

Nonspecific Response of Enzyme Immunoassay System to Myeloperoxidase Antigen Released From Blast Cells in Patients With Nonlymphocytic Leukemia

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Received August 31, 2000; accepted September 6, 2000

ABSTRACT

A substance exhibiting a nonspecific response in the ELSIA-F750 (International Reagent, Kobe, Japan) automatic immunoassay apparatus was found in patients with nonlymphocytic leukemia (NLL), but not in patients with acute lymphocytic leukemia. This substance exhibited both myeloperoxidase (MPO) antigenicity and peroxidase (POD) activity. Of the human myelocytic leukemia cell lines K562, KG-1, HL-60, Mo7E, and U937, it was detected only in the culture supernatant of HL-60 cells. The MPO antigen level correlated with POD activity ($r = 0.533$, $P < .01$) in the NLL group, but not in the infection group ($r = 0.284$). The number of blasts significantly correlated with POD activity in the NLL group ($r = 0.771$, $P < .01$). No substance exhibited nonspecific responses in normal neutrophils stimulated by phorbol myristate acetate and ionomycin. These results suggest that the substance yielding a nonspecific response may be specific to the NLL group. *Lab Hematol.* 2000;6:121-125.

KEY WORDS: Nonlymphocytic leukemia
Myeloperoxidase
Peroxidase activity

INTRODUCTION

Methods of measurement using antigen-antibody reactions have been widely used in attempts to measure biological substances in minute quantities with high sensitivity, and they are often employed in special tools using chemical emission and fluorescent substances. However, false-positive results due to nonspecific responses have been reported in antigen-antibody reactions [1,2]. The ELSIA-F750 automatic immunoassay apparatus (F750) (International Reagent, Kobe, Japan) based on enzyme immunoassay has been used routinely in our laboratory. Of 9142 serum samples in 8 months, 3 false-positive measurements of sickle cell hemoglobin antigen (HbS Ag) were found. These 3 samples were all from patients with untreated acute myelocytic leukemia (AML). The causal substance in the blood had myeloperoxidase (MPO) antigenicity, because it bound to the solid phase in the primary reaction and possessed peroxidase (POD) activity [3,4]. In the present study, we measured the POD activity of this nonspecific substance and the blood MPO antigen level in a nonlymphocytic leukemia (NLL) group and an infection group to determine whether the substance detected was specific to the NLL group.

SUBJECTS AND METHODS

Subjects

A total of 41 patients with untreated leukemia were studied. The diagnosis was based on cell morphology and genetic markers. The French-American-British (FAB) classification subtypes in the AML group were M0 (n = 2), M1 (2), M2 (10), M3 (2), M4 (1), M5 (2), and M6 (2). Eight cases of

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TABLE 1. Changes in Peroxidase (POD) Activity and Myeloperoxidase (MPO) Antigen in the HL-60 Culture Supernatants

HL-60, d	POD Activity, RFI*	MPO Antigen, ng/mL
0	5.8	1.2
1	25.9	265.9
2	36.9	270.2
3	53.1	232.9

*RFI indicates relative fluorescence index.

Isolation of Neutrophils and Method of Stimulation

Twenty milliliters of heparinized blood was mixed with 15 mL isotonic sodium chloride solution with 1% dextran and left to stand for 1 hour. The supernatant (7 mL) was overlaid on 3 mL Ficoll-Paque solution (Pharmacia, Uppsala, Sweden) and centrifuged for 30 minutes at 1500 rpm. After the supernatant was eliminated, the neutrophil and erythrocyte layers were washed with 30 mL phosphate-buffered saline and hemolyzed. After washing, neutrophils were adjusted to 10⁶/mL with Hank's solution (Wako Pure Chemical, Osaka, Japan). To 1 mL of the adjusted neutrophil suspension, 1 μL each of dimethylsulfoxide (DMSO) (Sigma, St. Louis, MO), 1 μmol/L ionomycin (IM) (Calbiochem, San Diego, CA), and 0.1 μg/mL phorbol myristate acetate (PMA) (Sigma) were added. After stimulation for 10, 30, or 60 minutes, the suspension was centrifuged for 5 minutes at 3000 rpm and used as a sample. DMSO was used as a diluting solution for IM and PMA and as a control.

Statistics

All values are presented as means ± standard deviation (SD). Statistical analysis of differences in POD activity among the groups of patients was performed using 1-way analysis of variance and multiple comparison for assessment of means. The relationship between variables was examined by determination of Spearman's rank-order correlation coefficients and by simple regression analysis. Differences were considered significant when *P* < .05. Analyses were performed using the StatView program (Abacus Concepts, Berkeley, CA).

RESULTS

POD Activity in Patients With Leukemia

POD activity in patients with leukemia is summarized in Figure 1. In patients with AML or ALL, POD activities expressed as RFI were 14.5 ± 12.5 and 5.6 ± 0.3, respectively. POD activity in patients with AML was significantly greater than that in patients with ALL (*P* < .05). By FAB classification, patients with M1, M2, or M3 subtypes had significantly higher POD activity than did ALL patients (*P* < .01). In patients with CML, POD activity was 13.4 ± 4.0, significantly greater than activity in patients with ALL (*P* < .01). In patients with infection, POD activity was 6.2 ± 0.7, lower than that in patients with AML or CML (*P* < .01).

Reproducibility of Measurements of POD Activity and MPO Antigen

The simultaneous reproducibilities of measurement of POD activity after 10 measurements using plasma samples with mean RFIs of 5.9 and 21.9 were 4.7% and 4.8%,

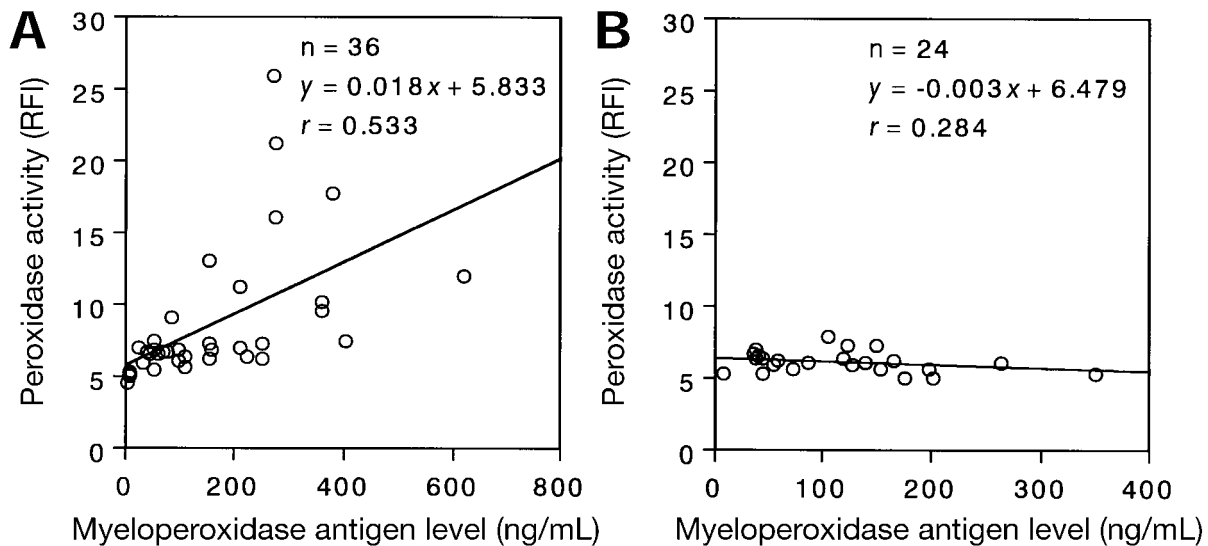


FIGURE 3. Correlation between peroxidase activity and myeloperoxidase antigen level in the nonlymphocytic leukemia (A) and infection (B) groups. RFI indicates relative fluorescence index.

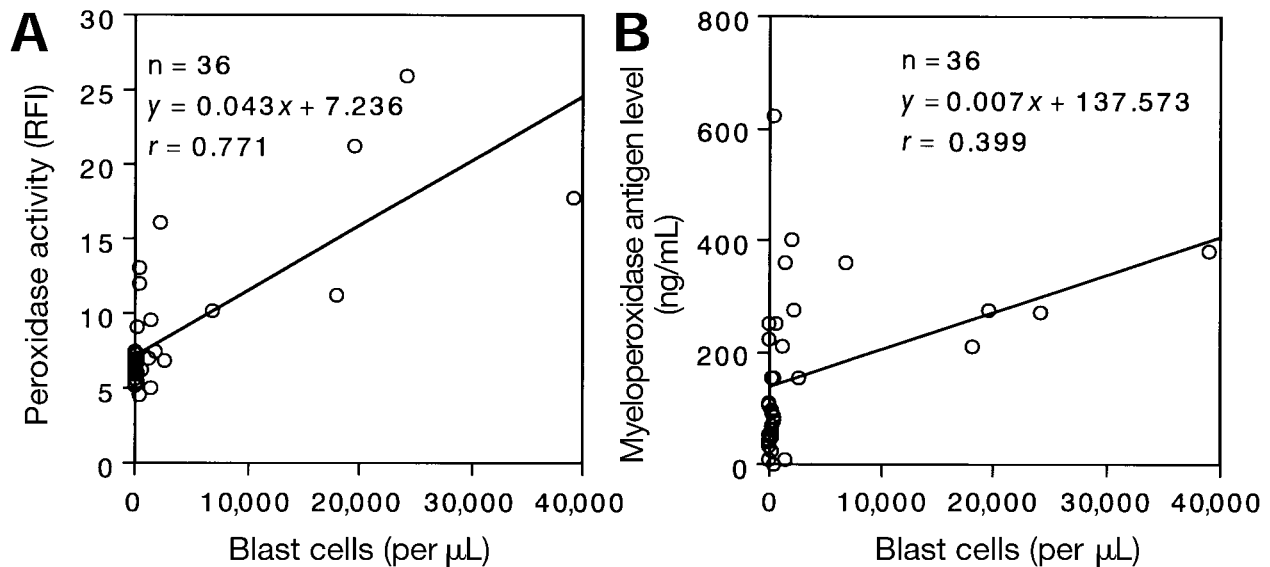


FIGURE 4. Correlations among blast cell count, peroxidase activity (A), and myeloperoxidase antigen level (B) in the nonlymphocytic leukemia group. RFI indicates relative fluorescence index.

respectively. For measurement of MPO antigen, the simultaneous reproducibility after 5 measurements using plasma samples with a mean RFI of 49.2 ng/mL was satisfactory at 10.9%.

Serum and Plasma POD Activity and MPO Antigen Level

Figure 2 shows POD activity and MPO antigen level in 24 samples of serum and plasma with K_2 -EDTA. POD activity, expressed as RFI, was 7.6 ± 3.6 in serum and 7.2 ± 2.6 in plasma; this difference was not significant. On the other hand, MPO antigen level was significantly higher ($P < .01$) in plasma (168.1 ± 117.4 ng/mL) than in serum (80.8 ± 77.5 ng/mL), but the rate of increase in serum varied. Thus, POD activity and MPO antigen were measured using blood samples with K_2 -EDTA added.

POD Activity and MPO Antigen in Culture Supernatants of Leukemia Cells

When measured in the culture supernatants of K562, KG-1, HL-60, Mo7E, and U937 cells after 3 days of culture, POD activity and MPO antigen level were detected only in HL-60 cells. POD activity and MPO antigen level were therefore measured between the first and third days for HL-60 cells, and results are shown in Table 1. The results demonstrated a slight difference in dynamics: POD activity increased linearly with time, whereas MPO antigen level increased on the first day and became low on the third day.

Relationship Between POD Activity and MPO Antigen Level in NLL and Infection Groups

The correlation between POD activity and MPO antigen level in the NLL group (Figure 3) had an r value of 0.533

($P < .01$) and was stronger than that in the infection group ($r = 0.284$). POD activity was negligible in cases with high MPO antigen titers in the infection group. Furthermore, r values for correlations of peripheral blast count with POD activity and MPO antigen level in the NLL group were 0.771 ($P < .01$) and 0.399 ($P < .05$), respectively (Figure 4), revealing a satisfactory relationship of peripheral blast count with POD activity.

POD Activity and MPO Antigen Level With Neutrophil Stimulation

Table 2 shows POD activity and MPO antigen level in the supernatant of neutrophils after isolation from healthy subjects and stimulation with IM and PMA. Stimulation with IM and PMA did not change POD activity from the control level. In contrast, MPO antigen increased to 568.1 ng/mL after stimulation with IM and to 128.9 ng/mL after stimulation with PMA, with a control value of 32.7 ng/mL. The MPO antigen level nearly peaked after 10 minutes of stimulation.

TABLE 2. Peroxidase (POD) Activity and Myeloperoxidase (MPO) Antigen in Neutrophils Stimulated by Ionomycin or Phorbol Myristate Acetate (PMA)

	POD Activity, RFI*			MPO Antigen, ng/mL		
	10 min	30 min	60 min	10 min	30 min	60 min
Control	4.8	4.7	4.9	32.7	66.1	32.1
Ionomycin	5.0	5.0	4.8	568.1	505.2	544.8
PMA	5.0	5.2	4.8	128.9	105.6	177.5

*RFI indicates relative fluorescence index.

DISCUSSION

We previously reported that a nonspecific response in the routine HbS Ag measurement system of the F750 automatic immunoassay apparatus using an antigen-antibody reaction was detected in 58.8% of patients with AML, but not in patients with ALL [3]. The Auszyme Monoclonal (Abbott, Chicago, IL) system indicated that these samples were negative for HbS Ag. This nonspecific substance was measurable using a noncoated tube instead of the reaction tube for HbS Ag. The nonspecific reaction was inhibited by the addition of 0.5% casein. This nonspecific substance had POD activity [3] and MPO antigenicity [4]. In the present study, we demonstrated that the POD activity of this substance was significantly higher in patients in the NLL group, especially in the AML M2 subgroup, than in the ALL and infection groups. The number of blasts in peripheral blood was significantly correlated with POD activity in the NLL group. These results suggest that this nonspecific substance may be present only in myelocytic tumor cells.

The culture supernatants of 5 myelocytic leukemia cell lines were then examined. POD activity and MPO antigen were detected only in HL-60 cells [5]. The HL-60 cell line was derived from a patient with AML M2 [6]. Four types of MPO antigen in HL-60 [7] and 3 types of MPO antigen in neutrophils [8] have been reported. It is unclear whether the nonspecific substance is different from these MPO antigens. To evaluate the similarity between the nonspecific substance with MPO antigenicity and neutrophil-derived MPO antigen [9], nonspecific POD activity and MPO antigen levels in the infection group and NLL group were compared. POD activity and MPO antigen level were significantly correlated in the NLL group, but not in the infection group. These results suggest that MPO derived from NLL cells had POD activity. However, even MPO antigen produced by neutrophils stimulated with IM and PMA had no POD activity. These findings indicate that MPO in the NLL group had characteristics different from those in the infection group, and was therefore specific to the NLL group.

Although high MPO antigen titer in the blood of patients with AML has already been reported [10,11], this finding is not specific to AML. In the future, measurement of the nonspecific substance tested here with the F750 will be made possible by identification of the substance and its mechanism

of production. This substance may also be found to be useful as a marker for myelocytic tumors.

CONCLUSION

Our results suggest that the POD-active substance exhibiting nonspecific F750 and MPO antigenicity may be useful as a myelocytic tumor marker.

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