Macroglobulinemia in a Child With Acute Leukemia

By Jan Čejka, Robert O. Bollinger, Henrica R.E. Schuit, Jeanne M. Lusher, Chung-Ho Chang, and Wolf W. Zuelzer

A 12-yr-old boy with acute leukemia was found to have paraproteinemia and Bence-Jones proteinuria. The paraprotein was characterized as immunoglobulin M, type \( \kappa \) and the Bence Jones protein as free \( \kappa \)-chains. Increased amounts of \( \beta_2 \)-microglobulin were found in the patient's serum and urine. Electron microscopic and immunofluorescence studies provided evidence of the presence of lymphoid leukemia cells apparently responsible for the production of the paraprotein.

Monoclonal gammopathy, usually found in multiple myeloma and Waldenström's macroglobulinemia, has been observed in other proliferative disorders including leukemias. The occurrence of paraproteins of the three main immunoglobulin classes in chronic lymphocytic and myelocytic leukemias is well documented. In children paraproteinemia is extremely rare. Only a few cases describing its association with childhood leukemia are on record, none of which are of the IgM class. This paper describes a unique case of \( \kappa \)-type macroglobulinemia with Bence Jones proteinuria in a child with acute leukemia.

CASE REPORT

K.W., a 12-yr-old white male, was admitted because of pallor, purpura, and fatigue of a few weeks' duration. The past history was noncontributory. The patient was one of twins whose mate had been stillborn. There was no family history of hematologic or immunologic disease. Physical examination revealed hepatosplenomegaly and generalized lymphadenopathy in addition to pallor and purpura.

Admission laboratory values were as follows: Hb 13.0 g/100 ml, hematocrit 39 vol %, WBC 25,800/\( \text{cu mm} \), and direct platelet count 13,000/\( \text{cu mm} \). The differential count showed immature granulocytes, 8% "blasts," and 32% lymphocytes. The blasts had abundant, deep blue, often vacuolated cytoplasm, sometimes containing irregular, streaky, gray areas, somewhat reminiscent of that of plasma cells. The nuclei had an open chromatin structure and one or more nucleoli. No Auer rods were found. The bone marrow showed almost complete replacement by similar blastic elements. Cytogenetic studies showed the leukemic cells in the bone marrow to be 46 XY without demonstrable abnormalities.

Initial therapy consisted of a combination of vincristine, 6-mercaptopurine, and prednisone. The bone marrow showed no change after 6 wk of this therapy, but the WBC dropped sharply. A second course of intensive therapy consisting of vincristine, cyclophosphamide, and cytosine arabinoside was given. Two
weeks later the patient complained of headaches and showed personality changes, fundal hemorrhages, and papilledema. Spinal fluid pressure was increased, and there were 107 cells/cu mm, identical in appearance to the blasts in the blood and bone marrow. Despite intrathecal amethopterin and irradiation, the patient expired soon thereafter.

The autopsy showed the findings expected in acute leukemia: massive leukemic infiltration of bone marrow, liver, spleen, kidneys, and lymph nodes, and in addition, a discrete intramural mass in the ascending colon composed of leukemia cells. The immediate cause of death was massive aspiration pneumonia.

MATERIALS AND METHODS

Serum was obtained at different times and stored at $-20°C$ until used. Urine specimens were ultrafiltrated through Amicon UM-2 membranes, dialyzed against distilled water, and lyophilized.

Antisera specific for heavy chains of IgG, IgA, and IgM, for light chains and fragments (Fab, Fc, and Fd) of IgG, as well as antisera against the individual serum proteins were purchased from Behring Diagnostics. Antiserum against $\beta_2$-microglobulin was raised in rabbits by immunization with purified protein isolated by the procedure described earlier.11

Agarose gel electrophoresis was performed in 1% agarose and 0.075 M barbital buffer, pH 8.6 containing 2 mM Ca-lactate at 20 V/cm under cooling according to Johansson.19 Immunoelectrophoresis was carried out on microscopic slides according to Scheidegger.18 Gel filtration was performed on a column of Sephadex G-200 (40–120 μ) in a 0.1 M phosphate buffer, pH 7.3 containing 1 M NaCl. The separation of the IgM from $\alpha_2$-macroglobulin was done by dialysis against diluted boric acid according to Badin and Levesque.4 Immunoglobulin quantitation was performed by the radial immunodiffusion method of Mancini et al. on plates purchased from Meloy Laboratories; the reference serum 67/95 obtained from NCI Immunoglobulin Reference Center was used as a standard. Quantitation of other serum proteins was done by the Mancini technique on plates purchased from Hyland Laboratories. $\beta_2$-microglobulin was quantitated by the radioactive single radial immunodiffusion method described by Čejka et al.19

Antemortem bone marrow aspirate, peripheral blood buffy coat, and postmortem lymph node were fixed for 2 hr in 2% glutaraldehyde17 buffered with 0.1 M sodium cacodylate, pH 7.2, and postfixed in veronal-acetate buffered 1% osmium tetroxide,14 pH 7.4. Dehydration and embedding in Epon 812 were carried out by the method of Luft.45 Ultrathin sections, stained with uranyl acetate and lead citrate,21 were examined with an RCA EMU-3H at 50 kV.

Cytocentrifuge slides were prepared from concentrated and washed bone marrow cells and from blood lymphocytes according to the technique described in detail elsewhere.22 The slides were fixed in acetic acid-ethanol and the direct immunofluorescent staining applied with fluorescein isothiocyanate (FITC)- or tetramethylrhodamine isothiocyanate (TRITC)-labeled monospecific antisera against the heavy chains of IgG, IgA, and IgM and the light chains of both types. Epi-illumination with narrow band excitation for the two-wavelength method23 and an HBO 100 light source were used for examination of the slides.

RESULTS

Agarose gel electrophoresis of patient's serum, shortly after admission, revealed an abnormal, homogeneous band in the cathodic part of the $\gamma$-region which persisted throughout the course of the illness (Fig. 1B). Immunoelectrophoresis also showed an unusual precipitin line (Fig. 2B). Using specific antisera, the serum paraprotein was classified as immunoglobulin M. Neither free light chains nor immunoglobulin fragments were found in serum by immuno electrophoresis. Quantitative analysis showed low IgG and IgA levels and an abnormally high concentration of IgM (Table I). The total concentration of immunoglobulins decreased with time in parallel with the WBC and the ratios of the three immunoglobulins remained the same.

To establish the light-chain type and size of the abnormal protein, the serum was separated on a Sephadex G-200 column. Three typical peaks were obtained, the fractions from the individual peaks were pooled and concentrated by
ultrafiltration. The IgM activity was detected only in the first peak (19S) eluted with the void volume of the column. Also eluted in this peak was α₂-macroglobulin as determined by immunoelectrophoresis and agarose gel electrophoresis. The two proteins were separated by dialysis against dilute boric acid. The precipitate thus obtained represented virtually pure IgM (Fig. 1C, 2F). In immunoelectrophoresis, the precipitated fraction reacted only with antisera specific for κ- and λ-chains (Fig. 2H, 2I); no reaction was obtained with anti-λ-chain antiserum (Fig. 2I).

In addition to immunoglobulins, α₁-antitrypsin, α₂-macroglobulin, ceruloplasmin, transferrin, C3 complement component, and β₂-microglobulin were
Table 1. Immunoglobulin and β₂-Microglobulin Quantitation of Serum K. W. Obtained on Dates Indicated

<table>
<thead>
<tr>
<th>Date</th>
<th>IgG (IU/ml)</th>
<th>IgA (IU/ml)</th>
<th>IgM (IU/ml)</th>
<th>β₂-Microglobulin (mg/100 ml)</th>
<th>WBC/cu mm</th>
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<tbody>
<tr>
<td>7-26-72</td>
<td>77.5</td>
<td>36.5</td>
<td>1.030</td>
<td>0.330</td>
<td>25,800</td>
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<td>8-14-72</td>
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<td>23.0</td>
<td>585</td>
<td>0.203</td>
<td>3,800</td>
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<tr>
<td>9-12-72</td>
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<td>6.0</td>
<td>220</td>
<td>0.420</td>
<td>600</td>
</tr>
<tr>
<td>10-6-72</td>
<td>37.0</td>
<td>28.0</td>
<td>340</td>
<td>1.270</td>
<td>31,000</td>
</tr>
<tr>
<td>Normal range</td>
<td>66–175*</td>
<td>36–170*</td>
<td>88–226*</td>
<td>0.098–0.310†</td>
<td></td>
</tr>
</tbody>
</table>

* Determined for the age group 9–12 yr.
† from Čejka et al. 16

Fig. 3. (A) Electron micrograph of leukemic cells in peripheral blood. The nucleus (N) shows little chromatin condensation except at the periphery where some segmentation at the nuclear pores is evident (arrows). The nucleolus is prominent (nu). The cytoplasm contains many free ribosomes, poorly developed golgi membranes (G), and some dilated endoplasmic reticulum (ER). A few nearly round mitochondria (m) were generally observed in each section. Bar equals 1 μ. (B) Electron micrograph of lymphoid cell exhibiting extensive development of endoplasmic reticulum (ER). Chromatin condensation is moderate in the nucleus (N), and a poorly developed nucleolus (nu) is evident. The golgi membranes (G) are also more developed than in the lymphoblast. Bar equals 1 μ.
quantitated in the serum. Elevated values were obtained for α₁-antitrypsin (510 mg/100 ml) and ceruloplasmin (70 mg/100 ml). Quantitation of β₂-microglobulin (Table 1) revealed increased concentrations of this protein; the highest concentration was found in a serum sample obtained 3 days before death.

The electrophoretic pattern of patient's urine (Fig. 1D) showed an intense cathodically migrating band which was shown to correspond to free light chains of the κ-type (Fig. 2K). No precipitin line was obtained in immunoelectrophoresis of the patient's urine with antisera specific for free λ-chains, nor for Fab, Fc, and Fd fragments. The presence of Bence Jones protein in the urine was confirmed by positive heat test. The remaining four intense bands in the anodic part of the electrophoretic pattern were identified by specific antisera as (in order of decreasing anodic mobilities): albumin, α₁-acid glycoprotein, inter-α trypsin inhibitor, and β₂-microglobulin, respectively.

Electron microscopic examination of bone marrow, lymph node, and peripheral blood showed lymphoid cells approximately 10–20 μ in diameter with moderate chromatin condensation at the periphery of the nucleus and a prominent nucleolus.
A wide range of cytoplasmic development was observed. The most frequently encountered cell type appeared lymphoblastic (Fig. 3A). Ribosomes were abundant with many aggregated into polysomes. The endoplasmic reticulum was limited to a few short profiles which were dilated and filled with material of moderate density. Approximately 5%–10% of the cells examined showed greater cytoplasmic development. This development included increased amounts of dilated endoplasmic reticulum and parallel arrays of long lamellae of endoplasmic reticulum at the outer border of the cell. A small proportion of the cells examined, less than 1%, showed extensive endoplasmic reticulum of the dilated form, but fewer non-membrane-associated monoribosomes and polyribosomes (Fig. 3B). Development of the golgi zone paralleled that of the endoplasmic reticulum.

Immunofluorescent investigation of the peripheral blood revealed very few cells positive for IgG, IgA, and λ-type of light chains. Numerous cells were positive for IgM of κ-type (Fig. 4). The fluorescence was more marked in the perinuclear zone and in addition patchily distributed through the cytoplasm. There was no significant staining of the cell membrane such as would be expected if the paraprotein was merely coating the surface. Thus while the staining was not as diffuse as usually seen in nonmalignant immunologically active cells, it appeared to be the result of intracellular production. No positive staining was obtained for IgM of λ-type. The same results were obtained in bone marrow; numerous cells were positive for IgM of κ-type; the IgM of λ-type was absent. Only one IgA positive plasma cell was seen; IgG positive cells were absent.

Fig. 4. Immunofluorescent picture of leukemic cells. (A) peripheral blood. (B) bone marrow (stained with anti-κ-TRITC, excitation at 546 nm). (C) peripheral blood (double staining with anti-κ-TRITC at left and anti-ε-FITC at right, excitation at 546 and 500 nm, respectively). Magnification 1250x.
DISCUSSION

The finding of a paraprotein of IgM class in association with acute leukemia represents an interesting and unique case. Two well-documented cases of childhood leukemia associated with the occurrence of IgG paraproteins in serum have been reported. In both, the paraproteins appeared at later stages of the disease. In the first case, the appearance of the paraprotein was interpreted as escape of one or more clones of immunoglobulin-producing cells from the effects of the immunosuppressive drug, and in the second case, as a result of a protracted intensive antigenic stimulation originating from viral infection. Neither of these alternatives is an acceptable interpretation in the present case since the paraprotein was found at the time of diagnosis before immunosuppressive therapy, and no evidence of infection was observed. This case is further distinguished from those reported previously in that the paraprotein belonged to the IgM class. The monoclonal protein was found to be composed of pentameric molecules with light chains of the κ-type only; the same type of light chains was found in patient’s urine. Clear evidence for monoclonal immunoglobulin synthesis came from immunofluorescence studies; whereas only few IgG, IgA, and λ-type light chain positive cells were found in both peripheral blood and bone marrow, numerous cells were positive for IgM of the κ-type. In addition, these results revealed the nature of the leukemic cells as cells capable of synthesizing complete immunoglobulin molecules, a property which is now generally attributed to cells of the lymphocyte-plasma cell series.

The characterization of the leukemic cells as lymphoid cells capable of immunoglobulin synthesis and release was also supported by electron microscopy. The majority of cells were lymphoblastic. The sparcity of endoplasmic reticulum and golgi membranes in these cells is inconsistent with the classical picture of a cell secreting protein, but is identical to that reported by Hummeler et al. for lymphocytes which could be shown by hemolytic plaque assay to be in fact doing so. No doubt more important in the observed production of serum paraprotein were those less common cells with more evident organelles associated with protein secretion. While these cells comprised only a few per cent of the population, this still represents a large number of competent cells, particularly during the times when the total WBC was high (Table 1). This type of cell, termed a lymphoplasmablast, is known to be associated with IgM production in Waldenström’s macroglobulinemia.

The decrease in the concentration of the paraprotein and other immunoglobulins with time (Table 1) can largely be attributed to the use of immunosuppressive drugs and paralleled the severe fall in the white count. In any case, the paraprotein was present in all the sera studied.

The level of β2-microglobulin in serum is of interest; increased concentrations of β2-microglobulin were found in three of the four sera tested. The β2-microglobulin concentration increased to nearly ten times the normal level in the serum sample drawn 3 days before death. Although the function of this protein and its relation to the malignant process are not known at the present time, it is interesting to note that increased β2-microglobulin levels were found by one of us in virtually all multiple myeloma patients studied and in certain groups of cancer patients. The increased serum concentration of α2-antitrypsin and the secretion of relatively high amounts of α2-acid glycoprotein, inter-α trypsin inhibitor, and β2-microglobulin
remain unexplained; no major pathologic changes in the patient’s kidneys or tubular damage have been found to explain the increased secretion of these proteins.

The case of childhood leukemia described in this paper appears to indicate the same relationship between lymphoid cell type and monoclonal macroglobulin production as found in Waldenström’s macroglobulinemia and also reported in other lymphoproliferative diseases such as chronic lymphocytic leukemia. In these disorders, the appearance of the abnormal protein often precedes other manifestations of the disease. In the present case an acute leukemia was fully established at the time when macroglobulinemia was discovered, and with the additional evidence presented, it seems certain that it is the product of the malignant cells themselves. The case is of interest because it may be regarded as an acute variant of Waldenström’s macroglobulinemia.

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