Transient IgA₁-λ Paraproteinemia During Treatment of Acute Myelomonoblastic Leukemia

By B. Van Camp, Ph. Reynaerts, J. P. Naets, and J. Radl

Monoclonal plasma cell proliferation with secretion of IgA₁-λ and free lambda light chains during a phase of bone marrow aplasia following intensive chemotherapy was observed in a patient suffering from acute myelomonoblastic leukemia. The clonal expansion and regression was investigated at the cellular level by immunofluorescence using an antisera against the idiotype of the paraprotein. Although a large panel of common antigens was used for testing, no antibody activity of the paraprotein could be demonstrated.

PARAPROTEINS have been observed in acute leukemia, and these could usually be attributed to a simultaneously occurring plasma cell proliferation. In some cases, electron microscopy and immunofluorescence studies on the blast cells revealed their plasma cell nature and indicated that they secreted a monoclonal immunoglobulin. Transient paraproteins have also been recognized during the course of malignant nonlymphoid hematologic diseases, especially in childhood. Although no antibody activity of the paraprotein could be demonstrated, the possible involvement of acute viral and bacterial infections during immunosuppressive treatment was considered. In one case, a transient paraproteinemia was thought to induce a spontaneous regression of an erythroleukemia.

We investigated a patient with acute myelomonoblastic leukemia who presented a clear-cut but transient paraproteinemia after intensive chemotherapy. An antiserum was raised against the idiotype of the paraprotein and used as a marker of monoclonality at the cellular level. A large panel of commonly occurring antigens was used in attempts to detect antibody activity of the paraprotein.

CASE HISTORY

A female patient (L’Ecl.), aged 72 yr, was admitted (2/5/76) because of fatigue, rheumatic symptoms, and generalized purpura (Fig. 1). Severe pancytopenia and the presence of peripheral blast cells led to the diagnosis of acute leukemia. The morphology and the cytochemistry of the bone marrow led to the diagnosis of acute myelomonoblastic leukemia. The total serum protein concentration was 62 g/liter with a normal electrophoretic profile. Initial treatment with 6-mercaptopurine and prednisolone was instituted. A soft painful ulceration on the metacarpophalangeal articulation of the right hand developed; this was 4 days before the day of detection of the paraprotein.

The hematologic picture, large numbers of atypical plasma cells as well as macrophages, some of which contained cocci, were seen in an otherwise depleted marrow. Agar and immunoelectrophoresis of serum on 2/2/77 indicated the presence of a monoclonal IgA₁-λ immunoglobulin, and a monoclonal light chain of lambda type was found only in the concentrated urine. Prior to the first date of the detection of the paraprotein, the electrophoretic pattern of the serum, obtained by zone electrophoresis on cellulose acetate, had been normal. A few days later, a second clinical remission, during which the paraprotein and the atypical plasma cells disappeared, was observed. Maintenance therapy with 6-mercaptopurine and prednisolone was instituted. A soft painful bilateral axillary adenopathy developed during the last 2 mo of the patient’s life. The bone marrow was infiltrated with blast cells; an increase in plasma cells was also observed. At this time, a polyclonal hypergammaglobulinemia was noted. Hemocultures revealed Escherichia coli. Treatment with daunomycin failed, and the patient died.

Postmortem examination disclosed a blastic infiltration mainly in the bone marrow, kidneys, tonsils, and to a lesser extent, in other tissues. Many lymph nodes were found to be enlarged, especially in the axillae, and showed an increased number of reactive plasma cells. A systemic Candida albicans infection infiltrated practically all organs. The finding of an unexpected paraproteinemia in this patient prompted us to undertake a study at the cellular level by using an antiserum to the idiotype of the paraprotein.

MATERIALS AND METHODS

Serum and Urine Analysis

Prior to the first detection of the paraprotein in the serum, electrophoretic patterns were obtained by zone electrophoresis on cellulose acetate. Agar electrophoresis was carried out according to Wieme and microimmunoelectrophoresis according to Radl. Monospecific antisera were used.

These methods were also applied on concentrated urine, starting from the day of detection of the paraprotein.

From the Experimental Medicine, Unit of Hematology of the Free University of Brussels (V.U.B.), the Department of Hematology, University Hospital Brugmann, and the Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands.

Supported in part by Grant 3.0019.75 FGWO Belgium.

Submitted January 17, 1979; accepted September 5, 1979.


© 1980 by Grune & Stratton, Inc.

0006–4971/80/5501–0004$01.00/0

Blood, Vol. 55, No. 1 (January), 1980

21
Fig. 1. Hematologic parameters during treatment. The paraprotein was present in the serum during the period indicated by the arced column.

Antiidiotype Antiserum

An antiserum against the idiotype of the IgA,,-X paraprotein (day 9/2/77) was raised in guinea pigs according to a technique described by Radl et al. Specificity testing was performed (A) by immunodiffusion techniques and immunoelectrophoresis in agar plates containing 3% PEG 6000, and (B) by indirect immunofluorescence on polyclonal and monoclonal bone marrow preparations.

Immunofluorescence Study of Cytoplasmic Immunoglobulins (CIg)

All preparations were viewed with a Zeiss fluorescence microscope with epillumination and equipped with a mercury 50-W light source and selective filters for simultaneous FITC and TRITC staining. Blood lymphocytes were isolated by Ficoll-Hypaque gradient separation. Bone marrow cell suspensions were obtained according to the technique of Hijmans et al. The cell suspension was thoroughly washed and sedimented on microscope slides by cytocentrifugation.

Anti-light and anti-heavy chain sera conjugated to FITC were routinely used with an albumin TRITC counterstain. In addition, the two wave length technique was applied for detection of light chains versus heavy chains (direct method) and idiotype versus isotype (indirect–direct method); interspecies cross-reactivity of the GP antiidiotype serum and of goat anti-human IgA, and kappa and lambda light chains conjugated to FITC were applied. Pooled sera and sera of young healthy adults were used as controls. In addition, heterologous blasts were incubated with the IgA,,-X paraprotein, after which the same conjugated antibodies were used.

Surface Immunoglobulin (slg) Study of Peripheral Blood Cells

Mononuclear cells were adjusted to 10^6 cells/ml. Vital staining of slg was obtained after incubation with antisera conjugated with FITC. On one occasion (day 6/7/77), indirect fluorescence was performed on the cells of the patient with the antiidiotype serum.

Phase contrast microscopy permitted the recognition of the mononuclear cells, and only small lymphocytes were examined for the presence of the slg.

Antibody Activity Determination on Serum (day 2/9/77)

Screening for antibody activity was performed in agar plates containing 3% PEG 6000. The antigens were derived from commonly occurring bacteria, as detailed elsewhere. A hemolytic assay was used for the determination of antistreptolysine and antistaphylolysin.

Complement fixation, indirect immunofluorescence, and radioimmunoassay methods provided viral and protozoan screening for the following diseases: cytomegalic disease, Herpes simplex, Herpes zoster, Coxsackie, Australia antigen-positive hepatitis, mononucleosis, toxoplasmosis, Rickettsia. Suspensions of the Staphylococcus albus, which were obtained from the patient’s positive hemocultures during the first phase of aplasia, were washed several times, sedimented on slides, fixed with methanol-ethanol at -20°C, and incubated with different dilutions (1/2–1/4000) of the serum of the patient. After washing, antisera against human Ig, IgA, and kappa and lambda light chains conjugated to FITC were applied. Pooled sera and sera of young healthy adults were used as controls. In addition, heterologous blasts were incubated with the IgA,,-X paraprotein, after which the same conjugated antibodies were used.

RESULTS

The agar and immunoelectrophoretic pattern of the serum (Fig. 2) on day 2/2/77 indicated the presence of a monoclonal IgA,,-X immunoglobulin. In the concentrated urine (day 9/2/77), a small amount of free light chains of lambda type was found.
The monoclonal expansion of the paraprotein-producing cells was followed with the antiidiotype marker. The decrease in the quantity of paraprotein in the serum was related to the disappearance of the monoclonal plasma cell population in the bone marrow (Table 1). Up to 90% of IgA-positive plasma cells and 66% of all plasma cells were part of this monoclonal proliferation at day 7/2/77. On day 8/2/77, a considerable increase in lymphoplasmacytoid cells in the peripheral blood was observed. Nine percent of these cells demonstrated an intracellular Ig, one-third of which belonged to the idiotype-positive clone. These cells disappeared from the blood within 2 wk, and small polyclonal IgM lymphocytes appeared. Less than 1% of all plasma cells in the bone marrow bore the idiotype on day 5/3/77.

The idiotype was restricted to the plasma cells containing IgA,\(\lambda\). No other isotype was observed simultaneously with the idiotype in the same cell in either the peripheral blood or the bone marrow. At day 8/2/77, a high concentration of surface IgA and a shift towards lambda type was found. This pattern disappeared 2 wk later. In addition, the indirect immunofluorescence test with the patient’s own blasts (6/7/77) incubated with antiidiotype antiserum gave negative results.

In the search for antibody activity of the paraprotein, only normal or negative results were obtained with the bacterial, protozoan, and fungal antigens, that were used in this study, and no evidence of an antibody activity against autologous or heterologous myeloblasts was found.

**DISCUSSION**

The simultaneous occurrence of a myelomonoblastic leukemia supervening on long-standing multiple myeloma after or without long-term chemotherapy with alkylating agents has been recognized several

**Table 1. Cytoplasmic Immunoglobulin Distribution (%) in Bone Marrow**

<table>
<thead>
<tr>
<th>Date</th>
<th>Ig +</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgD</th>
<th>k/(\lambda)</th>
<th>IgA,(\lambda)</th>
<th>IgA,(\kappa)</th>
<th>IgA-id</th>
<th>Ig-id†</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/02/77</td>
<td>38</td>
<td>20.5</td>
<td>78</td>
<td>1.5</td>
<td>0</td>
<td>0.26</td>
<td>95</td>
<td>5</td>
<td>90</td>
<td>66</td>
</tr>
<tr>
<td>23/02/77</td>
<td>3</td>
<td>66</td>
<td>27.5</td>
<td>6.5</td>
<td>0</td>
<td>0.6</td>
<td>82</td>
<td>18</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16/03/77</td>
<td>4</td>
<td>60</td>
<td>30</td>
<td>3.5</td>
<td>6.5</td>
<td>0.7</td>
<td>ND</td>
<td>ND</td>
<td>55</td>
<td>8</td>
</tr>
<tr>
<td>03/05/77</td>
<td>18</td>
<td>53.5</td>
<td>42.5</td>
<td>4</td>
<td>0</td>
<td>1.1</td>
<td>ND</td>
<td>ND</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.45 ± 2.2</td>
<td>53.2 ± 9.3</td>
<td>41.7 ± 15.3</td>
<td>8.9 ± 5</td>
<td>—</td>
<td>1.27 ± 0.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

ND, not done.

*Percent of bone marrow cells containing cytoplasmic Ig.
†Anti-total Fab,−TRITC was applied in addition to antiidiotype serum.
times.\textsuperscript{1,3} In such cases, however, clear-cut features of both malignant diseases were always present during life or at autopsy. In a few patients, immunofluorescence and electron microscopy revealed\textsuperscript{4-6} that the blast cells were part of the plasma cell proliferation.

Transient paraproteins were mentioned during the course of a few acute leukemias and attributed to intercurrent viral infections secondary to an immunosuppressive treatment.\textsuperscript{7-9,17} In one case,\textsuperscript{10} the sudden onset of paraprotein just before a spontaneous remission of an erythroleukemia was thought to be an immunologic response to the blastic proliferation. In none of these cases could antibody activity of the paraprotein be demonstrated. The association and relationship of the paraproteinemia with leukemia in our patient is not clear. Although the diagnosis of acute myelomonoblastic leukemia was well established, a transient monoclonal plasma cell proliferation developed during bone marrow aplasia following polychemotherapy.

The plasma cell proliferation had many characteristics of a malignant clone, such as plasma cell polymorphism, high paraprotein level, and Bence Jones protein excretion in the urine. Although its very short duration is an argument against malignancy, it may be speculated that the paraproteinemia represented a malignant transformation of a B-cell clone, (due to, for instance, chemical or viral mutagens) which, however, was “hit” again after initial expansion.\textsuperscript{18} The new mutation might have caused changes incompatible with further survival of the clone. In this respect, it seems appropriate to emphasize that the monoclonal expansion was visible not only in the bone marrow but also in the peripheral blood. The shift towards IgA-lambda at the surface of small peripheral B lymphocytes and the presence of lymphoplasmacytoid cells containing the monoclonal immunoglobulin are in accord with recent findings on monoclonal precursor cells in multiple myeloma.\textsuperscript{19,20} The question as to whether the paraprotein-producing clone in our case was triggered by an antigen or was a “nonspecific” clonal escape remains unanswered. In any event, its antibody activity was directed neither to membrane antigen of the myeloblasts nor against a number of infectious agents.

An alternative explanation of the paraproteinemia in our patient may be offered. In recent studies on the reconstitution of the immune system after bone marrow transplantation in patients with immunodeficiencies,\textsuperscript{15,22} aplastic anemia with pretreatment with an immunosuppressive regimen,\textsuperscript{23} in lethally irradiated monkeys\textsuperscript{24} and mice,\textsuperscript{25} transient paraproteins were often seen. Some of these could be shown to have antibody activity.\textsuperscript{26} Explanation of these findings has been sought in analogy to what happens in normal ontogenesis. In the early stages of the development as well as during early stages of reconstitution, the immune response shows a restriction in its heterogeneity as compared to that in a mature individual.\textsuperscript{27} In the presence of excess stimulation, insufficient numbers of the precursor cells of different clones may be available to respond, resulting in the appearance of homogeneous antibodies. More recently, a delay in maturation and consequently dysfunction in T-cell cooperation and control functions over the B cells, was considered to be responsible for such clonal expansions.\textsuperscript{28} Only after complete reconstitution of the immune system was a normal heterogeneous Ig spectrum observed.\textsuperscript{21,26} An analogous situation might be assumed in the present case. Aggressive chemotherapy could have led to immunosuppression and unequal depletion of the T and B precursor cell compartments, with a severely disbalanced immune response to an unknown antigenic stimulus as a consequence.

ACKNOWLEDGMENT
We wish to thank Prof. A. Capron for Aspergillus and Candida antibody testing. Prof. W. Hjimans for critical revision of the text and inspiring discussions, P. Stroobants and L. Broodtaerts for expert technical assistance.

REFERENCES
\begin{enumerate}
\item Osserman EF: Proceedings of the Nobel Symposium on Gamma Globulins. Stockholm, Almqvist and Wiksell, 1967, p 573
\item Danon F, Seligmann M: Serum monoclonal immunoglobulins in childhood. Arch Dis Child 48:207, 1973
\item Wieme RJ: Agarel Electrophoresis. Amsterdam, Elsevier, 1965, p 142
\item Radl RJ: Light chain typing of immunoglobulins in small samples of biological material. Immunology 19:137, 1970
\item Radl J, De Glopper E, De Groot G: A rapid, simple and
reliable technique for preparation of antisera against idiotypes of homogeneous immunoglobulins. Vox Sang 35:10, 1978


Dooren LJ, Vossen JJ, Radl J: unpublished observations


Transient IgA-lambda paraproteinemia during treatment of acute myelomonoblastic leukemia

B Van Camp, P Reynaerts, JP Naets and J Radl