Paraproteinemia in a Child with Leukemia

By KAARE J. LINDQVIST, ABDELSALAM H. RAGAB AND C. KIRK OSTERLAND

MONOCLONAL gammopathies are commonly associated with multiple myeloma and Waldenström's macroglobulinemia. These diseases generally occur only in older age groups. Paraproteinemias have also been observed in myeloproliferative disorders, as well as in association with lymphomas. However, paraproteinemia in children is an extremely rare condition, and only very few cases have been recorded in the literature. Danon et al. recently described this condition in four children. Stoop et al. described the development of a monoclonal gammopathy in a child with leukemia.

In the course of an extensive study of serum protein levels in leukemic children, one patient who initially showed a normal serum protein pattern, developed changes suggestive of a monoclonal gammopathy.

The protein changes, characterizations of the abnormal protein and the clinical course of this patient are the subjects of this report. The immunological competence of the patient was also investigated.

MATERIALS AND METHODS

Case Report

C.W., a four-year-old Caucasian boy, presented to us in January 1967 with anemia, purpura and hepatosplenomegaly. His hemogram revealed: hemoglobin 7.6 Gm. per cent, hematocrit 24 per cent, white blood cell count 4195/mm.³ with 90 per cent lymphocytes, two per cent segmented forms, two per cent eosinophiles and six per cent lymphoblasts. Platelet count was 38,000/mm.³. The bone marrow was completely replaced by lymphoblasts. He was given 150 ml. packed red cell transfusions twice, and started on a five-week course of vincristine (2.0 mg./m.²) weekly and prednisone (60 mg./m.²) daily; this brought about complete remission. He was then maintained on 6-mercaptopurine. Between August 1967 and March 1968 he had repeated remissions and relapses of his disease. Therapy with cytoxan, vincristine brought about successful remission on one occasion, daunomycin and prednisone on a second, and methotrexate plus prednisone on a third. Serum protein electrophoresis in March 1968 revealed a normal pattern though the total protein was decreased. In spite of the good clinical response to prednisone and methotrexate, repeat bone marrow examinations showed a gradual increase in lymphoblasts. Only very rare plasma cells were seen in any of the marrow specimens. During the period March–July 1968 there was a decrease in his gammaglobulin content from 0.58 Gm. per cent to 0.16 Gm. per cent. Quantitative determinations revealed low levels of γG, γA and...
γM globulins. On September 10, 1968, the bone marrow revealed 12 per cent lymphoblasts and 1–2 per cent plasma cells. The serum protein electrophoresis at this time showed a pronounced increase, and a sharp spike in the gammaglobulin fraction. The child was maintained on methotrexate alone at this time. In January 1969 he was seen again and was observed to be in a very good remission. Bone marrow aspiration revealed only three per cent lymphoblasts and plasma cells now numbered over three per cent. Serum protein electrophoresis pattern again showed the distinct elevation in γ-globulin with the appearance of a discreet band on the stained electrophoresis strip. Repeat bone marrow examination in April 1969 showed similar findings. A skeletal survey was negative, except for some metaphyseal sclerosis felt to be secondary to the methotrexate therapy.

Attempts were made to study the patient’s immune mechanism. He received an intradermal injection of 0.1 ml. candida antigen (Dermatophytin “O”, Hollister-Stier Laboratories, Spokane, Wash.) on April 3, 1969, and in 48 hours he developed an 18-mm. induration. He also received 0.5 ml. of standard tetanus toxoid subcutaneously on the same date. Antitoxin titrations prior to this booster dose showed < 0.64 antitoxin units (AU) per ml. Serum samples obtained one and three weeks after the injection contained five AU per ml. The antitoxin levels after eight and 11 weeks were 6–10 AU/ml.

Culture of his lymphocytes with the addition of 0.1 ml. of phytohemagglutinin (Difco) revealed 50 per cent blastic transformation in 72 hours.

Immuno-electrophoresis was performed according to Scheidegger using antisera obtained commercially (The Hyland Laboratories, Los Angeles, Calif.) or specific antisera prepared in this laboratory.

Immunodiffusion tests were performed in one per cent agarose in 0.1 M phosphate-buffer pH 8.00, layered on microscope slides. The slides were treated as described above for immuno-electrophoresis.

Electrophoresis of serum was done with the Beckman Microzone equipment on cellulose acetate strips, and scanned with a Beckman analytrol scanner.

Total serum protein was determined with the Biuret method using a human serum standard supplied by the Hyland Laboratories.

DEAE-cellulose chromatography of serum was performed using stepwise elution with phosphate-buffers of increasing molarity at constant pH 7.4. The first protein fraction eluted from the column with 0.01 M phosphate-buffer was concentrated by vacuum dialysis at 4°C, dialyzed against saline and used for further studies.

Starch gel electrophoresis of serum was performed according to Smithies.7

Analytical ultracentrifugation was done in the Spinco Model E ultracentrifuge at 59780 rpm.

Quantitation of immunoglobulins and C3 was performed with the method of Fahey and McKelvey using specific antisera prepared in this laboratory and reference standards obtained from the Hyland Laboratories.

Results

The patient was diagnosed as having lymphoblastic leukemia in January 1967. Serum electrophoresis of a sample obtained 14 months (March 15, 1968) after the diagnosis was made, showed a normal pattern. However, total serum protein was low (4.75 Gm. per cent). The subsequent three serum samples (April 11, May 17, and July 9, 1968) revealed very low gamma-globulin values as well as low total protein. Then, on September 10, 1968 the serum protein level was found to be elevated to 6.75 Gm. per cent and electrophoresis revealed a sharp spike in the slow gamma region. The gammaglobulin content estimated from the electrophoresis strip was 1.27 Gm. per cent. Quantitation by the radial diffusion method revealed an increase of γ-G from 3 mg./ml. in the previous sample to 13.4 mg./ml. The complete results of serum electrophoresis and immunoglobulin and C3 quantitations are re-
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>4.75</td>
<td>5.52</td>
<td>5.85</td>
<td>5.41</td>
<td>6.75</td>
<td>7.62</td>
<td>6.67</td>
<td>6.96</td>
<td>6.92</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.89</td>
<td>3.61</td>
<td>3.79</td>
<td>3.96</td>
<td>3.97</td>
<td>3.05</td>
<td>3.23</td>
<td>3.45</td>
<td>3.48</td>
</tr>
<tr>
<td>a1</td>
<td>0.18</td>
<td>0.21</td>
<td>0.33</td>
<td>0.16</td>
<td>0.25</td>
<td>0.20</td>
<td>0.22</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>a2</td>
<td>0.66</td>
<td>0.57</td>
<td>0.53</td>
<td>0.49</td>
<td>0.51</td>
<td>0.53</td>
<td>0.49</td>
<td>0.59</td>
<td>0.61</td>
</tr>
<tr>
<td>β</td>
<td>0.49</td>
<td>0.78</td>
<td>0.80</td>
<td>0.64</td>
<td>0.76</td>
<td>0.93</td>
<td>0.93</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>γ</td>
<td>0.58</td>
<td>0.35</td>
<td>0.40</td>
<td>0.16</td>
<td>1.27</td>
<td>2.92</td>
<td>1.81</td>
<td>1.73</td>
<td>1.65</td>
</tr>
<tr>
<td>γG</td>
<td>4.30</td>
<td>2.80</td>
<td>3.00</td>
<td>3.00</td>
<td>13.40</td>
<td>24.40</td>
<td>32.40</td>
<td>21.80</td>
<td>22.90</td>
</tr>
<tr>
<td>γA</td>
<td>0.55</td>
<td>0.72</td>
<td>0.65</td>
<td>0.41</td>
<td>0.62</td>
<td>2.59</td>
<td>0.79</td>
<td>0.97</td>
<td>1.04</td>
</tr>
<tr>
<td>γM</td>
<td>0.18</td>
<td>0.12</td>
<td>0.13</td>
<td>0.12</td>
<td>0.53</td>
<td>2.59</td>
<td>0.46</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>C3</td>
<td>1.48</td>
<td>1.17</td>
<td>1.64</td>
<td>1.56</td>
<td>1.64</td>
<td>1.72</td>
<td>1.83</td>
<td>3.32</td>
<td>3.55</td>
</tr>
</tbody>
</table>
Fig. 1.—Zone electrophoresis patterns of serum from patient C.W. obtained on dates indicated. For percentage distribution of electrophoretic fractions see Table 1.

corded in Table 1. The changes occurring in the serum electrophoresis pattern in the course of time are shown in Fig. 1.

Starch gel electrophoresis revealed a sharp band in the slow gamma region. Subsequent serum samples showed changes suggesting multiple banding in the gammaglobulin area (Fig. 2). However, this was a transient phenomenon.

Characterization of the abnormal protein. Immunoelectrophoresis of the patient's serum obtained at the time when the electrophoresis pattern exhibited a spike in the gamma region, showed that a gammaglobulin of limited electrophoretic mobility was present (Fig. 3). This protein reacted only with a anti-γG serum, and was shown to possess light chains of the K type only (Figs. 4 and 5). The protein was isolated by DEAE-cellulose chromatography and examined in the ultracentrifuge. The isolated protein sedimented as a homogeneous peak with a sedimentation coefficient of 6.3 S (Fig. 6). This value is uncorrected for protein concentration.

An immunodiffusion test using anti-γG serum specific for γ-chain showed complete identity with purified γG isolated from a pool of normal human serum (Fig. 7).

A urine specimen obtained in April 1969 was concentrated 100-fold by vacuum dialysis and was examined for Bence Jones protein with negative results.

Discussion

The role of lymphoid cells in both normal and abnormal immunoglobulin production is well established. In lymphoblastic leukemia with vastly in-
PARAPROTEINEMIA IN A CHILD WITH LEUKEMIA

Fig. 2.—Alkaline starch gel electrophoresis of serum samples from C.W. obtained on dates below:
#2 — 4/11/68
#3 — 7/9/68
#4 — 9/10/68
#5 — 1/9/69
#6 — 3/5/69
#7 — 4/3/69; #1 and #8: normal human serum.

Fig. 3—Immunoelectrophoresis analysis of serum C.W. Upper well: serum C.W. 9/10/68. Lower well: normal human serum. Antiserum: antihuman serum (Hyland Laboratories).

Increased numbers of potentially immunoglobulin-producing cells, a corresponding hypergammaglobulinemia is rarely seen. In fact, most children with this disease exhibit a marked hypogammaglobulinemia. It therefore appears that malignant lymphoid cells have lost some or all of their capacity to produce immunoglobulins. However, this interpretation is complicated by the use of immunosuppressive drugs in these patients, and it becomes difficult to dis-
tistinguish between inherently abnormal cellular functions and drug-induced changes.

In rare instances of lymphomatous disorders, myelomalike proteins appear, some even possessing antibody activity of an autoimmune nature. This paradoxical situation of antibody production in the presence of a general deficiency in immunological capacity may be analogous to that observed in patients with congenital and acquired agammaglobulinemia. It is also interesting to note that there is a higher incidence of leukemia and lymphoma in patients with agammaglobulinemia.

Various antimetabolites are known to inhibit the immune responses in man. In most cases the effect of these drugs has been examined in cancer patients and also in patients with immunological diseases. In a study by Swanson and Schwartz it was found that the primary response was affected in every patient and therapy with azathioprine (imuran) or amethopterin. In addition to complete inhibition of the antibody response, three types of abnormalities were found: prolongation of the induction time, delayed or absent synthesis of yM antibodies (although yM antibodies were found in normal amounts), and enhancement of antibody synthesis. The enhancement of antibody synthesis appeared to be limited to the yM class. The authors also noted a marked effect on the immunoglobulin levels, in that eight of 20 patients had IgG levels decreased by 20 per cent or more, six of 18 and seven of 18 patients had decreases in IgA and IgM levels, respectively.

A recent study by Bagab et al. on immunoglobulin levels in leukemic children indicates that the disease process itself is not the all important factor in the development of hypogammaglobulinemia of these patients. The fluctua-
PARAPROTEINEMIA IN A CHILD WITH LEUKEMIA

Fig. 6.—Ultracentrifuge analysis of monoclonal protein isolated from serum C.W. obtained on 4/9/69. Purified protein sedimented as homogeneous peak with \( S_{20,w} = 6.3 \) at protein concentration of 10 mg. per ml. Sedimentation left to right. Picture taken 48 minutes after reaching 59,780 rpm.

Fig. 7.—Immunodiffusion analysis of isolated paraprotein (1 mg./ml.) in well #1 using anti-human-\( \gamma \)G absorbed with K and lambda chains (well A). Well #2 normal \( \gamma \)G (1 mg./ml.).

...tions of the immunoglobulin levels with time could largely be attributed to the use of immunosuppressive drugs. Cessation of therapy would result in a substantial increase in immunoglobulin levels, which would subsequently fall when therapy was reinstituted.

The appearance of a monoclonal gammopathy in leukemic children under treatment with immunosuppressive drugs may be a rare occurrence, although perhaps not unexpected. It is possible that one or more clones of cells escape the effect of the drug, remain resistant and function normally in terms of immunoglobulin synthesis and response to antigenic stimulation. It is apparent (Table 1) that in the patient reported here, all immunoglobulins showed increased levels at one time (January 9, 1969). At that time there were indications of multiple monoclonal \( \gamma \)G bands on starch electrophoresis (Fig. 2), and a marked increase in levels of \( \gamma \)A and \( \gamma \)M as well. However, only the \( \gamma \)G immunoglobulin possessed properties characteristic of a monoclonal protein. Subsequently, the \( \gamma \)A and \( \gamma \)M levels returned to low or normal levels.
It seems evident that the increased level of $\gamma$-globulin appearing in September 1968 is due to the presence of a $\gamma G$ immunoglobulin of slow and limited electrophoretic mobility. This protein appears to be a complete molecule of 6.3 S (uncorrected for protein concentration) and exhibits most characteristics of a myeloma protein. The multiple banding in the gamma region appearing in January 1969 later disappeared leaving only the slow band observed previously. The C'3 levels were initially within normal range. After the appearance of the monoclonal protein, the C'3 level also increased to more than twice the normal level (Table 1). Interestingly, such increases in the C'3 levels have been observed in patients with multiple myeloma. However, no explanation can be offered at the present time for this protein anomaly.

Bence Jones proteinuria was described in the leukemic patient with monoclonal gammopathy reported by Stoop et al. However, a urine sample obtained from our patient in April 1969 when the serum level of the $\gamma G$ paraprotein was still high (approx. 22 mg./ml.), did not contain Bence Jones proteins. However, it is possible that Bence Jones proteinuria may be of a transient nature and could have been missed.

The relationship between the appearance of a paraprotein and the favorable course in this patient remains obscure. It may be purely coincidental. Apparently, the patient possesses normal immunological competence as determined by his response to antigenic stimulation with candida skin test antigen, tetanus toxoid and a good response of his lymphocytes to phytohemagglutinins.

The patient has thus been able to keep up his antitoxin levels (0.64 AU/ml.) in spite of prolonged immunosuppressive therapy. He also showed an excellent response to secondary stimulation with tetanus toxoid.

The 50 per cent blast transformation obtained with this patient's lymphocytes is somewhat lower than normal, which is in the range of 70-90 per cent. The reason for this decrease of percentage blast transformation is uncertain. It has been found that lymphocytes from patients with acute lymphatic leukemia respond normally to phytohemagglutinin. It is possible that administration of the immunosuppressive drugs played a role in depressing this response. It is remarkable that in spite of continuing therapy with cytotoxic and immunosuppressive drugs and a persisting leukopenia, this child has never had problems with infections. The "spontaneous" remission in this boy occurred almost synchronously with the appearance of changes in the gamma-globulins. Whether the patient's paraprotein represents an antibody response to his own leukemic cells and is involved in controlling the formation of malignant cells is purely speculative.

**SUMMARY**

A four-year-old boy was diagnosed as having lymphoblastic leukemia in January 1967. On immunosuppressive therapy he had several clinical remissions and relapses. In mid-1968 he entered a period of sustained clinical remission. At that time a monoclonal gamaglobulin peak appeared in the electrophoresis pattern of his serum. The paraprotein which was present in concentrations above 44 mg. per ml., was characterized as $\gamma G$ with K-chain specificity. There was no evidence of multiple myeloma, and no urinary
PARAPROTEINEMIA IN A CHILD WITH LEUKEMIA

Bence-Jones protein was detected. The patient was investigated for cellular and humoral type immunologic responsiveness during the sustained remission period with normal results. The possible relationship between the paraprotein and the favorable clinical course in this patient is discussed.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Geoffrey Edsall, Massachusetts State Laboratory Institute, Boston, Massachusetts for the tetanus antitoxin determinations, and Dr. Teresa Vietti for continued support.

REFERENCES

Paraproteinemia in a Child with Leukemia

KAARE J. LINDQVIST, ABDELSALAM H. RAGAB and C. KIRK OSTERLAND