Case Report

The Discrepancy between Chemotaxis and Leukotriene B₄ Production in a Patient with Chronic Neutrophilic Leukemia

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Chronic neutrophilic leukemia (CNL) is a rare type of leukemia. We diagnosed a 81-year-old woman as CNL because she showed that sustained leukocytosis dominated by mature neutrophils, hepatosplenomegaly, high neutrophilic alkaline phosphatase (NAP) score, absence of the Ph¹ chromosome and no evidence of leukemoid reaction. During the clinical course, she did not manifest hemorrhagic tendency or infection. We also examined neutrophil function including chemotaxis, chemiluminescence, nitroblue tetrazolium (NBT) dye reduction, which all indicated normal neutrophil function. Using a reversed phase-high pressure liquid chromatography (HPLC), we detected the production of leukotriene B₄ (LTB₄) in neutrophils. We found that the LTB₄ production was decreased in neutrophils whereas they showed normal chemotaxis. This discrepancy has never, to our knowledge, been reported before in case of CNL.

Key words: Neutrophil, Migration, Leukotriene

Chronic neutrophilic leukemia (CNL) is a rare type of leukemia, which is classified among chronic myeloproliferative disorders (CMPD) (1), and is considered to be a different clinical entity from chronic myelogenous leukemia (CML) (2-4). Only a few reports (6-8) describing the role of neutrophils in CNL are found possibly because CNL is infrequently associated with infection in contrast to myelodysplastic syndrome (MDS) (5). Indeed, the morphology of neutrophils in the peripheral blood of CNL patients is normal by light microscopic examination except for the presence of Döhle bodies and toxic granules which are also found in other conditions (6, 9). We describe here a patient with CNL whose neutrophil function test showed a discrepancy between chemotaxis and leukotriene B₄ (LTB₄) production.

CASE REPORT

A 81-year-old woman was admitted to our hospital because of leukocytosis and

hepatosplenomegaly after cardiomegaly and atrial fibrillation were noted during a prior physical examination. The patient visited a nearby hospital, where leukocytosis (18,600/ μ l, neutrophil 89%) and hepatosplenomegaly were noted in April 1985. Because leukocytosis was sustained (22,700/ μ l, neutrophil 94%) for a year, she was suspected of suffering from CML, and introduced to our hospital. Since hyperuricemia was detected in the first examination, she was treated with allopurinol at our out-patient clinic. Though she did not manifest fever or hemorrhaging during this period, she was admitted to our hospital for further investigations in September 1986.

The results of a physical examination on admission were as follows: height 154 cm, body weight 53.2 kg, blood pressure 120/68 mmHg, pulse 72/min, irregularly, body temperature 35.4°C. Skin and mucous membrane were not icteric and petechial. Surface lymph nodes were not palpable. Heart and respiratory sounds were normal. She had

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marked splenomegaly (12 cm below the left costal margin) and hepatomegaly (7 cm below the right costal margin). Extremities were not edematous. Neurological findings were normal.

Laboratory findings: The WBC count was markedly high at $31,900/\mu\text{l}$ consisting mainly of mature neutrophils (96%) (Fig. 1). Blastic cells were not evident in peripheral blood smears. Some of these neutrophils showed dense granules and Döhle bodies. NAP score was high (450) compared with a control subject (252). Morphological changes were not apparent in RBC. The Hb level was 14.2 g/dl, the Ht was 46.6% and the RBC count was $5.20 \times 10^6/\mu\text{l}$. The platelet count was $131 \times 10^3/\mu\text{l}$. The prothrombin time (PT) was 17.2 second (control 16.8 sec). The activated partial thromboplastin time (APTT) was elongated (63.8 sec) compared with a control (44.5 sec). The bleeding time and the platelet aggregation (induced by epinephrine, ADP and collagen) were normal. In a bone marrow specimen, the number of nucleated cells (NCC) was high and consisted mainly of mature granulocytes (48.5×10^4 cells/ μl). The M/E ratio was 13.25. The number of megakaryocytes was within the normal range. Chromosome analysis revealed normal karyotype. Serum levels of LDH and vitamin B₁₂ were 803 IU/l and 14,914 pg/ml respectively. The concentrations of C₃, C₄, CH₅₀ and immunoglobulin were normal. No monoclonal peak was found in the serum protein separation. The liver function and electrolytes were normal. C-reactive protein, RA test and ANA were negative.

Fig. 1. The neutrophils in the peripheral blood. They are mature and have typical granules in the cytoplasm. One of them (below) has a few vacuoles.

Clinical course after admission: We suspected CNL because of the patient's advanced age, sustained leukocytosis dominated by mature neutrophils, hepatosplenomegaly, high score of NAP and absence of Ph¹ chromosome. To rule out leukemoid reaction due to chronic infections or malignant disorders, serological examinations and imaging procedures were performed. We found a polypoid lesion in the stomach, which was resected using an endoscope. The only pathological finding in this specimen was a hyperplastic polyp. Using abdominal echo and gallium scintillation scanning, there was no evidence of malignancy, but dilated and tortuous splenic vein were noted which suggests portal hypertension. A liver biopsy specimen showed invasion of many mature neutrophils to sinusoids without lobular reconstruction and fibrous change (Fig. 2). We diagnosed CNL based on above clinical findings adaptable to the criteria proposed by You et al and Miura et al (3, 4). Although no treatment except medication for hyperuricemia was administered, the patient had no complaints related to hemorrhaging such as petechia, hemoptysis or melena. Because the WBC count was stably high and hepatosplenomegaly was not progressive, she was discharged on October 15th in 1986. Since that time, the patient has shown neither a change in peripheral blood features nor progression of hepatosplenomegaly. On October 24th 1988, the peripheral blood showed WBC $38,000/\mu\text{l}$ (neutrophil 90%), RBC $4.35 \times 10^6/\mu\text{l}$ and platelet $95 \times 10^3/\mu\text{l}$.

Neutrophil function test: Preparation of neutrophils for use in these experimental studies is

Fig. 2. The liver biopsy specimen. The sinusoids are full of mature neutrophils. Reconstruction and fibrous change are not evident.

described briefly. One vol. heparinized venous blood was mixed with 1/3 or 1/4 vol. of 6% dextran and incubated 20 to 30 minutes at room temperature. The supernatant only was then centrifugated at 1,400 rpm for 10 minutes at 4°C. Residual RBCs in the pellet were removed by hypotonic lysis by adding 0.2% saline and 1.6% saline sequentially. After centrifugation, the pellet was suspended in Hank's balanced salt solution without calcium chloride or magnesium sulfate (HBSS), then Ficoll-Hypaque was added to separate the neutrophil-rich supernatant by centrifugation. The number of neutrophils was calculated in 2 ml HBSS, and after centrifugation they were adjusted to appropriate neutrophil concentration (10). In the assay for chemotaxis the final concentration was 5×10^7 cells/ml and in the chemiluminescence test, the final concentration was 1.5×10^6 cells/ml. The neutrophil migration under agarose (11) was measured with various concentrations of N-formil-methionyl-leucyl-phenylalanine (fMLP) and LTB_4 as chemoattractant. The chemotactic activity was expressed as a chemotactic index (CI), being the ratio of chemotaxis/random mobility. All assays were performed in duplicate.

Table 1 shows the effect of fMLP and LTB_4 on neutrophil chemotaxis. There was no difference in chemotactic activity between the patient and a control (normal volunteer). The luminol-dependent chemiluminescence of neutrophils phagocytosing

opsonized zymosan was studied by ATP-photometer Lumiphotometer TD-4000 (Labosience, Japan). The peak value was 25.75 RLU (relative light unit) and peak time was 560 second. Both results were within the normal range. We performed the NBT dye reduction test using a microcapillary tube (12). This activity was normal (10%). To measure the production of LTB_4 , minimal essential medium (MEM) added to the neutrophil suspension prepared as above to a final concentration of 5×10^6 cells/ml. This suspension was stimulated by calcium ionophore A23187 (final concentration $10^{-5}M$) for 15 min at 37°C. Released LTB_4 into the medium was measured with a reversed phase-HPLC (13). The production of LTB_4 in the patient was 3.1 ng/ 5×10^6 cells. This value was remarkably less than that of control (19.8 ± 8.1 ng/ 5×10^6 cells).

DISCUSSION

At first, CML was suspected in this patient because of leukocytosis accompanied by hepatosplenomegaly. Mature neutrophilia were sustained for over one year and blastic cells did not increase in the peripheral blood, we diagnosed her as CNL rather than CML. Though it is doubtful whether CNL is a subtype of CML, the absence of the Ph^1 chromosome and high score of NAP are different from CML. By these criteria, CNL has recently been considered to be an independent clinical entity from CML. Until now the diagnostic criteria of CNL acceptable in general was proposed by You et al (3), including 1) severe sustained mature neutrophilic leukocytosis, 2) hepatosplenomegaly, 3) elevated leukocytic alkaline phosphatase, 4) elevated serum vitamin B_{12} , 5) elevated serum uric acid, and 6) the absence of fever or an underlying infection or disease sufficient to deem the findings a reactive leukemoid state. In our patient, especially on the basis of the sixth item, there was no evidence for chronic infection, connective tissue disease or malignant neoplasia as the causative disorder. Recently, modified diagnostic criteria for CNL have been suggested by Miura et al (4) as follows; 1) severe sustained mature neutrophilic leukocytosis ($>20 \times 10^9/l$), 2) the absence of Ph^1 chromosome, 3) the absence of other chronic myeloproliferative disorders (polycythemia vera and essential thrombocythemia),

Table 1. The neutrophil function tests

	Patient	Control
Chemotaxis chemo- attractant	CI*	CI
fMLP 10^{-7} M	1.78	1.54
LTB_4 10^{-7} M	1.33	1.22
Chemiluminescence		
peak value	25.75 RLU+	Not done
peak time	560 sec	
NBT Reduction	10%	Not done
LTB_4 Production		
	3.13 ng / 5×10^6 cells	19.76 ± 8.07 / 5×10^6 cells

*CI: chemotactic index, + RLU: relative light unit

4) high neutrophilic alkaline phosphatase, and 5) the absence of a reactive leukemoid state, with all of these being fulfilled for the diagnosis. Hepatosplenomegaly, Döhle bodies and toxic granules in neutrophils, elevated vitamin B₁₂, lysozyme and uric acid in the serum are not essential, but they are common in such patients. Since all essential items described above were satisfied by our case, we diagnosed the patient as definitely having CNL.

During the clinical course, CNL often shows hemorrhagic tendency such as subcutaneous hematoma, bloody stool, intra-articular hemorrhage and petechia (14–17), but no hemorrhaging was noted in our patient. She presented elongated APTT, but bleeding time, PT, fibrinogen and fibrinogen degradation products (FDP) were all within normal ranges. Although there was no evidence for liver dysfunction, we thought that there might be some deficiency of intrinsic coagulation factors or circulating anticoagulants, but the concentration of clotting factor VIII, IX, XI and XII were all within normal ranges. We subsequently performed mixing experiments of normal (control) plasma and the abnormal (patient) plasma after 37°C incubation for 30 minutes to decide whether the abnormal plasma is deficient in another coagulation factor or contains an inhibitor of coagulation. When 1 vol. of patient plasma was added to 9 vol. of control plasma, the prolongation of APTT was almost completely corrected, but at ratios 1:1 and 9:1 of patient to control plasma, the APTT prolongation not corrected (data not shown). This result indicates that the patient's plasma likely contained an inhibitor. Some other cases of CNL (14, 18–20) have been reported to show an elongated APTT and most of these patients died due to bleeding accidents without a decrease in platelets. However, there have been no descriptions regarding the circulating anticoagulants. The hematological results from our patient may explain the bleeding disorders noted previously among other CNL patients and requires additional clinical evaluation.

Utilizing imaging procedures, portal hypertension was demonstrated in our patient as well as an enlarged and tortuous splenic vein. The cause of this portal hypertension was thought to be due to leukocyte infiltration to the liver and the spleen. The

findings from the liver biopsy specimen which did not show reconstruction or fibrosis of the hepatic lobule confirmed that the vascular blood flow and vascular resistance had increased (19–23).

There are few CNL cases associated with infection during the clinical course. Most of case reports indicate that the neutrophil function is normal but a few cases showed below normal neutrophil function (6–8). On the other hand, severe infection has been implicated in MDS (24). One of the most important causes is a deficiency in superoxide production as well as in the intracellular killing system such as phagocytic activity or chemotaxis (25). In our patient, infection did not occur and neutrophilic function was also normal. We investigated the neutrophilic functions such as phagocytic activity against zymosan detected by chemiluminescence, reducing activity to NBT and chemotaxis stimulated by fMLP and LTB₄. All of these assays showed normal results. Although chemotaxis examined by exogenous fMLP and LTB₄ was normal, the production of endogenous LTB₄ was remarkably decreased. This result has never, to our knowledge, been reported before in case of CNL.

Normal chemotactic function and decreased production of LTB₄ in neutrophils in a patient with CNL were reported here. Palmblad et al (26) has shown that LTB₄ enhanced neutrophil chemotaxis in vitro. And Sun et al (27) indicated that neutrophils themselves were capable of producing large quantities of LTB₄ when stimulated with calcium ionophore A23187. In addition, neutrophils not only responded when exposed to LTB₄ but also were a major source of this factor when stimulated by the calcium ionophore (28). Thus LTB₄ may serve as an intracellular second messenger in neutrophils evoked by other chemoattractants (29). It has been postulated, therefore, that LTB₄ is an intermediate in the inflammatory responses such as chemotaxis. However our observation does not correspond with this hypothesis because of the discrepancy between chemotaxis and the production of LTB₄. Recently Clancy et al (30) showed that neutrophils did not produce LTB₄ after experimental administration of fMLP or C5a and they concluded that chemotaxis to an exogenous factor was independent from the production of LTB₄ in neutrophils. We have found that the neutrophils of

our CNL patient showed normal chemotaxis but their productive ability declined notably. This discrepancy supports the findings of Clancy et al clinically.

For the future we will determine whether the discrepancy between chemotaxis and LTB₄ production observed in our case is usual in CNL or the selected clone is proliferated by chance.

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