Monoclonal Gammopathy in a Child with Leukemia

By Jan W. Stoop, Ben J. M. Zegers, Cees Van Der Heiden and Rudy E. Ballieux

Paraproteins* are generally found in the serum in patients suffering from multiple myeloma or Waldenström's macroglobulinemia. Recently, however, there have been a number of reports on patients in whom paraproteinemia* was found in association with a myeloproliferative disorder such as polycythemia, erythroleukemia, acute myeloblastic and lymphoblastic leukemia, and chronic myelocytic leukemia.1

Paraproteinemia in children is exceedingly rare. Stoop et al.2 observed a clear-cut case of paraproteinemia in a child; Hochwald and Thorbecke3 described a child whose CSF contained a paraprotein. The child described by Schaller et al.4 was probably suffering from paraproteinemia also, although these investigators failed to establish this with certainty. Dalloz et al.5 observed a child with Aldrich's syndrome in whom transient paraproteinemia was demonstrated. Harhoe et al.6 described a patient who developed a donor-type paraproteinemia following a thymus gland transplantation. In a recent publication Danon et al.7 mention four children with paraproteinemia, including two who had been previously discussed in the literature; the two new cases concerned a 6 month old boy with dual paraproteinemia and a 3 year old girl with acute myeloid leukemia and (transient?) paraproteinemia.

This report describes a well-documented case of paraproteinemia in association with an acute leukemia disorder in a child.

Case Report

The patient was a boy, born at term on October 21, 1963 after an uneventful pregnancy; there were no perinatal or neonatal difficulties. The boy was the first child of healthy, nonconsanguineous parents. The family history includes no significant diseases.

In June 1965 the boy developed tonsillitis and otitis media; he made no swift recovery: the body temperature remained too high, he continued to be listless and became pale. A pediatrician was consulted, who found that the throat and ears were disturbed; many enlarged lymph glands were palpable, and there was considerable enlargement of the liver and spleen. The hematologic data obtained are summarized in Table 1. The

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*In this report the terms "paraproteins" and "paraproteinemia" are only used for descriptive purposes as a synonym of M-component and monoclonal gammopathy.
bone marrow was highly cellular and showed proliferation of cells probably lymphoblasts, including some with nucleoli. Occasional cells of the red and white system were seen. (Fig. 1).

The condition was diagnosed as acute (lympho)blastic leukemia. The patient responded favourably to transfusion of 400 ml. blood and administration of prednisolone and 6-mercaptopurine: a complete remission occurred, i.e., blood count and bone marrow showed complete normalization and the enlargement of the lymph glands, liver and spleen disappeared entirely. Treatment was continued with 5 mg. prednisolone and 30 mg. 6-mercaptopurine daily. No problems were encountered until prednisolone was discontinued late in December 1965 because of a varicella contact. One week later the patient began to complain about headaches and nausea and started to vomit.

On January 20, 1966 the boy was referred to the University Children’s Hospital. At physical examination he gave an impression of illness. No abnormalities were found in the ears, nose, throat, heart and lungs. The abdomen was neither distended nor hard. There was no enlargement of liver and spleen, and no enlarged lymph glands were palpable.

Percussion of the skull elicited a cracked pot sound; the coronal suture showed a dehiscence of about 4 mm. Both optic discs were vaguely outlined. There were no signs of meningeal irritation. No neurologic signs were observed.

The hematologic data obtained at admission are presented in Table 1. Bone marrow, liver and renal functions were normal. Urine (albustix negative) and feces were normal. The CSF contained 145/3 white cells, including 98 per cent lymphocytes, and 10/3 erythrocytes; its protein concentration was 12 mg./100 ml. and its glucose level was 92 mg./100 ml.

The condition was diagnosed as an intracranial localization of the leukemic process, which otherwise seemed to be in good remission.

Prednisolone medication was resumed while 6-mercaptopurine was continued at a daily dosage of 30 mg. Five intrathecal injections of 6 mg. methotrexate each were given. The symptoms of increased intracranial pressure disappeared rapidly. Blood count and bone marrow continued to be normal also when the prednisolone was gradually reduced and finally discontinued; but the initially normalized ESR showed some increase again (Table 1).

On March 9, 1966 the patient was discharged on a maintenance dose of 30 mg. 6-mercaptopurine daily.

His condition remained stationary until early in June 1966, when headaches and vomiting recurred. Early in July the boy showed the same signs of increased intracranial pressure as in January 1966. Lymph glands, liver and spleen were not enlarged. The blood count did not indicate a relapse of the leukemic process elsewhere. The CSF contained 350013 white cells, including 95 per cent lymphocytes (70 per cent mature and 30 per cent blasts with nucleoli); the protein concentration was 22 mg./100 ml. and the glucose concentration was 54 mg./100 ml.

After two intrathecal injections of 7.5 mg. methotrexate each, the CSF changes disappeared.

Late in September 1966 the patient suffered a generalized relapse of the leukemic process with high fever (40 C.), enlargement of all lymph glands and hepatomegaly and splenomegaly (both 3 cm. below the costal margin). The hematologic findings are given in Table 1.

The bone marrow was highly cellular with numerous mitoses and contained 95 per cent blasts, no megakaryocytes and sporadic erythroblasts or myelocytes (Fig. 2). The CSF showed no abnormalities; urine (albustix negative) and feces were normal.

The patient was treated with methotrexate and prednisolone, and received two blood transfusions of 500 ml. each. As judged by blood picture (Table 1) and bone marrow, a remission occurred fairly soon. Subsequently, however, there were numerous complications. In mid-November 1966 the boy developed purulent meningitis caused by Listeria monocytogenes; this was cured by antibiotic medication. Late in November the boy developed extensive necrotic herpes simplex of the sacral region; this infection con-
### Table 1.—Hematological Data Obtained at Various Dates

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb (Gm. per cent)</th>
<th>Hematocrit</th>
<th>Reticulocytes</th>
<th>Thrombocytes</th>
<th>Leucocytes</th>
<th>Stabs</th>
<th>Segm. Neutroph.</th>
<th>Lymphocytes</th>
<th>Blasts</th>
<th>ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/25/65</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>115,000</td>
<td>3%</td>
<td>1%</td>
<td>97%</td>
<td>2%</td>
<td>100 mm</td>
</tr>
<tr>
<td>1/20/66</td>
<td>14.8</td>
<td>43</td>
<td>9%</td>
<td>11%</td>
<td>6700</td>
<td>68%</td>
<td>68%</td>
<td>29%</td>
<td>—</td>
<td>36 mm</td>
</tr>
<tr>
<td>3/9/66</td>
<td>13.4</td>
<td>40</td>
<td>10%</td>
<td>33%</td>
<td>6300</td>
<td>43%</td>
<td>43%</td>
<td>53%</td>
<td>—</td>
<td>35 mm</td>
</tr>
<tr>
<td>7/6/66</td>
<td>12.3</td>
<td>38</td>
<td>11%</td>
<td>33%</td>
<td>11100</td>
<td>56%</td>
<td>56%</td>
<td>33%</td>
<td>—</td>
<td>48 mm</td>
</tr>
<tr>
<td>9/30/66</td>
<td>8.6</td>
<td>28</td>
<td>3%</td>
<td>95%</td>
<td>46000</td>
<td>1%</td>
<td>1%</td>
<td>95%</td>
<td>—</td>
<td>88 mm</td>
</tr>
<tr>
<td>11/11/66</td>
<td>12.1</td>
<td>43</td>
<td>10%</td>
<td>31%</td>
<td>6400</td>
<td>62%</td>
<td>62%</td>
<td>31%</td>
<td>—</td>
<td>3 mm</td>
</tr>
<tr>
<td>12/2/66</td>
<td>11.2</td>
<td>34</td>
<td>13%</td>
<td>13%</td>
<td>5600</td>
<td>87%</td>
<td>87%</td>
<td>13%</td>
<td>—</td>
<td>9 mm</td>
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<td>2/8/67</td>
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<td>20</td>
<td>10%</td>
<td>51%</td>
<td>30800</td>
<td>44%</td>
<td>44%</td>
<td>51%</td>
<td>—</td>
<td>71 mm</td>
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continued in spite of all measures taken. Early in December 1966 the boy suffered from R.S. virus and adeno-virus infections and late in December the protein pattern showed increasing changes.

The η-globulin concentration increased and a narrow-banded protein fraction became visible; proteinuria was now found. A severe relapse of the leukemic process occurred early in February 1967 (Table 1).

The patient died on February 12, 1967; request for postmortem examination was denied.

MATERIAL AND METHODS

Agar electrophoresis and immunoelectrophoresis (IE) were carried out according to Wieme8 and Scheidegger9 respectively. Paper electrophoresis was carried out as indicated by Meulemans.10 Immunoglobulins were quantitatively determined by the radial diffusion technic.11,12

The antisera specific for IgG, IgA and IgM, used in immunoelectrophoretic experiments and quantitative determinations, were produced in rabbits against heavy chains of pool IgG, against pool IgA (kindly supplied by Dr. P. J. J. van Munster, University Children’s Hospital, Nijmegen) and against a purified macroglobulin from a patient with Waldenström’s macroglobulinemia, respectively. After absorption (with L-chains, IgG

Fig. 1.—Bone marrow smear of June 1965. Highly cellular with many cells resembling lymphoblasts. May-Grünwald Giemsa stain. × 125
and serum from a patient without IgM, respectively), specificity was tested in IE. The anti-IgD used was a gift from Dr. J. F. Fahey of the National Cancer Institute, Bethesda, Md.

Anti-κ and anti-λ were obtained in rabbits by immunization with purified κ and λ Bence Jones proteins.

Antihuman antisera of horse origin was obtained from the Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross.

The control serum for IE and quantitative determinations was made up of about 20 serum samples from healthy donors. Immunoglobulin levels of this reference serum, as determined by the radial diffusion technique, were 1038 mg./100 ml. for IgG, 86 mg./100 ml. for IgM, and 194 mg./100 ml. for IgA. Sera obtained from our patient at different dates were evaluated against this reference serum. Details on techniques of quantification and standardization of our reference serum will be presented in separate papers.13,14

Autoantibodies against thyroid (colloid and cytoplasm), gastric mucosa, smooth muscle tissue and nuclear components were kindly determined in our laboratory by Dr. N. A. J. Mul, who used commercial available fluorescent antiglobulin anti-sera (Roboz) in the indirect immunofluorescence microscopy.

Immunofluorescence studies on bone marrow cells were carried out by Dr. W. Fig. 2.—Bone marrow smear of September 1966. Highly cellular, containing predominately blasts. May-Grünwald Giemsa stain. × 250
MONOCLONAL GAMMOPATHY

Table 2.—Serum Protein Concentrations in Gm. per 100 ml. of Serum at Various Dates

<table>
<thead>
<tr>
<th></th>
<th>1/24/66</th>
<th>12/12/66</th>
<th>12/24/66</th>
<th>1/20/67</th>
<th>1/27/67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein content</td>
<td>6.4</td>
<td>4.4</td>
<td>6.0</td>
<td>6.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.2</td>
<td>1.9</td>
<td>2.5</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>α1-globulins</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>α2-globulins</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>β-globulins</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>γ-globulins</td>
<td>0.8</td>
<td>0.7</td>
<td>1.8</td>
<td>2.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Hijmans (Research Laboratories, Department of Rheumatology, University of Leyden) with the aid of a technic described elsewhere.\textsuperscript{15}

Pathologic protein fractions were isolated from the patient's serum and urine by precipitation with 30 per cent ammonium sulfate. The precipitate was dissolved and dialysed against phosphate buffer at pH 8.0, 0.01 M. Final purification was achieved by chromatography on DEAE-cellulose with step-wise elution using phosphate buffer at pH 8.0 of increasing molarity. Ultracentrifugal analyses of protein preparations were made in the Spinco analytical ultracentrifuge.

Gm.-typing was done by Dr. Erma van Loghem (Central Laboratory of the Blood-transfusion Service of the Netherlands Red Cross, Amsterdam).

Isohemagglutinins were determined by Dr. C. Dudok de Wit (Blood Transfusion Laboratory, University Hospital, Utrecht).

Virus isolations and determination of the complement-fixing antibodies against the viruses isolated were carried out at the Department of Virology (head, Prof. Dr. R. Gispen) of the National Institute of Public Health, Bilthoven.

RESULTS

The serum protein study with the aid of paper electrophoresis disclosed that hypogammaglobulinemia existed early in December 1966. In the latter half of this month, the γ-globulin concentration of the serum increased considerably and this increase continued until early in February 1967—the time of the patient's death (Table 2). These findings were confirmed by the results of quantitative determination of the immunoglobulins by the radial diffusion technique. The IgG level increased from 324 mg./100 ml. on December 12, 1966 to 1972 mg./100 ml. on December 24, 1966; the IgA and IgM concentrations likewise increased (Table 3). Immunoelectrophoretic analysis of the serum samples obtained on these dates disclosed that the increase in IgG concentration was not of a purely polyclonal nature. The course of the IgG precipitation line in the IE pattern suggested a selective increase of a subpopulation of the G-immunoglobulin class. This slight selective increase, however, became quite apparent at serum analysis on January 27, 1967, at which time both agar gel electrophoresis and IE revealed a monoclonal gammopathy (Fig. 3). The serum paraprotein was classified as IgG1.

Table 3.—Immunoglobulin Levels in Serum Determined by the Radial Diffusion Method

<table>
<thead>
<tr>
<th></th>
<th>1/24/66</th>
<th>12/12/66</th>
<th>12/24/66</th>
<th>1/27/67</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>not determined</td>
<td>27 mg. %</td>
<td>110 mg. %</td>
<td>82 mg. %</td>
</tr>
<tr>
<td>IgA</td>
<td>not determined</td>
<td>54 mg. %</td>
<td>111 mg. %</td>
<td>62 mg. %</td>
</tr>
<tr>
<td>IgG</td>
<td>not determined</td>
<td>324 mg. %</td>
<td>1972 mg. %</td>
<td>2000 mg. %</td>
</tr>
</tbody>
</table>
Fig. 3.—Immunoelectrophoretic analysis of the patients serum obtained at various dates and of the urine.

(A) Development of monoclonal gammopathy as demonstrated in the immunoelectrophoretic pattern of the serum samples obtained on December 12, 1966, on December 24, 1966 and on January 27, 1967.

(B) Presence of a monoclonal protein component in the γ-globulin region of the patients serum of January 27, 1967, and in the patients urine (70 × concentrated) of January 27, 1967. For comparison the agar gel-electrophoretic pattern of normal human serum is included.

(C) The M-component in the patients serum and the Bence Jones protein in the urine were typed as type L.
The IgA and IgM concentrations in this patient's serum at that time were lower than on December 24, 1966 (Table 3). IgD was not demonstrable by IE. In the ultracentrifuge the serum produced a sharp, high G-peak (Fig. 4). Analysis of the patient's (70 × concentrated) urine of January 27 disclosed a monoclonal band in the medium-speed γ-globulin range. In IE this fraction reacted specifically with an anti-λ antiserum, while it did not react with an antiserum specific for the H-chain of immunoglobulins G, A, M and D. The M component could be isolated from the serum as well as from the urine. Ultracentrifuge analysis showed that the abnormal serum fraction (Fig. 4a) had a sedimentation coefficient \( s_{20,w} = 6.3 \) S. The abnormal urinary protein fraction (Fig. 4c) had a sedimentation coefficient \( s_{20,w} = 3.3 \) S and, on the basis of the results of the immunologic analysis, could be identified as a type L Bence Jones protein. The purified M component from the serum was typed as Gm. a(+). In this isolated serum protein it was even in high dilutions impossible to demonstrate complement-fixing antibody activity against the isolated viruses.

A study of bone marrow punctates (latter half of January 1967) by the immunofluorescence technic (report by Dr. W. Hijmans) disclosed that the number of plasma cells was not unequivocally increased but that IgG-positive plasma cells were decidedly predominant: IgA-positive 4.0 per cent; IgM-positive 5.1 per cent; IgG-positive 90.9 per cent. The ratio of \( \kappa \)-type to \( \lambda \)-type L-chains was 0.11 (adult standard = 3.0). Dual staining showed that 94 per cent of the IgG was of the \( \lambda \)-type (Fig. 5). The serum isohemagglutinin titre (blood group B) was anti-A 1:64.

Several viruses were isolated early in December 1966. Herpes simplex virus was cultured from sacral skin lesion; respiratory syncytial virus was cultured from the throat, and adeno-virus type 12 from the feces. The course of titres of complement-fixing antibodies against the viruses isolated, is shown in Table 4. Auto-antibodies against cell nuclei, thyroid antigens, smooth muscle and gastric parietal cells were not demonstrable.

**DISCUSSION**

The case history of this boy shows some important features. The symptoms of acute lymphoblastic leukemia became apparent following an upper respiratory infection. They were conventionally treated with steroids and antimetabolites, and this led to a remission. About two years later a generalized relapse, which again responded to therapy, was followed by listerial meningitis and, a few weeks later, by very refractory necrotic herpes simplex infection of the sacral skin and infections with R.S. virus and adeno-virus type 12, manifested in gastroenteritis and bronchopneumonia, respectively.

The occurrence of these infections coincided with a period of unequivocal

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(D) No reaction between the M-component in the patients serum and the Bence Jones protein in the urine and anti-\( \kappa \) antiserum. The precipitation line formed between the (70 × concentrated) urine and the anti-\( \kappa \) antiserum is due to residual IgG in the urine.
Fig. 4.—Ultracentrifuge analysis of the serum and the monoclonal protein components of December 24, 1967. The isolated M-component from serum (protein concentration 0.6 Gm./100 ml.) sedimented as a homogeneous peak (A) with $S_{20,w} = 6.3$ S. The serum pattern (protein concentration 1.3 Gm./100 ml.) is characterized as having a sharp high G (6.5 S) peak (B). The isolated Bence Jones protein from the urine (protein concentration 0.6 Gm./100 ml.) sedimented as a homogeneous peak (C) with $S_{20,w} = 3.3$ S. Sedimentation from right to left. Pictures taken at 44 minutes after reaching 59780 rpm.
diminution of all serum immunoglobulins (Table 3); this period was characterized also by lymphocytopenia (Table 1).

During December 1966 the protein pattern changed considerably: the IgA and IgM fractions increased and a significant monoclonal IgG component developed.

The patient finally died during a relapse of the leukemic process.

The clinical picture of this patient suggests an inadequate immunologic defense especially against viruses.

A combination of immunologic deficiency with lymphoreticular malignancy has been described several times; this concurrence has been observed so frequently that coincidence can be ruled out.18,24

In this patient we found no distinct clues suggestive of inadequate humoral immunity. The patient showed normal isohemagglutinin titers and responded to various virus infections by normal synthesis of specific antibodies (Table 4). The serum immunoglobulin levels during these infections were very low (Table 3), but not so low as to warrant a prediction of increased suscepti-

| Table 4.—Serum Titres of Complement-Fixing Antibodies Against Isolated Viruses |
|---------------------------------|-----------|-----------|-----------|
|                                 | 12/12/66  | 12/24/66  | 1/31/67   |
| Herpes simplex virus            | 1 : 8     | 1 : 32    | 1 : 8     |
| Adeno virus                     | 1 : 16    | 1 : 64    | 1 : 16    |
| R.S. virus                      | neg.      | 1 : 8     | 1 : 8     |
bility to infection. It is possible that the longterm therapy with antimetabolites led to these low serum immunoglobulin levels.23,26

The frequent occurrence of virus infections and the unusual course of the sacral herpes simplex infection rather suggest a disturbed cellular immunity, although this might be unexpected in lymphoblastic leukemia.21 In support of this suggestion is the lymphocytopenia which developed at the time of the virus infections, and the absence of the usual relative lymphocytosis in response to these infections (Table 1).

Shortly after a number of virus infections, and while severe herpes simplex infection persisted, the immunoglobulins showed striking changes. The most prominent feature was the occurrence of IgG paraproteinemia. The blood count at that time showed no abnormality. The bone marrow was characterized by slightly inhibited maturation of the white cell system but otherwise showed no indications of a relapse of the leukemic process. The number of plasma cells was normal, but immunofluorescence disclosed that the majority of these cells produced type L IgG. The paraprotein had no antibody activity against the viruses isolated.

In the urine, a Bence Jones protein was demonstrated which was likewise typed as type L. The immunofluorescence study of the bone marrow revealed that (within limits of error) there were no plasma cells which exclusively contained \( \lambda \)-chains. Since the majority of the cells contained type L IgG, it is a plausible assumption that overproduction of \( \lambda \)-chains in (a number of) these cells gave rise to the Bence Jones proteinuria.

There is possibly a casual relation between intensive antigenic stimulation originating from the virus infection, and the occurrence of paraproteinemia (actually the second malignant degeneration of the lymphoreticular system in this child). Osserman and Takatsuki27 have pointed out that protracted intensive antigenic stimulation can give rise to a polyclonal reaction which later assumes a monoclonal form. Dameshek28 has mentioned the possibility of a virus-induced form of self-perpetuating proliferation of immunocytes. Herpes virus like particles have been demonstrated in lymphoma cells, cultured from patients with malignant diseases. These cells produced homogeneous immunoglobulins and probably represent one single clone of malignant cells.29 In studies on virus-induced Aleutian disease, Porter et al.30 demonstrated that “the \( \gamma \)-globulins of mink affected with this disease are initially heterogeneous but that some animals late in the course of the disease show a transition to homogeneous myeloma-like hypergammaglobulinemia together with the excretion of Bence Jones proteins in the urine.” The changes in protein pattern observed in our patient closely resemble those in mink with Aleutian disease. In the homogeneous \( \gamma \)-globulin fraction of mink thus affected, antibody activity against the viral agent was no more demonstrable than in our patient.31

The paraproteinemia which occurred in our patient was possibly based on a combination of factors, viz., genetic predisposition, immunologic deficiency, protracted intensive antigenic stimulation and infection with viruses known to have carcinogenic properties.
Summary

This paper describes the case history of a boy found to suffer from acute blastic leukemia at the age of two years. Manifestations of an intracranial localization of the leukemic process occurred several times in the course of the next 18 months; a generalized relapse responded well to therapy.

The boy subsequently developed a severe refractory virus infection of the skin and suffered a few other virus infections, without any of the usual changes in the morphology of the blood. A few weeks later he developed a monoclonal gammopathy of the IgG class type L with Bence Jones proteinuria, likewise of type L.

The possible causes of this clinical course and of the paraproteinemia are discussed.

SUMMARIO IN INTERLINGUA

Le presente communication describe le historia clinic de un puero suifrente de acute leucemia blastic al etate de duo annos. Manifestationes de un localisation intracranial del processo leucemic occurreva plure vices in le curso del sequente 18 menses. Un recidiva generalisate respondeva ben al therapia. Subsequentemente le puero disveloppava un sever e refractori infection virusal del pelle e suifreva varie altere infections virusal sin ulle del usual alterationes in le morphologia del sanguine. Plure septimanas plus tarde ille disveloppava un gammopathia monoclonal del classe IgG, typo L, con proteinuria de Bence Jones, equalmente del typo L.

Es commentate le causas possibile de iste curso clinic e del paraproteinemia associate con illo.

Acknowledgments

The authors wish to thank Dr. George M. Bernier, Walter Reed Army Institute of Research, Washington, for his comments and assistance in the preparation of this manuscript. We are indebted to Dr. W. Hijmans, University of Leyden, for his great cooperation in the immunofluorescence studies of the bonemarrow smears, and to Dr. Erna van Loghem, Amsterdam, for the Gm-typing.

References


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