Significance of the detection of antineutrophil antibodies in children with chronic neutropenia

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We evaluated the clinical characteristics of 41 children with chronic neutropenia based on the quantitative analysis of antineutrophil antibodies in serum by flow cytometry. According to the strength of antineutrophil antibodies, the patients were divided into 3 groups: 12 patients presented negative antibodies, 13 patients showed weak positive antibodies, and 16 patients showed strong positive antibodies. No significant differences were seen in age of diagnosis, severity of neutropenia, and infectious complications associated with neutropenia among the 3 groups. The spontaneous resolution of neutropenia was observed in all patients with negative antibodies and in 22 of 29 patients with positive antibodies. The age of the recovery of neutropenia and the duration until spontaneous resolution of neutropenia were significantly dependent on the antibody strength at the time of diagnosis. These results demonstrate that the quantification of antineutrophil antibodies at the time of diagnosis may be useful in considering the clinical course of chronic neutropenia in childhood.

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Introduction

Chronic neutropenia is defined as low absolute neutrophil count (ANC) of less than 1500/μL blood, lasting for at least 6 months.1-3 The pathophysiology of chronic neutropenia in childhood remains unclear because of the heterogeneity of this disorder. Chronic benign neutropenia (CBN) and primary autoimmune neutropenia (AIN) are common forms of neutropenia in infants and early childhood.1-11 Both types of neutropemia have shown similar hematologic findings along with their clinical features. The definitive distinction is the presence or absence of circulating antineutrophil antibodies in serum. Most of these cases actually are considered antineutrophil antibody–mediated neutropenia, because technical difficulties exist in the detection of antineutrophil antibodies.3,4,7 Several methods are used in the detection of serum antineutrophil antibodies.5-8,12-17,18 However, the specificity and sensitivity of these methods have not been well established. In this study, we have developed a method to semiquantify the strength of antineutrophil antibodies by flow cytometric analysis of the granulocyte indirect immunofluorescence test (GIIFT) by using same donor neutrophils. The results demonstrate the efficacy of the quantification of antineutrophil antibodies at the time of diagnosis and its relevance to the duration of neutropenia.

Study design

Patients

All patients or their sera were referred to our hospital for examining antineutrophil antibodies from June 1995 to October 1999. Forty-one patients were included in this study when their ANC was less than 1500/μL blood for more than 6 months without underlying diseases, a history of drug administration causing neutropenia, or a history of blood transfusions. Approval was obtained from the institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Preparation of monoclonal antibodies to neutrophil-specific antigens and the typing of neutrophils

Human neutrophils were prepared by previously described method.9 Monoclonal antibodies, TAG1, TAG2, and TAG4 that recognize neutrophil-specific antigens, HNA-1 and HNA-2, were originally established. The characteristics of TAG1 and TAG2 were identified by comparing the binding properties of MG38 (Nichirei, Tokyo, Japan), GRM1 (a kind gift from Dr Garrido, Granada University, Granada, Spain), and 3G8 (Pharmingen, San Diego, CA).18,19 The binding characteristic of TAG4 was almost identical to that of 7D8 (a generous gift from Dr Stroncek, National Institutes of Health, Bethesda, MD).20,21,22 The genotypes of HNA-1 antigens were determined by using a modification of polymerase chain reaction methods previously reported.23,24

GIIFT and micro-leukocyte agglutination test

GIIFT and micro-leukocyte agglutination test (MLAT) were performed by using a modification of the previously described method.5 In GIIFT, fluorescence isothiocyanate–labeled Fab′(x)2 fragments of goat antihuman polyclonal immunoglobulin, immunoglobulin (Ig)G, IgM, and IgA (Tago, Burlingame, CA) were used as the second antibody. The cell suspension was examined by using flow cytometry (FACS Vantage; Becton Dickinson).
Immunocytometry Systems, San Jose, CA) with 4-W argon ion laser and/or ORTHO CYTRON ABSOLUTE (Ortho-Clinical Diagnostics K.K., Tokyo, Japan) by using a 15-mW argon ion laser.

To avoid variability in the flow cytometric analysis, the same pooled sera from AB-type healthy male subjects and the neutrophils prepared from same donors were used throughout this study. Neutrophils from a FcγRIIIb-deficient donor were used as a control, if necessary. To confirm the specificity of antibodies against target antigens, monoclonal antibody-specific immobilization of granulocyte antigens was performed according to the previously reported method.25 Lymphocyte cytotoxicity test was also performed to exclude the antibodies associated with HLA antigens.

Results and discussion

Monoclonal antibodies established by our laboratory, TAG1 or TAG2, reacted with HNA-1a– or HNA-1b–homozygous
neutrophils, respectively (Figure 1A). The bindings of monoclonal antibodies, TAG1 or TAG2, to the same HNA-1a– or HNA-1b– homozygous donor neutrophils gave rise to almost consistent fluorescence intensity by setting the mean fluorescence channel of the control sample from 50 to 100. The ratio of the mean fluorescence channel of each sample to that of control serum was expressed as relative fluorescence intensity (RFI) to quantify the strength of the antibodies. The bindings of antibodies to neutrophils and the RFI of each antibody depended on the concentrations of antibodies (Figure 1B). The representative flow cytometric findings of strongly and weakly positive antibodies to 2 different HNA-1a–homozygous donors are shown in Figure 1C. The data describing the RFI value for each sample were expressed as the mean of the results against 2 or 3 different donors probably possessing the target antigens of the antibody. As shown in Figure 1D, patients were classified into 3 groups based on the RFI value of antineutrophil antibody test in serum at the time of the diagnosis. Eighty-five percent of patients with positive antibodies showed IgG, and the remaining showed IgM antibodies. The distribution of immunoglobulin class did not differ between group B and group C (data not shown). No patients with negative antibodies by flow cytometry showed a positive MLAT in this study.

The clinical characteristics of patients in the 3 groups are presented in Figure 2A. There were no significant differences in the age of patients, the severity of leukopenia and neutropenia, and the frequency of infectious complications during the neutropenic period among 3 groups as determined by chi-squared test and Kruskal-Wallis test. These observations suggest that the clinical findings are similar in presentation among patients, irrespective of the results of antineutrophil antibody test. Most children with chronic neutropenia have a benign clinical course because neutropenia often spontaneously resolves. It has been reported that some antibodies such as anti-HNA-3a are better detected by agglutination than by GiIFT. Therefore, the combination of semiquantified GiIFT and agglutination test for detecting antineutrophil antibodies provide information for predicting the clinical course of children with chronic neutropenia. Further, a large prospective study is necessary to demonstrate that measurement of autoantibodies at the time of diagnosis will aid in observations of the natural history of this disorder.

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