INTERNAL MEDICINE

□ CASE REPORT □

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

(Intern Med 56: 1415-1419, 2017)
(DOI: 10.2169/internalmedicine.56.7619)

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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Received for publication April 25, 2016; Accepted for publication October 3, 2016
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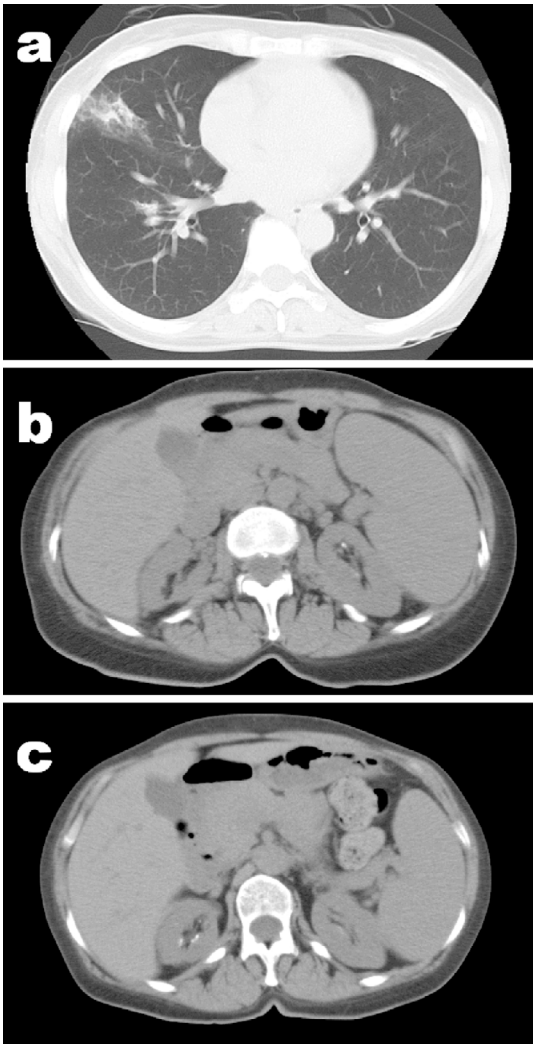

Figure 1. Computed tomography (CT) scans. CT scans taken during the initial visit to a local hospital showed mild pneumonia in the right upper lobe (a) and mild-to-moderate splenomegaly (b). An abdominal CT scan 31 days after starting prednisolone showed improvement of the splenomegaly (c).

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 4.23×10^6/μL, hemoglobin was 12.2 g/dL, hematocrit was 36.3%, mean corpuscular volume was 85.8 fL, reticulocyte count was 85×10^3/μL, and platelet count was 141×10^3/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

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

Antigens	Before treatment	28 days after starting prednisolone
HNA-1a/a	3.65	0.78
HNA-1a/b	3.77	1.21
HNA-1b/b	8.05	1.46

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.

decrease in mature segmented neutrophils (0.4%) with a relative increase in myelocytes without an increase in blastic cells (Fig. 2b, Supplementary Table 2). Phenotypically abnormal lymphoid populations were not detected by flow cytometry. Morphological dysplasia was not detected in any of the hematological cell lineages. The myeloid:erythroid ratio was 2.27. Flow cytometric analyses detected no abnormal cell populations in the bone marrow cells, and a chromosomal analysis showed a normal karyotype.

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-

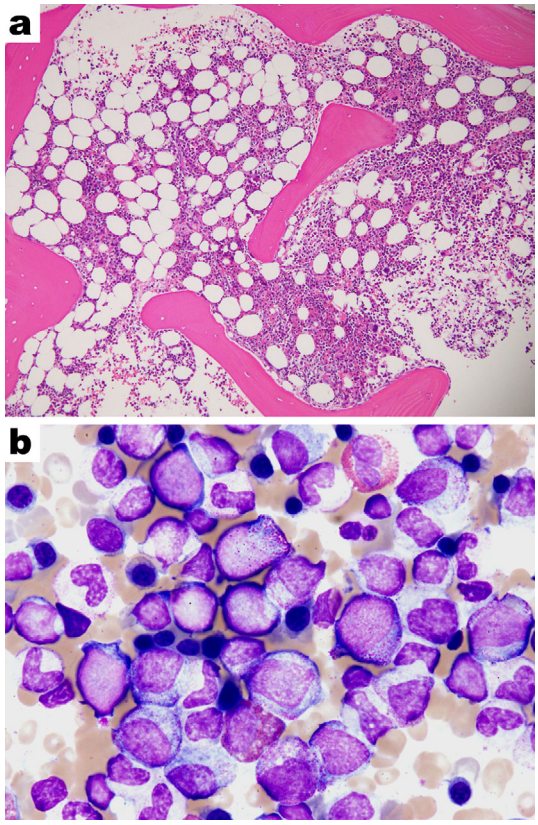

Figure 2. A bone marrow biopsy (a) and smear (b). A bone marrow biopsy revealed a slightly hypercellular marrow (a). Bone marrow smears revealed a decrease in the numbers of segmented neutrophils without morphological dysplasia in the hematological cell lineages (b). Original magnifications: (a)×40, (b)×1,000.

admission day 3. However, her neutrophil count, which was 130/μL on admission, did not respond to G-CSF therapy through 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 serum (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 autoimmune hemolytic anemia, AIN is a disease entity with autoantibody-induced cytopenia. Anti-neutrophil antibodies, mostly the IgG isotype, can generally be detected in the serum 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 pathogenesis 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 mature neutrophils from the stages of metamyelocytes to segmented neutrophils, and its expression is strongest in segmented neutrophils (16); this may explain the relative increase 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 malignancies are common courses of AIN in adults (17). She had chronic symptoms of upper respiratory tract inflammation, which suggested viral infections, although we were unable to identify any pathogens. These symptoms may have been due to a respiratory bacterial infection caused by neutropenia, as the intravenous administration of antibiotics resolved 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 rheumatoid arthritis that can cause secondary AIN. At the final visit, she was apparently healthy without any signs of systemic autoimmune diseases, but careful follow-up will be necessary to guard against the possible development of hidden underlying diseases. Other possible causes of her neutropenia 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 histological 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 pathological 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-

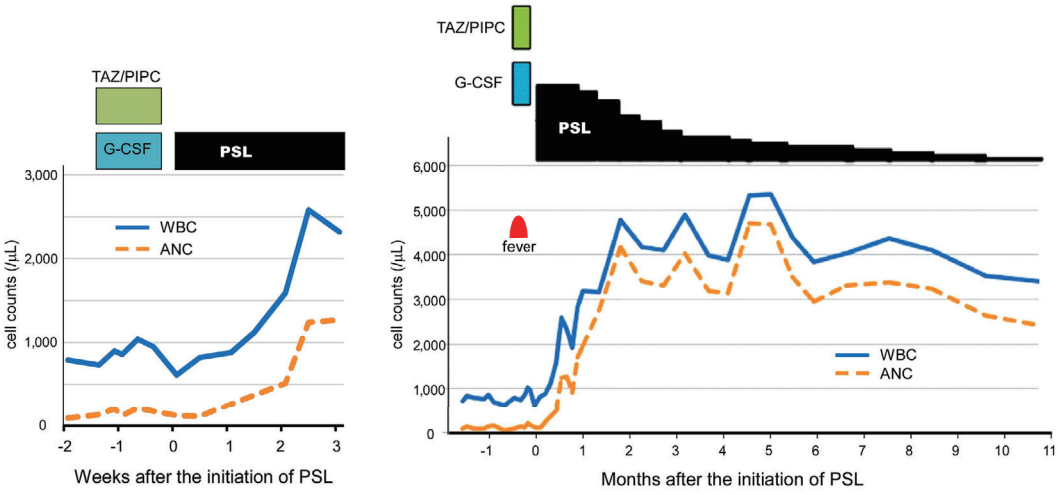

Figure 3. **Clinical course. The treatments and white blood cell counts (per μL) are shown. The left and right panels show the clinical course of the first 3 weeks and 11 months after the initiation of prednisolone, respectively. TAZ/PIPC: tazobactam/piperacillin, G-CSF: granulocyte-colony-stimulating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

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

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

Ref: reference, HNA: human neutrophil antigen, G-CSF: granulocyte-colony stimulating factor, CyA: cyclosporine A, PSL: prednisolone, IVIG: intravenous immunoglobulin

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, the anti-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

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).

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