, multi-staining procedures and the analysis of the expression of various molecules in abnormal cells may also be helpful for elucidating the pathogenesis of this rare disease.
The authors state that they have no Conflict of Interest (COI).

References


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