CONCISE REPORT

Alterations of Polypeptide Composition of Mature Granulocytes Obtained From Patients with Chronic Myelogenous Leukemia

By Jun Yokota, Shigetaka Asano, Sachiko Teshima, Kazuhiro Morishita, Akichi Iwamoto, Hiroshi Yoshikura, and Shiro Miwa

In order to compare the polypeptide composition of the CML mature granulocytes with that of the normal whole cell lysates, mature granulocytes from four healthy volunteers and six CML patients were analyzed by two-dimensional gel electrophoresis. In the normal subjects, 60 major spots were commonly and reproducibly identified. In the CML, there were constant alterations in some of these major spots. Four spots were totally absent in all the CML samples, and another four spots were absent in five of the six samples. In addition, one spot was larger in CML than in normal cells, and another spot, which was only faintly visible or not detectable in the normal samples, was massively present in all the CML samples. Our data suggest specific changes in the polypeptide compositions of CML granulocytes. This method could be clinically applied for the analysis of granulocytic disorders.

CIRCULATING mature granulocytes (both banded and segmented neutrophils) in patients with chronic myelogenous leukemia (CML) appear morphologically normal. Decreased alkaline phosphatase activity, loss of granulocyte-specific alloantigen normally found in the mature granulocytes, etc., are among the few reported alterations in the CML granulocyte. It was postulated that these and other yet unidentified alterations could be reflected in the polypeptide compositions of the CML granulocytes. In order to test this possibility, polypeptide patterns in normal and CML mature granulocytes were compared by two-dimensional gel electrophoresis, which had been successfully applied in the analysis of differentiation of the mouse granulocytes.

MATERIALS AND METHODS

Sources of Materials

Blood samples were obtained with heparinized syringes from six patients with CML in chronic phase and from four healthy volunteers who gave informed consent. Five of the CML patients were positive for the Ph chromosome, while the other one was negative. Four patients were under the treatment of busulfan (2–4 mg/day) when their blood samples were collected and the other two were not treated at all (Table I).

Isolation of Peripheral Mature Granulocytes

Each of the blood samples was mixed with an equal volume of isotonic saline containing 4% dextran (average mol wt 161,000; Sigma Chemical Company, St. Louis, Mo.) and was left at 37°C for 30–60 min. The leukocyte-rich plasma was layered on a sucrose cushion (specific gravity 1.077, Nyegaad & Co. A/S, Oslo) and centrifuged at 400 g for 30 min at room temperature. The cell pellet, composed of erythrocytes and granulocytes, was then suspended in approximately 10 volumes of ice-cold 0.2% NaCl for 3 min to lyse most of the erythrocytes. Immediately after the incubation, the same volume of cold 1.6% NaCl was added to restore the isotonicity. The intact cells were then washed twice in cold, Ca++, Mg++-free Hanks’ balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.). The hypotonic shock could not remove all of the erythrocytes, but the remaining erythrocytes were consistently spun down just on top of the white granulocyte layer during washing and could be easily removed by a Pasteur pipette. The purity of mature granulocytes in the final cell suspension was 88–98.5% (Table I), and the viability of the cells usually exceeded 90% in dye exclusion test.

Two-Dimensional Gel Electrophoresis

The washed cell pellets (2×10⁶ cells) were lysed in 40 μl of lysis buffer (9.5 M urea, 2% (w/v) Nonidet P40, 2% ampholines (1.6% pH 5–7 and 0.4% pH 3–10), and 5% mercaptoethanol). The lysates were stored at -70°C until electrophoresis. The electrophoresis was carried out as described by O’Farrell. All the gels were loaded with a constant volume (40 μl) of the lysates. The condition for the first-dimension isoelectric focusing produced a linear pH gradient that ranged from pH 4 to pH 7. The second-dimension SDS separating gel contained a uniform concentration of 10% acrylamide. As the gels were silver-stained as described by Oakley et al., the intensity of the spots expectedly paralleled the amount of the polypeptide in the spot.

RESULTS AND DISCUSSION

Figure 1 shows the typical polypeptide patterns of the mature granulocyte fraction in the normal and in the CML subjects. About 150 spots were detected in each gel. In the 4 normal subjects, the overall pattern was almost identical, though there were minor variations, especially in the small-sized spots. In this investigation, the 60 major spots (spot nos. 1–60 in Fig. 1B), which were reproducibly identified in all the normal samples, were examined extensively.

In the CML subjects, though the overall pattern was similar to that of the normals, there was a...
Table 1. Polypeptide Analysis of Normal and CML Mature Granulocytes

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<th>CML</th>
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*(+)* Treated, (-) untreated.
†(+)* Positive, (-) negative.
‡Differentia] counts of isolated mature granulocyte fraction.
§(+)* Present, (+++) increased in the CML relative to the normal, (-) absent or greatly decreased in the CML relative to the normal.

Fig. 1. Two-dimensional gel electrophoresis of normal and CML mature granulocytes. Gels were silver-stained. (A) Normal (case 3); (B) diagram of normal; (C) CML patient treated with busulfan (2 mg/day) (case 5); (D) CML patient who received no therapy (case 9). Arrows that point up indicate proteins that increased in all the CML samples (spots 30 and 61), and arrows that point down indicate proteins that decreased in all the CML samples (spots 7, 8, 38, 39), relative to the normal.
constant alteration in some of these major spots. Four spots (spots 7, 8, 38, 39) were totally absent in all the CML samples, and another 4 spots (spots 13, 26, 27, 28) were absent in 5 of the 6 samples. These changes could not have been artificially derived from the variable amount of the lysates loaded to the gels, because almost the same numbers of spots were detected in the normal and in the CML subjects, and also because the major spots in the CML and the corresponding spots in the normals were almost of the same size. In addition, spot 30 of the CML subjects was larger in size than that of the normals, and spot 61, which was only faintly visible or not detected in the normal samples, was massively present in all the CML samples. The alteration in the polypeptide pattern could not have been derived from the minor contaminating cells. For example, the normal samples 2 and 4 were contaminated by cells other than granulocytes in amounts of 1.5% and 9.0%, respectively; yet, the pattern was identical at least in the 60 major spots.

Furthermore, this is also the case with CML; the pattern of sample 6 was almost the same as that of sample 5, although the population of nongranulocytic cells was 5.0% and 12.0%, respectively. Thus, these results indicate quantitative changes in the specific polypeptides in the granulocytes during CML leuke-mogenesis.

Among the CML subjects, the one pH'-negative case (case 8) showed a much lower number of spots than the other Ph' positive ones; only 39 of the 60 spots were detected. The deleted spots were not detected even if twice the amount of the material was loaded to the gel or when the samples obtained at the different instance were used for the gel analysis. The two spots, 4 and 46, were absent in the untreated patients (case 9 and 10), while they were present in the treated ones.

Our data with two-dimensional gel electrophoresis showed that there were specific changes in the polypeptide pattern in the mature CML granulocytes. The differences among the CML subjects may reflect the types of CML and the effect of busulfan treatment, although further investigations will be necessary, as the number of examined cases was so few. This method could be clinically applied for the analysis of granulocytic disorders.

ACKNOWLEDGMENT

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REFERENCES

Alterations of polypeptide composition of mature granulocytes obtained from patients with chronic myelogenous leukemia

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