CONCISE REPORT

Mixed Lymphoblastic-Myelomonoblastic Leukemia in Treated Hodgkin’s Disease

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In a case of acute leukemia occurring 5.5 yr after diagnosis of Hodgkin’s disease, a mixture of lymphoid and myelomonocytoid blasts was identified by morphological, cytochemical, and immunologic methods. The majority of blasts were characterized as lymphoid by their strong expression of nuclear terminal transferase (TdT) enzyme. In contrast, a minor blast population was identified as myelomonocytoid by the strong nonspecific esterase positivity and lack of TdT. The two distinct blast cell populations showed different sensitivity to the various chemotherapeutic protocols used.

IT IS NOW evident that mixtures of lymphoid and myeloid blast cells may be simultaneously present in the acute blast crisis of Philadelphia chromosome positive (Ph1-positive) chronic myeloid leukemia1,2 (CML). These cell mixtures have been identified by a number of methods, including conventional morphological, cytochemical reactions, immunologic surface membrane markers, and the biochemical identification of elevated levels of the nuclear enzyme terminal deoxynucleotidyl transferase (TdT), which is present in most cases of acute lymphoblastic leukemia.3,4

Strong evidence has been presented that mixtures of lymphoid and myeloid blast cells are also present in some cases of Ph1-negative acute leukemia.3,4 Furthermore, studies of large numbers of cases of acute nonlymphocytic leukemia (ANLL) have demonstrated that, while the majority are TdT-negative, approximately 6% are associated with elevated levels of TdT, suggesting the presence of a lymphoblastic component in these cases.6

Recently, purified antibodies to TdT have become available for the detailed study of the distribution of this enzyme in hematologic disorders.7,10 In this report, immunofluorescent studies for TdT and surface membrane leukemia-associated antigens have been combined with cytochemical analysis to investigate a case of acute leukemia occurring in an adolescent female. This patient had previously received intensive combination chemotherapy and radiotherapy for nodular sclerosing Hodgkin’s disease (HD). The leukemia was shown to consist of a mixed population of blast cells, which were predominantly lymphoblastic with a minor myelomonoblastic component. The response of the disease to treatment further underlined the heterogeneous nature of the blast population.

MATERIALS AND METHODS

Leishman-stained smears were made of peripheral blood and bone marrow aspirate, and blasts classified according to the FAB proposals.11 Bone marrow aspirate and peripheral blood white cell concentrate smears were stained for Sudan Black, periodic acid-Schiff (PAS), acid phosphatase, chloroacetate (specific) esterase (SE), and alpha-naphthyl-acetate (nonspecific) esterase (NSE) by standard techniques.12,14

For membrane immunofluorescence (IF) studies, aspirated bone marrow was separated on Ficoll-Isoaque. Rabbit anti-ALL serum (made against non-T, non-B ALL blasts15), chicken anti-1a serum (made against human la-like p28,33 antigens16), and rabbit anti-human thymocyte/T-cell antigens (made against monkey thymocytes17) were used in indirect IF. A quantity of 1–2 × 106 cells in phosphate-buffered saline plus 0.5% bovine serum albumin and 0.02% sodium azide (PBSA) were incubated with antisera for 10 min at 20°C. After washing 3 times in PBSA, cells were incubated with goat anti-rabbit Ig conjugated with fluorescein (FITC), or sheep anti-chicken Ig conjugated with tetrarhodamine isothiocyanate (TRITC), as appropriate, for 10 min at 20°C and washed 3 times. Surface membrane immunoglobulin (SmIg) was stained for with goat anti-human Ig–FITC (Behringwerke) in direct IF. Cells were examined in suspension with a standard Zeiss microscope equipped with IV F epifluorescence and a 63 Ph Oid objective.

Cytocentrifuge smears of cells, prestained for membrane la antigens with TRITC, were stained for nuclear TdT. Smears were fixed in methanol (4°C, 30 min), rehydrated in phosphate-buffered saline (PBS) for 30 min, and incubated with rabbit anti-TdT (a gift from Dr. F. J. Bollum) in a humidified chamber for 15 min, washed in PBS for 30 min, and stained with purified goat anti-rabbit Ig–FITC (15 min). After a further 30-min wash in PBS, slides were sealed in formol-glycerol with a glass coverslip and examined as above.

Separated bone marrow mononuclear cells were also examined for TdT enzyme activity18 by Dr. K. Ganeshaguru and Professor A. V. Hoffbrand, Royal Free Hospital, London. A quantity of 5 × 106 cells were used per test, and results were expressed as U/106 cells.18

CASE REPORT

A 14-yr-old girl presented in December 1972 with stage IIIb nodular sclerosing Hodgkin’s disease (HD) for which she received repeated courses of MVPP (mustine hydrochloride, vinblastine, procarbazine, and prednisolone). Clinical remission was obtained,
Table 1. Course of Acute Leukemia

| Date      | Hb (g/dl) | WBC (x 10^3/liter) | Platelet Count (x 10^3/liter) | Marrow Blasts | Marrow Cellularity | Percentage | Type (%) | TdT (U/l) | TdT (%) | Type (%) | TdT (%) | Biochemical Type | Chemotherapy |
|-----------|-----------|-------------------|-------------------------------|---------------|--------------------|------------|---------|---------|---------|---------|---------|---------------|--------------|-------------|
| 9/6/78    | 10.0      | 2.7               | 32                            | Maximum       | 100                | 70         | 30      | 70      | 30      | 38.0    | TAD*    |               |              |             |
| 10/6/78   | 10.8      | 0.6               | 15                            | Aplasia       | 0                  | 0          | 0       | 0       | 0       | -       | TAD     |               |              |             |
| 10/27/78  | 8.7       | 0.6               | 16                            | Hypoplasia    | 60                 | 60         | 0       | 60      | 0       | 38.5    | TAD     |               |              |             |
| 11/22/78  | 10.7      | 1.0               | 10                            | Hypoplasia    | 100                | 100        | 0       | Not done | Not done | 5.0     | MTX/ASP† |               |              |             |
| 1/22/79   | 11.5      | 2.8               | 40                            | Normal        | 12                 | 12         | 0       | 15      | 0       | 1.0     | MTX/ASP |               |              |             |
| 3/7/79    | 7.8       | 6.8†              | 70                            | Normal        | 2                  | 2          | 0       | 1       | 0       | 0.3     | MTX/ASP |               |              |             |
| 5/10/79   | 9.3       | 102               | 15                            | Maximum       | 100§               | 15         | 85      | 15      | 85      | 0       | MTX/ASP |               |              |             |

*TAD: thioguanine, cytosine arabinoside, and daunorubicin.
†MTX/ASP: methotrexate and asparaginase.
‡Normal differential white count.
§Marrow cellularity assessed at autopsy 2 wk later; typing done on peripheral blood blasts.

RESULTS

At the time of diagnosis of acute leukemia, three types of blasts were seen in Leishman-stained blood and bone marrow smears. Seventy percent resembled lymphoblasts (L1 in FAB classification), and were small (10–12 µ diameter) with scanty cytoplasm, well condensed nuclear chromatin, and 1 or 2 nucleoli (Fig. 1A: cell type 1). Another group, comprising 20%, resembled myeloblasts (M1, FAB), being larger (18–22 µ diameter) with more abundant cytoplasm and a finer nuclear chromatin pattern with 2 or more nucleoli (Fig. 1A: cell type 2). Finally, a small number of cells (10%) were large (25–30 µ diameter) with abundant cytoplasm and 3 or more large prominent nucleoli in nuclei that were folded and indented. This last group was monocytoid (M5, FAB) in appearance (Fig. 1B: cell type 3). No multinucleate blasts or cytoplasmic inclusions were seen.

All blasts were negative for Sudan Black, PAS, acid phosphatase, and specific esterase. Cell types 2 and 3 were strongly and diffusely NSE positive, while cell type 1 was predominantly negative for NSE with occasional weakly positive cells. (Fig. 2).

Immunologic marker studies revealed that over 95% blasts expressed surface la-like antigens. All cells were negative for the common ALL antigen, human T-lymphoid antigens, and SmIg. In smears, the smaller...
blasts (type 1) were strongly positive for nuclear TdT by IF, while larger type 2 and 3 blasts had very little or no detectable TdT (Fig. 3). TdT enzyme activity was grossly elevated at 38.0 U/10⁸ cells (range for normal bone marrow 0–3.0 U/10⁸), confirming the presence of TdT-positive cells.

Chromosome analysis revealed that the Ph¹ chromosome was absent in all metaphases, but hyperdiploidy of all cells was noted in four separate marrow samples.

TREATMENT AND SUBSEQUENT COURSE (TABLE 1)

Since the leukemia had arisen during therapy with vinblastine and prednisolone, the patient was treated with TAD (thioguanine, cytosine arabinoside and daunorubicin). The first course eradicated the blast cells, and bone marrow aplasia was induced. Following the second course of TAD, at reduced doses, the marrow was hypoplastic, but contained 60% blasts, virtually all of which were type 1 morphologically and Ia⁺, TdT⁺ by IF (TdT level 38.5 U/10⁸ cells). A third course of TAD was given with no change in marrow appearances.

Treatment was then changed to intravenous methotrexate and L-asparaginase (MTX/ASP) in increasing doses at 10-day intervals. On this regimen, the blast content of the marrow was slowly reduced, with return of normal hemopoietic elements. The Hb, WBC, and platelets rose, and the patient was discharged from hospital. In March 1979, the marrow had regained normal cellularity, with only 1% abnormal blasts (Ia⁻, TdT⁻) being detectable. However, in May 1979 she was readmitted in florid relapse with 50 × 10⁹/liter circulating blasts. Bone marrow smears confirmed relapse with predominant type 2 blasts. IF studies demonstrated 85% Ia⁺, TdT⁺ cells, consistent with type 2 morphology, and 15% Ia⁺, TdT⁺ type 1 cells. TdT expression in the type 1 population was weak, as judged by IF and the low level on biochemical assay. No further therapy was given, and she died with fulminant Staphylococcus aureus septicemia 9 mo after the initial diagnosis of acute leukemia. Autopsy revealed replacement of the normal structure of lymph nodes in all areas by leukemic blasts similar to those seen in the peripheral blood before death; evidence of HD was found in only one small node.

DISCUSSION

The greatly increased risk of acute leukemia in long-surviving patients with treated Hodgkin's disease is well recognized. The majority of patients reported had ANLL, although detailed cytochemical studies were done in only 7 of the 122 cases reported. There appears to be no increased risk of developing ALL, of which only 7 cases have been described in association with HD, and no cases of mixed lymphoid-myeloid leukemia have been described.

In our patient, the combined morphological, cytochemical, and immunologic evidence strongly suggested a mixed acute leukemia. The predominant cell type was lymphoblastic, of the non-T, non-B type, resembling the L1 blast of the FAB classification. These cells expressed surface Ia-like antigens and nuclear TdT enzyme, but were cytochemically unreactive. Although the type 2 cells resembled M1 blasts and type 3 resembled M5 blasts, the strong diffuse NSE positivity using alpha-naphthyl-acetate and hexazonised pararosaniline indicated a monocytic type in both. The expression of membrane Ia antigens in
both types 2 and 3 without a significant nuclear TdT, is consistent with these cytochemical data, since approximately 60% of cases of acute myeloid leukemia (AML) and most cases of acute myelomonocytic leukemia (AMOL) are known to express Ia antigens. It is most unlikely that the large type 3 cells were circulating Reed-Sternberg cells (RSC), since RSC-leukemia has been reported only in advanced and uncontrolled HD, whereas our patient had only minimal evidence of residual HD. Furthermore, the reported cytochemical and immunologic features of exfoliated RSC are unlike those described in this case.

The behavior of the disease following cytotoxic chemotherapy clearly indicated that separate lymphoblastic and myelomonoblastic populations existed. These populations exhibited markedly different sensitivities to the various drug protocols used. Furthermore, the terminal relapse constituted a major phenotypic shift from the original dominant blast population, although it seems likely that the blasts in relapse represented the regrowth of the type 2 cells originally present as a minor population, rather than the emergence of a new subclone. A similar phenomenon in Ph1-negative acute leukemia has also been reported, with the dominant emergence of originally minor populations. In addition, Mertelsmann et al. have described a case with mixed lymphoid-monocytoid features in which considerable fluctuations in the expression of the tumor markers were observed during therapy. This phenomenon is well documented in blast crisis of Ph1-positive CML. Further studies of similar cases of mixed lymphoid-myeloid acute leukemia with sensitive single cell techniques may prove useful in evaluating the effects of different drug combinations on leukemic subpopulations.

ACKNOWLEDGMENT

We are indebted to the following people for their cooperation in this case: Dr. F. J. Bollum for anti-TdT serum; Dr. G. Janossy for surface membrane marker antisera and his criticisms of the manuscript; Dr. K. Ganeshaguru and Prof. A. V. Hoffbrand for TdT biochemical analysis; Dr. J. H. Dagg and Dr. N. P. Lucie for encouraging us to report this case.

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