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A Rare Case of Adult Autoimmune Neutropenia Successfully Treated with Prednisolone

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Abstract

Autoimmune neutropenia (AIN) is a rare disorder that may cause life-threatening infections. In adults, most cases are secondary to other pathological conditions, and primary AIN is extremely rare. We herein report a case involving a 57-year-old woman diagnosed with AIN. A granulocyte immunofluorescence test detected autoantibodies against human neutrophil antigens in her serum, while various examinations revealed no other causes of neutropenia, suggesting her AIN was primary. She was refractory to granulocyte-colony-stimulating factor but responded to prednisolone. Her neutrophil count remained normal after gradual discontinuation of prednisolone. Diagnostic procedures and optimal treatments for this disorder need to be established.

Key words: human neutrophil antigen, autoimmune neutropenia, granulocyte-colony-stimulating factor


Introduction

Autoimmune neutropenia (AIN) is a rare hematological disorder characterized by the autoantibody-induced destruction of neutrophils. The primary mechanism for this is opsonization, which accelerates the phagocytic clearance of neutrophils. Additionally, anti-neutrophil antibodies affect the functions of target proteins, causing impairment of the neutrophil functions (1). Such sustained severe neutropenia and impairment of the neutrophil functions resulting from AIN can cause life-threatening severe infections.

AIN is classified into two categories: primary and secondary. Primary AIN is relatively frequent in infants and children and is mostly a benign condition with a self-limited course (2), while secondary AIN is relatively frequent among adults and is associated with various pathological conditions, such as infectious diseases, autoimmune diseases, hematological malignancies, transplantation, and drug allergies (3-5). Primary AIN among adults is extremely rare, and only a few cases have been reported to date. In addition, although the efficacy varies by case, granulocyte-colony-stimulating factor (G-CSF) has been reported to be effective for both primary and secondary AIN (6, 7).

However, we herein report an adult case of AIN that developed without any apparent cause and did not respond to G-CSF; the case was successfully treated with low-dose prednisolone.

Case Report

A previously healthy 57-year-old woman visited a local hospital for a cough and nasal discharge persisting for 3 months. Blood tests revealed severe leukopenia (700/μL), and a computed tomography (CT) scan revealed pneumonia in the right lung and mild-to-moderate splenomegaly (Fig. 1a and b). She was therefore suspected of having a hematological disorder and was referred to our hospital.

Upon the initial visit to our hospital, she was afebrile, and her vital signs were unremarkable. No skin lesions were observed. She had a history of pregnancy, and she had no family history of hematological or autoimmune diseases. She

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had taken no medication for at least three months. Her white blood cell count was 710/μL (neutrophils: 11%; lymphocytes: 57%), red blood cell count was 4.23x10^6/μL, hemoglobin was 12.2 g/dL, hematocrit was 36.3%, mean corpuscular volume was 85.8 fl, reticulocyte count was 85x10^3/μL, and platelet count was 141x10^3/μL. Her findings for serum biochemical tests were all normal (Supplementary Table 1). C-reactive protein was weakly positive (0.5 mg/dL), and soluble interleukin-2 receptor (sIL-2R) was elevated (1,880 U/mL). Serological tests excluded infection with hepatitis B and C viruses, HIV, Mycoplasma pneumoniae, Chlamydia pneumoniae, and Mycobacterium tuberculosis, and tests for Epstein-Barr virus indicated a previous but not recent infection (supplementary Table 1). Fluorodeoxyglucose (FDG) positron emission tomography-CT revealed diffuse and mild FDG uptake in the spleen and the bone marrow of the trunk and proximal extremities. A bone marrow biopsy revealed a slightly hypercellular marrow with mild reticulin fibrosis (Fig. 2a). Bone marrow smears revealed a decrease in mature segmented neutrophils (0.4%) with a relative increase in myelocytes without an increase in blasts. An abdominal CT scan 31 days after starting prednisolone showed improvement of the splenomegaly (c).

Table 1. The Results of the Indirect Granulocyte Immunofluorescence Test (GIFT).

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Before treatment</th>
<th>28 days after starting prednisolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1a/a</td>
<td>3.65</td>
<td>0.78</td>
</tr>
<tr>
<td>HNA-1a/b</td>
<td>3.77</td>
<td>1.21</td>
</tr>
<tr>
<td>HNA-1b/b</td>
<td>8.05</td>
<td>1.46</td>
</tr>
</tbody>
</table>

The indirect GIFT was performed as previously described by Kobayashi, et al. with some modifications (9). Patient sera was incubated with newly isolated blood cells from healthy donors possessing HNA-1a/a, HNA-1a/b, or HNA 1b/b alleles. After washes with phosphate-buffered saline, the cells were incubated with anti-human immunoglobulin antibodies conjugated with FITC, and the cells were analyzed by flow-cytometry. Mean fluorescence intensities (MFI) of the neutrophil fraction gated by forward and side scatter profiles were measured. Reactivity against HNA-1a/a, HNA-1a/b, and HNA-1b/b was assessed using relative fluorescence intensity (RFI), i.e., the ratio of MFI with patient sera to that obtained with control sera. An RFI>2 is considered to be positive. A significant elevation of RFI for these neutrophil fractions was observed before treatment. We also assessed the RFI of monocyte and lymphocyte fractions and found no increase of it, indicating that the antibodies that we detected in her sera were not against human leukocyte antigen or non-specific cell surface antigens.

To screen for autoimmune diseases, a series of autoantibodies were measured. The results indicated that anti-single stranded DNA antibody was positive (209 AU/mL), rheumatoid factor was weakly positive (11.7 IU/mL), and antinuclear antibody was weakly positive, with a titer of 1:40. Other autoantibodies, including anti-double stranded DNA, anti-ribonucleoprotein (RNP), and anti-SS/A antibodies were negative. The indirect granulocyte immunofluorescence test (GIFT) detected antibodies that reacted with both human neutrophil antigen (HNA)-1a and HNA-1b in her serum (Table 1) (8-10). HNA typing by the GIFT revealed that the patient had HNA-1a/1b alleles, indicating that the antibodies detected in her serum were autoantibodies. Because her symptoms and signs did not meet the diagnostic criteria for common autoimmune disorders, such as rheumatoid arthritis, Sjögren’s syndrome, or systemic lupus erythematosus (11-13), the patient was diagnosed with primary AIN.

Oral levofloxacin and itraconazole were administered for pneumonia, and, because spontaneous recovery of her neutrophil count was expected, she was followed as an outpatient. However, 5 weeks later, severe neutropenia persisted, and we admitted the patient to our hospital and began daily subcutaneous injections of G-CSF at a dose of 5 μg/kg. She developed a high-grade fever on the day of admission, and oral levofloxacin was switched to intravenous tazobactam/piperacillin. Her body temperature normalized on post-

Figure 1. Computed tomography (CT) scans. CT scans taken during the initial visit to a local hospital showed mild pneumonia in the right upper lobe (a) and mild-to-moderate splenomegaly (b). An abdominal CT scan 31 days after starting prednisolone showed improvement of the splenomegaly (c).
admission day 3. However, her neutrophil count, which was
130/μL on admission, did not respond to G-CSF therapy
to day 8, so we discontinued G-CSF therapy and
started oral prednisolone at a dose of 0.5 mg/kg/day
(Fig. 3). Her neutrophil count began to increase immediately
(Fig. 3). Chest X-ray taken 15 days after the initiation of
prednisone showed that the pneumonia had improved.
Twenty-eight days after starting prednisolone, autoantibodies
against HNA-1a and HNA-1b had disappeared from her se-
rum (Table 1). Abdominal CT revealed improvement in her
splenomegaly (Fig. 1c), and she was discharged four days
later. The prednisolone dosage was gradually tapered and
was discontinued 10 months after its initiation (Fig. 3). Her
neutrophil count remained normal at her last visit (two
months after discontinuation of prednisolone).

Discussion

Similar to immune thrombocytopenic purpura and autoim-
mune hemolytic anemia, AIN is a disease entity with
autoantibody-induced cytopenia. Anti-neutrophil antibodies,
mostly the IgG isotype, can generally be detected in the se-
rum of patients with AIN. Neutrophil-specific alloantigens
are divided into five groups: HNA-1, HNA-2, HNA-3,
HNA-4, and HNA-5. HNA-1 is located on the Fcγ receptor
IIIb and is polymorphic (HNA-1a, HNA-1b, and HNA-1c).
In many AIN cases, the target antigen is HNA-1a or HNA-
1b (14). A substantial proportion of healthy parous women
have anti-neutrophil antibodies (10, 15), so the detection of
such antibodies does not necessarily imply pathogenicity.
In this case, however, sera from our patient obtained before the
treatment contained antibodies that reacted with both HNA-
1a-positive neutrophils and HNA-1b-positive ones, and these
reactivities completely disappeared after the recovery of the
neutrophil count, implicating these antibodies in the patho-
genesis of our case. The high relative fluorescence intensity
for HNA-1b/1b neutrophils suggested that her autoantibody
had high specificity for HNA-1b. HNA1 is expressed in ma-
ture neutrophils from the stages of metamyelocytes to seg-
mented neutrophils, and its expression is strongest in seg-
mented neutrophils (16); this may explain the relative in-
crease in the numbers of myelocytes and the disappearance
of segmented neutrophils in her bone marrow.

We diagnosed the present patient’s neutropenia as primary
AIN based on various examinations and her clinical course.
Infections, drugs, autoimmune diseases, and lymphoid ma-
lignancies are common courses of AIN in adults (17). She
had chronic symptoms of upper respiratory tract inflamma-
tion, which suggested viral infections, although we were un-
able to identify any pathogens. These symptoms may have
been due to a respiratory bacterial infection caused by neu-
ropenia, as the intravenous administration of antibiotics re-
solved these symptoms before the initiation of prednisolone
(Fig. 3). She was not taking any medications, so drug-
induced neutropenia was ruled out. Her signs and symptoms
did not meet the criteria for common systemic autoimmune
diseases, such as systemic lupus erythematosus and rheuma-
toid arthritis that can cause secondary AIN. At the final
visit, she was apparently healthy without any signs of sys-
temic autoimmune diseases, but careful follow-up will be
necessary to guard against the possible development of hid-
den underlying diseases. Other possible causes of her neu-
ropenia included lymphoid malignancies, such as large
granular lymphocyte (LGL) leukemia and splenic marginal
zone B-cell lymphoma, indicated by the elevation of the
sIL-2R level and uptake of FDG in the spleen. However, the
lack of LGL or lymphocytosis in her peripheral blood and
in the bone marrow, the lack of M-protein, her clinical
course, as well as the complete disease response to low-dose
steroid (Fig. 3) suggested that the presence of lymphoid
neoplasms was unlikely (although we did not perform a his-
tological examination of her spleen to verify this fact).
In addition, the splenomegaly improved with the recovery of
the neutrophil count (Fig. 1c), suggesting that phagocytosis
of opsonized neutrophils in the spleen was a main patho-
logical process in this case.

Patients with primary AIN are usually affected with mild
to moderate bacterial infections of the skin, otitis media, or
upper respiratory tract (3). A fever of unknown origin occurs
in about 20% of cases, and severe infections such as pneu-
Effective, and the effect was observed within a few days (7). In AIN cases, the administration of G-CSF (5 μg/kg daily) was found only four case reports (Table 2) (6, 18-20). In most cases, the administration of G-CSF (5 μg/kg daily) was effective, and the effect was observed within a few days (7).

The results of these studies as well as the current clinical course indicate that measuring anti-HNA antibodies is important for a prompt diagnosis of AIN, although a certain proportion of parous women possess non-pathologic anti-HNA antibodies, and there are some monia, meningitis, or sepsis occur in 15-20% of cases (3). In such cases, prompt treatment to increase the neutrophil count is crucial. However, in our case, the neutrophil count did not respond to G-CSF therapy, and, because of an infectious complication, we initially hesitated to start immunosuppressive therapies. We therefore first brought her fever under control with antibiotics and then started prednisolone. With the steroid therapy, her neutrophil count normalized, and HNA antibodies disappeared, and her spleen size decreased. After tapering and discontinuing prednisolone, her neutropenia did not recur (Fig. 3), supporting the diagnosis of primary AIN.

We also conducted a literature review to examine the characteristics of primary AIN in adults. A PubMed search found only four case reports (Table 2) (6, 18-20). In most AIN cases, the administration of G-CSF (5 μg/kg daily) was effective, and the effect was observed within a few days (7). Indeed, in all four cases of adult AIN mentioned above, the initial treatment consisted of G-CSF, and this treatment was effective in three. However, one case did not respond to G-CSF therapy (as in our case), and so the patient was treated successfully with a combination of prednisolone and cyclosporine A. In addition, three of the four reported cases (including the one that did not respond to G-CSF) were treated with steroids after the initial G-CSF treatment. Therefore, the results of these studies as well as the current case show that steroids and immunosuppressants seem to be effective in treating AIN, although their efficacy varies case by case (Table 2 and Fig. 3).

The results of this case as well as the information from the literature review indicate that measuring anti-HNA antibodies with GIFT is important for a prompt diagnosis of AIN, although a certain proportion of parous women possess non-pathologic anti-HNA antibodies, and there are some

![Figure 3](image_url) Clinical course. The treatments and white blood cell counts (per μL) are shown. The left and right panels show the clinical course of the first 3 weeks and 11 months after the initiation of prednisolone, respectively. TAZ/PIPC: tazobactam/piperacillin, G-CSF: granulocyte-colony-stimulating factor, PSL: prednisolone, WBC: white blood cell count, ANC: absolute neutrophil count

### Table 2. Clinical Findings from Reported Cases of Adult Primary Autoimmune Neutropenia.

<table>
<thead>
<tr>
<th>Age/sex (Ref)</th>
<th>Autoantibody</th>
<th>Splenomegaly</th>
<th>Bone marrow examination</th>
<th>Response to G-CSF</th>
<th>Response to other treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>22/M (19)</td>
<td>Anti-HNA-1a</td>
<td>Moderate</td>
<td>Normocellular; reduced granulocyte precursors</td>
<td>Did not respond</td>
<td>Responded to CyA (5-12 mg/kg/day) in combination with PSL (2 mg/kg/day)</td>
</tr>
<tr>
<td>75/F (6)</td>
<td>No HNA specificity</td>
<td>None</td>
<td>Normocellular; severe decrease of mature neutrophils</td>
<td>Responded</td>
<td>Responded to PSL (40 mg/day)</td>
</tr>
<tr>
<td>90/F (20)</td>
<td>Not described</td>
<td>Not described</td>
<td>Not described</td>
<td>Transiently responded</td>
<td>Responded to PSL (1 mg/kg/day)</td>
</tr>
<tr>
<td>69/M (18)</td>
<td>Anti-HNA-1a</td>
<td>None</td>
<td>Increased number of promyelocytes</td>
<td>Transiently responded</td>
<td>Transiently responded to IVIG</td>
</tr>
<tr>
<td>57/F (Current case)</td>
<td>Anti-HNA-1a and HNA-1b</td>
<td>Moderate</td>
<td>Slightly hypercellular; severe decrease of mature neutrophils</td>
<td>Did not respond</td>
<td>Responded to PSL (30 mg/day)</td>
</tr>
</tbody>
</table>

false positive results for this test. However, because this test can be performed only in select institutions, a considerable number of adult AIN cases may be overlooked. Our findings also suggest that G-CSF and prednisolone are therapeutic options that can induce durable disease remission. To establish the optimal treatment strategy for AIN in adults, further research is needed.

The authors state that they have no Conflict of Interest (COI).

References


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