Syndrome of Neutrophil Agranulocytosis, Hypogammaglobulinemia, and Thymoma

By Laurent Degos, Annick Faille, Martin Housset, Laurence Boumsell, Claire Rabian, and Teresa Parames

The clinical, hematologic, and immunologic findings of a syndrome of agranulocytosis, hypogammaglobulinemia, and thymoma are described. Neutrophil agranulocytosis predisposing to severe infectious disease resulted from a deficiency of mature cells in bone marrow. Autologous and heterologous stem cell growth in vitro was inhibited by the patient's serum. Immunoglobulin deficiency was secondary to the absence of peripheral blood B lymphocytes, while T-cell subpopulations and cellular immunity were present. Surgical removal of a spindle cell thymoma had no effect on the agranulocytosis and B-cell deficiency. The hematologic findings did not respond to steroid therapy and cyclophosphamide. However, the agranulocytosis improved with repeated plasmapheresis and the patient achieved a clinical remission.

Several disorders associated with a thymic tumor are well documented. Some of them involved hematologic features such as erythroid hypoplasia, pancytopenia, acquired hypogammaglobulinemia, and pernicious anemia. The rare syndrome of agranulocytosis, hypogammaglobulinemia, and thymoma seems to carry a poor prognosis; all three published cases died acutely from severe infection. The diagnosis of thymoma in these cases was made postmortem. The rapid course and fatal outcome in these three cases precluded their analysis with detailed clinical and laboratory studies.

We report the case of a patient diagnosed with (1) a chronic severe agranulocytosis and a deficiency of mature cells in bone marrow, (2) hypogammaglobulinemia with absence of B lymphocytes, and (3) a thymoma detected by tomodensitometric scanning. Agranulocytosis did not respond to thymectomy but improved with plasmapheresis. The patient is alive and well, free of infections, 18 mo after diagnosis.

CASE REPORT

A 52-yr-old woman was admitted to the hospital on December 2, 1980 with a history of repeated, severe infectious diseases due to agranulocytosis. No hematologic disease was recorded in the family. Her previous medical history revealed that she initially became ill in October 1978 with stomatitis, diarrhea, and fever due to a Candida albicans infection. Laboratory studies then showed a white blood cell count of 2500/cumm, with 2% neutrophils, 70% lymphocytes, and 28% monocytes. Other parameters of the hemogram were normal. The patient had received lithium carbonate (800 mg daily) for 15 mo after diagnosis. However, episodes of diarrhea occurred during that period. Beginning in March 1980, severe bacterial, mycotic, and parasitic infections occurred repeatedly. The patient received antibiotics almost constantly, and she was admitted to several hospitals in France and Spain. During these hospital admissions, laboratory findings revealed agranulocytosis (0–500 neutrophils/cumm) associated with a mild anemia (10 g hemoglobin/100 ml) and hypogammaglobulinemia (5 g/liter). More recently, chronic Giardia lamblia infection required metronidazol treatment (5 cycles of treatment). Lithium carbonate (800 mg daily) and prednisone therapy (30 mg daily) were ineffective. The patient was admitted to Hospital Saint Louis for clinical and laboratory studies.

On physical examination, the patient appeared to be in ill health during the short periods without infection. The lymph nodes, liver, and the spleen were not enlarged. The tonsils were involved. Laboratory studies were used to investigate three areas: agranulocytosis, immunologic defects, and etiology.

Hematologic Findings

Hematologic findings revealed a complete neutrophil agranulocytosis. The hemogram showed 1000 white blood cells/cumm with 0% neutrophils, 3% eosinophils, 4% basophils, 60% lymphocytes, and 33% monocytes. There was a normochromic, normocytic mild anemia (10 g hemoglobin/100 ml), with a reticulocyte count of 60,000/cumm. The platelet count was 200,000/cumm platelets. Examination of aspirated bone marrow smears disclosed a normal cellularity and a deficiency of mature forms after the promyelocytic stage. Bone marrow GM-CFC cultures were performed using the previously described technique. Normal bone marrow and patient bone marrow cells at 6 x 10⁶/ml were suspended in 15% heat-inactivated fetal calf serum in McCoy's 5A medium (GIBCO, UK). To 0.1 ml of cells was added 0.1 ml of AB control serum, test serum, or 6 x 10⁴ autologous lymphocytes. Cells were incubated for 2 hr. At the end of the incubation, the cells were washed, sufficient agar was added to establish a concentration of 0.3%, and the cell suspension was plated in triplicate and incubated at 37°C. Colonies were counted on day 10. All aggregates containing more than 50 cells were recorded as colonies. The results are reported in Table 1. In the patient’s bone marrow culture, a dramatic decrease of colonies was found in the presence of autologous serum. No effect of autologous lymphocytes was detected. In the control bone marrow cultures, no difference was noted when considering the presence or absence of autologous sera or lymphocytes. The control culture was also inhibited by the patient’s serum (Table 1) but not by her lymphocytes.

IgG fraction was purified from the patient’s defibrinated plasma. An ammonium sulfate precipitation at 33% saturation was performed. After centrifugation at 50,000 g in a Sorval centrifuge, the precipitate was dialyzed first in 0.15 M NaCl until no more NH₄⁺ ion was detected, and then against two batches 2:1 each of a 0.05 M Tris-HCl buffer, pH 8.0. The globuline fraction was then chromatographed on a DEAE Sephacel (Pharmacia, Uppsala, Sweden)
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column equilibrated in the same buffer and the unretained peak was collected. All the operations were effected at +5°C. The chromato-
graphed fraction was tested by immunoelectrophoresis and found to contain pure polyclonal IgG at 13 mg/ml concentration. The normal human IgG employed was Cohn fraction II (Squibb), and the stock solution at 11 mg/ml in 0.15 M NaCl, tested as mentioned above, was devoid of any significant contaminant. The heterologous bone marrow cultures were inhibited by the patient's IgG and not by control IgG (Table 2). It was not possible to obtain a new specimen of patient's bone marrow to test it with the IgG fractions.

Using the indirect fluorescence technique on a panel of granulocytes, the patient's serum did not react and was also negative on granulocytes obtained from the patient in remission.

Immunologic Findings

Immunologic findings confirmed the hypogammaglobulinemia (IgG 4.7 g/liter; IgA 0.12 g/liter; no detectable IgM and IgE). Anti-B blood group isoagglutinins (the patient being A+ -) and tetanus toxoid and cytomegalovirus antibodies were weakly positive. A weak anti-Hbs antibody was noted.

In lymphocyte subpopulations, the B lymphocytes were evaluated by staining with indirect fluorescence of surface immunoglobulins (Slg), by EAC rosettes after the removal of monocytes, and by both the lymphocytotoxicity technique and indirect immunofluorescence using an anti-B-cell monoclonal antibody (B1, kindly provided by L.M. Nadler) on cell suspensions depleted in E-rosette-forming cells and iron particle phagocytosis. No B cells were detected in the patient's blood with these three techniques. T lymphocytes were counted by E rosettes and with anti-T monoclonal antibodies T3, T4, T8 (kindly provided by E. Reinerz), and A50 using the lymphocytotoxicity technique. T-lymphocyte populations and sub-populations were normal: among peripheral blood lymphocytes, there were 80% E-rosette lymphocytes, 90% of cells positive with A50 (an anti-T-lymphocyte antibody), 85% positive with T3, 40% positive for T4 (helper T lymphocytes), and 20% positive for T8 (cytotoxic and suppressor T lymphocytes). In vitro stimulation of the patient's peripheral blood lymphocytes in AB normal serum and in autologous serum with candida antigens, CMV antigens, and PPD induced normal proliferative responses. Stimulations with alloantigens (mixed lymphocyte reaction) and various mitogens (PHA, pokeweed, and concanavalin-A) in AB normal serum were similar to the controls. T lymphocyte proliferation was measured by H-thymidine incorporation. However, the patient was unresponsive to intradermal injection of candida and tuberculin (I. Pasteur).

<table>
<thead>
<tr>
<th>Table 1. Bone Marrow GM-CFC Culture</th>
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<tr>
<td>Colonies</td>
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<td>Patient's bone marrow</td>
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<tr>
<td>Alone</td>
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<tr>
<td>With peripheral lymphocytes</td>
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<tr>
<td>With non-T lymphocytes</td>
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<td>With T lymphocytes</td>
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<td>With patient's serum</td>
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<td>Normal bone marrow</td>
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<td>Alone</td>
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<tr>
<td>With AB serum</td>
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<td>With patient's serum</td>
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* Non-T lymphocytes correspond to non-T, non-B lymphocytes in this case.
† After E rosetting.
‡ Serum from an AB (red blood cell group) normal individual.

The patient's serum, after absorption with platelets, reacted in a lymphocytotoxicity test with B cells from 40% of a panel of individuals. The reactions did not correlate with any known HLA-DR specificities. The patient's activated T cells (after stimulation by phytohemagglutinin) normally expressed the patient's HLA-DR specificities. The patient's serum did not react with autologous activated T lymphocytes (cytotoxicity technique). Studies of the patient's serum revealed weakly positive (1:10) antinuclear activity using the indirect fluorescence technique, negative Coombs test, and no evidence of rheumatoid factors. Total serum hemolytic complement was normal.

The absence of mature cells associated with a toxic effect of the patient's serum in bone marrow culture and the absence of B lymphocytes suggested an autoimmune disease. This suggestion was supported by the response of plasmapheresis (continuous flow blood cell separator, IBM Corp., White Plains N.Y.); the WBC count was 5000/cumm with 70% neutrophils (Fig. 1) and reticulocytes increased to 150,000/cumm. Three percent of peripheral blood lymphocytes reacted as B cells and the patient developed a weakly positive skin test to candida and tuberculin. Immunoglobulin concentrations could not be evaluated accurately when heterologous normal plasma or albumin plus gammaglobulins were used for plasmapheresis. Cycles of plasmapheresis were also performed with albumin alone. Immunoglobulin concentrations were not improved after these procedures.

Etiology

Investigations for an etiology included x-rays of the chest, which were considered to be normal. Lymphangiography and bone marrow biopsy excluded any apparent lymphoproliferative disorder. Tomodensitometric scanning (C.G.R., France) disclosed a tumor of the thymus (38 x 25 mm) (see Fig. 2). A thymectomy was performed (Laennec Hospital) and a spindle cell thymoma was diagnosed. Although thymectomy had been performed, the granulocyte count continued to decrease and further plasmaphereses were performed every 3 wk according to the count of granulocytes. Prednisone (1.5 mg/kg daily) and cyclophosphamide (2 mg/kg daily) were then administered for 3 mo without any effect and were stopped. Three months later, normal count of granulocytes remained stable without plasmapheresis for 4 mo. However, a new decrease in granulocytes was cured by a cycle of three plasmaphereses.

The patient is now alive and free of any infectious disease because of plasmapheresis. The hypogammaglobulinemia was not reversed by the proposed therapy.

DISCUSSION

The three patients with agranulocytosis, hypogammaglobulinemia, and thymoma reported in the litera-
tured died from infectious disease. The present patient, with a similar syndrome, is alive probably because of periodic plasmapheresis. Clinical examination demonstrates an involution of the tonsils, which was also seen in one of the three reported cases of this syndrome. Hematologic findings confirmed that neutrophil agranulocytosis was due to a deficiency of mature neutrophil granulocytic cells, while monocytes and eosinophilic granulocytes were present. Studies with bone marrow cultures suggested the patient's serum (specially IgG fraction) was toxic to myeloid cells. Repeated plasmapheresis without infusion of normal plasma confirmed the role of the patient's plasma in the agranulocytosis, since here neutrophil counts were normal after this treatment. Neither studies with bone marrow culture nor plasmapheresis were performed in the other three cases.

Hypogammaglobulinemia does not seem to be associated with deficiencies of cellular immunity. Most immunodeficiency syndromes appear to result from an arrest in the differentiation of immunocompetent cells or a defect in the function of T cells, either a lack of helper function or an activation T-suppressor cells. The lack of helper T-cell function in immunodeficiency syndromes with hypogammaglobulinemia has been noted to be associated with anti-helper-T-cell antibody and the loss of the T4+ inducer T-cell function. In all cases due to a functional T-cell defect, the B cells appeared to be normal. The absence of B lymphocytes in our adult female patient suggests the presence of an autoantibody against B cells. Data on the occurrence of such antilymphocyte antibodies in hypogammaglobulinemia patients are rare. One case showed a definite anti-B antibody involved in the pathogenesis of hypogammaglobulinemia. The results of lymphocytotoxicity tests showed the presence of an antibody in the serum of our patient reacting on a panel of B cells. It was neither an anti-HLA-A, B, C antibody, since it remained positive after extensive platelet absorption, nor an anti-HLA-DR antibody, because the positive reactions were not correlated with any known HLA-DR specificities. Further, the reaction was negative with autologous HLA-DR determinants expressed on T-activated cells. It was not possible to test the patient's serum on autologous B lymphocytes.

Surface immunoglobulin-bearing cells were absent in five cases of hypogammaglobulinemia and thymoma. Patients with thymoma and hypogammaglobulinemia, in agreement with the case reported here, have a normal number of T cells and normal proliferative T-cell function, except in one patient whose antigen-stimulated lymphocytes were inhibited. The T-lymphocyte subpopulations (helper T4 versus suppressor and cytotoxic T8 cells) was in normal proportions in our patient. Skin tests were normal in some patients and not in others, as reported here. After plasmapheresis, the patient's skin tests became weakly positive.

In our patient the thymoma was not visible on chest
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Fig. 2. Tomodensitometric scan of the chest. The thymoma is indicated by a line (+ ———— +).

x-ray. Only the tomodensitometric scan was abnormal, demonstrating the thymic tumor. In one patient with agranulocytosis, hypogammaglobulinemia, and thymoma, x-rays revealed no evidence of the tumor, which was discovered at autopsy. In the two other cases, the mediastinal mass was detected by x-ray examination and the diagnosis of thymoma confirmed at autopsy. Tomodensitometric scan not only suggests the diagnosis of thymic tumor, but also provides information about size and localization of the tumor that is useful for the surgeons.

After restoration of the neutrophil counts to normal with plasmapheresis, a thymectomy was performed in our patient with no complication. The spindle cell thymoma diagnosed is the most common histologic type of tumor found in the cases with aplastic anemia and hypogammaglobulinemia. Generally, autoimmunity is reported in these patients. In the present case, the agranulocytosis and the B-cell defect with hypogammaglobulinemia could represent one aspect of the various autoimmune features associated with thymoma. If so, it would be interesting to know if a single antibody interfered with neutrophil granulopoiesis and was cytotoxic to B cells. The scarcity of B cells did not allow absorptions with the serum to be performed. The finding and characterization of such an autoantibody is clinically significant because the severity of this syndrome is clearly related to agranulocytosis and hypogammaglobulinemia. Previously proposed treatment, such as splenectomy, a single dose of cyclophosphamide, or antibiotics, have had no effect on the outcome of the syndrome.

In the present case, the effect of plasmapheresis on the agranulocytosis was transient (3 wk) during the first 6 mo. It was not extended when used in association with prednisone and cyclophosphamide therapy. Hypogammaglobulinemia was not affected by plasmapheresis. Thymectomy had no immediate effect on the progress of agranulocytosis and B-cell deficiency. Thymectomy is helpful in 14% of patients with aplastic anemia, some of them requiring steroid therapy.

Plasmapheresis played a major role in preserving the quality of life for our patient; she has now returned to Spain and is living normally. This therapeutic modality must be emphasized, because it could be used in other severe thymoma-associated disorders, as presently for myasthenia gravis.

REFERENCES

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