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LETTER TO THE EDITORS

Neutropenia associated with antihuman neutrophil antibodies following allogeneic hematopoietic stem cell transplantation

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Dear Sirs,

Although detection of antibodies reactive with neutrophils (ARN) following hematopoietic stem cell transplantation (HSCT) is not uncommon [1,2], neutropenia because of antihuman neutrophil antigen (HNA) antibody is rare, and very few cases have been reported to be associated with anti-HNA-2 antibody [3,4]. We herein present a case of anti-HNA antibody-associated neutropenia following allogeneic HSCT. By monitoring representative anti-HNA antibodies, the patient showed a variety of anti-HNA antibodies; we speculated that the anti-HNA-2 antibody played a crucial role in the development of neutropenia.

A 58-year-old man with chronic myeloid leukemia carrying T315I mutation received a bone marrow transplantation from an unrelated, human leukocyte antigen identical donor on March 3, 2011. The initial post-transplant clinical course was unremarkable except for mild acute graft-versus-host disease of the gut, which was improved by low-dose (0.5 mg/kg) prednisolone (PSL). Neutrophil engraftment was confirmed on day 15 after HSCT. However, the neutrophil counts gradually decreased on day 240 and finally fell below 500/μl on day 285. Then, the patient was admitted to our hospital because of acute pharyngitis on day 303. At that time, the white blood cell count was 1800/μl with 130/μl of neutrophils, hemoglobin 8.3 g/dl, and platelets 7.3×10^4/μl. The pharyngitis was subsided with tazobactam/piperacillin (TAZ/PIPC); however, the neutrophil counts remained below 100/μl despite the administration of granulocyte colony-stimulating factor (G-CSF). Bone marrow aspiration performed on day 309 showed normocellular marrow with marked decrease in metamyelocytes (2.0%) and mature granulocytes (3.8%). Bcr/abl chimeric transcripts were not detected. G-CSF was stopped on day 322 because of no response. With a suspicion of immune-mediated neutropenia, the patent received a screening test for both anti-G-CSF and anti-HNA antibody. Although anti-G-CSF antibody was not detected, anti-HNA antibody tests were positive for anti-HNA-2 and -5a/b (Table 1). Therefore, the patient was diagnosed as immune-mediated neutropenia and received 1 mg/kg of PSL after day 336. As early as the next day after starting PSL, the neutrophil counts increased to 525/μl and returned to normal range in a week and then discharged on day 346. However, 1 month later, although the patient was put on 1 mg/kg of PSL, the neutrophil counts again abruptly decreased to 310/μl. The further dose escalation of PSL was considered to be less effective; instead, PSL was tapered. On day 406, the patient developed urinary tract infection by *Enterococcus faecalis*, which was successfully treated with TAZ/PIPC and vancomycin. During this clinical event, the neutrophil counts were gradually recovered and eventually returned to normal range without any specific interventions. Anti-HNA antibodies tests performed on day 636 were negative for HNA-2, but positive for HNA-4a/b, -5a/b (Table 1).

Our patient had no obvious infection, which might induce the neutropenia, and neutropenia progressed despite the discontinuation of possible drugs that might cause cytopenia. Moreover, anti-HNA-2-antibody level well-traced the clinical course. Based on these findings, the patient was eventually diagnosed as immune-mediated neutropenia, and we assumed that anti-HNA-2-antibody might play a pivotal role. HNA-2 was highly expressed on metamyelocyte and mature neutrophil in both adult and fetal marrow cells, whereas the high expression of HNA-2 on myelocyte was observed only in adult marrow cells [5]. This implies that myelocytes at 10 months after HSCT in our patient might have expressed HNA-2 at a low level and been slightly affected. We performed extracted granulocyte immunofluorescence assay for assessment of anti-HNA antibodies, which was modified from monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA) and had superior sensitivity and specificity to granulocyte indirect immunofluorescence test (GIFT) [6]. Briefly, HNAs were extracted from gene-transfected cell

Table 1. Anti-neutrophil antibodies.

Antigen	HNA-1a			HNA-1b			HNA-1c		HNA-2			HNA-4a		HNA-4b		HNA-5a		HNA-5b	
MoAb	3G8	TAG3	LNK16	3G8	TAG3	LNK16	3G8	LNK16	TAG4	7D8	MEM166	CD11b	BD CD11b	CD11b	BD CD11b	CD11b	BD CD11b	CD11b	BD CD11b
Index*																			
January 2012	0.8	1.3	0.5	0.7	1.2	1.1	1.0	1.4	1.2	2.8	0.7	1.0	1.6	1.1	0.8	2.1	2.9	2.3	22.4
December 2012	1.8	1.6	0.7	1.4	1.0	0.8	1.4	0.7	1.2	0.9	1.3	5.8	5.5	1.1	2.8	4.3	1.7	3.8	11.0

MoAb, monoclonal antibody; HNA, human neutrophil antigen.
*Index was calculated as the ratio of fluorescence intensity of MoAb to that of control IgG. Index >2 considered positive.

lines (extracted HNAs: eHNAs) [7,8] and were bound to microsphere-binding specific monoclonal antibody (MoAb) for generating eHNA/MoAb/microsphere complexes (eHNA-microspheres). The eHNA-microspheres were incubated with patient's serum for 30 min at room temperature. After washing, antibody/eHNA-microsphere complexes were incubated with PE-conjugated goat antihuman IgG $F(ab')_2$ antibodies for 30 min at room temperature. After washing, fluorescence intensity (FI) was calculated using Luminex 100™ (Luminex Corporation, Austin, TX, USA). Relative FI (RFI) was calculated as the ratio of FI of eHNA-microspheres to that of control IgG1-microspheres and The RFI >2 considered positive. We also use two or three antibodies recognizing different epitopes of each HNA because of avoiding false-negative results by competing reaction, and this seemed to be useful for the detection of anti-HNA-2 antibody in our patient (Table 1).

We used the same MoAbs for the detection of HNA-4a and HNA-4b (HNA-5a and HNA-5b) (Table 1), which indicates that anti-HNA-4a/b (or -5a/b) antibody observed in our patient recognized common epitope. Although a possible influence of antibody against CD 18, which forms a complex with CD 11a and 11b, cannot be entirely denied in our case in December 2012, we consider that our patient developed autoantibody against both allelic variants in HNA-5a/b in January 2012. Furthermore, our patient maintained remission at the onset of neutropenia. Based on these results, we consider that the neutropenia was caused by an autoimmune reaction.

Although ARN could be frequently observed after HSCT, their real contribution on the neutrophils remains controversial. In one report, ARN was associated with delayed neutrophil engraftment and post-engraftment neutropenia [2]. Conversely, in another report, ARN had little impact on neutrophil engraftment [1]. Our patient showed various antibodies other than the anti-HNA-2 antibody, and these antibodies seemed to be irrelevant to the development of neutropenia. Thus, the appearance of these nonpathogenic antibodies may have affected the results in these previous reports. Our patient did not respond to G-CSF, while immune neutropenia in infancy well-responded to G-CSF [9]. As G-CSF promoted the expression of HNA-2, it seemed to be ineffective in some patients with anti-HNA-2 antibody. Thus, these results indicate that administration of G-CSF could negatively affect the neutrophil counts in some immune neutropenic patients.

In summary, our patient developed autoimmune neutropenia by anti-HNA-2 antibody following allo-HSCT and showed various anti-HNA antibodies other than anti-HNA-2 antibody. Thus, encompassing, sequential anti-HNA antibody measurements are necessary for the identification of pathogenic antibody and for the development of a treatment strategy.

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Conflict of interest

The authors declare no competing financial interests.

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