Successful Treatment of a Patient with Posthepatitic Severe Aplastic Anemia with Bolus Methylprednisolone

Shuji TOHDA, Toshiya SUZUKI, Kaoru NAGATA, Nobuo NARA and Nobuo AOKI

A 31-year-old female developed severe aplastic anemia following acute non-A non-B hepatitis. Because of the impossibility of bone marrow transplantation, bolus methylprednisolone therapy was done. Soon after the therapy, pancytopenia improved. She has since taken mepitiostane and no longer needs blood transfusions. Good response to the immunosuppressive therapy suggests that the pathogenesis of this anemia is related to the immune system. In vitro culture demonstrated that the patient’s serum contained a factor which suppressed hematopoietic activity, possibly gamma-interferon.

Key words: Hepatitis, Interferon, Colony assay

The pathogenesis and clinical course of aplastic anemia (AA) is highly variable. Posthepatitic AA is usually very severe and refractory to various treatments. Allogenic bone marrow transplantation (BMT) (1) has been approved as the only effective therapy. However, BMT is not feasible for all patients. In this article, we have described a patient with posthepatitic AA who recovered from severe pancytopenia after bolus methylprednisolone (mPSL) therapy (2). The fact that immunosuppressive therapy such as bolus mPSL was effective suggests that an abnormal immune response played a role on the pathogenesis of anemia in this patient. To elucidate the pathogenesis of posthepatitic AA, we studied an in vitro colony assay of the patient’s bone marrow cells, serum and peripheral lymphocytes.

CASE REPORT

A 31-year-old female was hospitalized for acute non-A non-B hepatitis on September 1, 1987. The serum GPT was 1476 U/l on admission. The maximum bilirubin was 20.9 mg/dl. The findings of the liver biopsy were compatible with acute hepatitis. Although the clinical course of hepatitis had been uneventful, pancytopenia with marked hypoplasia of bone marrow developed in the middle of November. Furthermore she developed acute pneumonia and was transferred to our hospital on November 27 (Fig. 1).

Physical examination on admission revealed high grade fever and multiple petechiae on the lower legs. Laboratory findings were as follows: white blood cells 1,600/μl (neutrophils 1%), hemoglobin 10.5 g/dl, reticulocytes 3,000/μl, platelets 4,000/μl, nucleated bone marrow cell count 34,000/μl (myeloid cells 3%, erythroid cells 27%), megakaryocytes 0/μl, serum GOT 66 U/l, GPT 138 U/l, bilirubin 1.0 mg/dl, total protein 7.8 g/dl, A/G ratio 0.81, IgG 2,025 mg/dl. Chest X-ray revealed soft exudative infiltration in the left mid-lung field.

In accordance with the history and laboratory findings, she was diagnosed as having severe posthepatitic AA complicated by pneumonia. Although antibiotics were not effective in treating the pneumonia, antifungal drugs improved the infiltrative shadow. She also suffered massive gastrointestinal bleeding and needed multiple transfusions of red blood cells and platelets. Initially, BMT was considered as a treatment for the AA. However,
BMT was given up because of poor response to platelet transfusion, even HLA-matched transfusion. The poor response was attributed to frequent transfusions. Therefore, she was treated with bolus mPSL therapy; mPSL was given at a dose of 1,000mg per day for 3 days and then tapered and ceased within 4 days. Soon after the therapy, reticulocytes and neutrophils increased in number and two months after the therapy, pancytopenia improved as follows: neutrophils 650/μl, reticulocytes 60,000/μl and platelets 13,000/μl. Because GPT increased to 1,419 U/l and bilirubin increased transiently to a maximum of 13.4 mg/dl after therapy, mPSL therapy was not repeated and instead she received mepitiostane.

Now, 16 months after mPSL therapy, she does not need blood transfusions and is in good hematological condition: neutrophil 1,500/μl, hemoglobin 15g/dl and platelets 32,000/μl.

**In vitro colony assay**

To elucidate the pathogenesis of AA in this patient, we studied the effects of the patient's serum and lymphocytes on colony-forming units in culture (CFU-C) of autologous and normal bone marrow cells as previously described (3). Bone marrow cells were taken from the patient after treatment and from a normal volunteer with informed consent. Mononuclear cells separated through a Ficoll-Hypaque density gradient (1.077 g/ml) were used as target cells.

Bone marrow mononuclear cells were plated in triplicate at $8 \times 10^4$ cells for the patient and $4 \times 10^4$ for the normal volunteer in 0.4ml of α-MEM with 20%FCS, 0.8% methylcellulose, 1ng/ml of recombinant human GM-CSF and 10ng/ml of G-CSF. In this assay system, the patient's peripheral lymphocytes ($4 \times 10^4$ cells/well) before treatment, the patient's serum before or after treatment (4 months after bolus mPSL), or the serum preincubated with 1.5NU (neutralizing unit)/ml or 15NU/ml of anti-γ-interferon(IFN) antibody(AB) were added. After 7 days incubation, colonies consisting of more than 40 cells and clusters consisting of more than 10 cells were counted (Table 1).

The patient's lymphocytes did not significantly suppress CFU-C of the normal volunteer. In contrast, the patient's serum before treatment suppressed autologous CFU-C. Preincubation with anti-γ-IFN Ab nullified the suppressive effect of the patient's serum. These results indicate that the pathogenesis of this patient's AA was mediated by humoral rather than cellular immunological factors,
Table 1. Effects of patient's lymphocytes and serum on autologous and normal CFU-C.

<table>
<thead>
<tr>
<th>Target</th>
<th>Added PBMNC or serum</th>
<th>Added antibody</th>
<th>CFU-C/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM(N1)</td>
<td>–</td>
<td>–</td>
<td>25.0</td>
</tr>
<tr>
<td>BM(N1)</td>
<td>PBMNC(N2)</td>
<td>–</td>
<td>24.0</td>
</tr>
<tr>
<td>BM(N1)</td>
<td>PBMNC(P,B)</td>
<td>–</td>
<td>17.7</td>
</tr>
<tr>
<td>BM(P)</td>
<td>–</td>
<td>–</td>
<td>6.0</td>
</tr>
<tr>
<td>BM(P)</td>
<td>serum(P,B)</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>BM(P)</td>
<td>serum(P,B)</td>
<td>IFN Ab. 1.5NU/ml</td>
<td>8.3</td>
</tr>
<tr>
<td>BM(P)</td>
<td>serum(P,B)</td>
<td>IFN Ab. 15NU/ml</td>
<td>11.3</td>
</tr>
<tr>
<td>BM(P)</td>
<td>–</td>
<td>–</td>
<td>9.0(^a)</td>
</tr>
<tr>
<td>BM(P)</td>
<td>serum(P,B)</td>
<td>–</td>
<td>4.7(^a)</td>
</tr>
<tr>
<td>BM(P)</td>
<td>serum(P,A)</td>
<td>–</td>
<td>6.3(^a)</td>
</tr>
</tbody>
</table>

Value represents the mean CFU-C per well of triplicate cultures. \(a\): Value represents cluster number per well. BM: bone marrow cells, PBMNC: peripheral mononuclear cells, (N1): normal volunteer 1, (N2): normal volunteer 2, (P): patient, (B): before treatment, (A): after treatment, N.S.: not significant.

possibly \(\gamma\)-IFN. The suppressive effect of the serum after treatment was weaker than that before treatment. The difference, however, was not significant.

**DISCUSSION**

The pathogenesis of AA is not well understood. The role of autoimmune mechanisms has been reported previously (4, 5). In the case reported herein, the effectiveness of immunosuppressive therapy suggests a possible immune role in the pathogenesis of posthepatitic AA.

To further analyze the pathogenesis of AA in the present case, we have tested the effects of the patient's lymphocytes and serum on hematopoietic precursor cells. We have shown that humoral rather than cellular immunity was a factor in this case of AA. The finding that the suppressive effect of the serum was cancelled by the addition of anti- \(\gamma\)-IFN Ab indicates that the humoral factor in the serum was \(\gamma\)-IFN. Zoumbos et al (6) have reported that \(\gamma\)-IFN was overproduced by cultured lymphocytes of AA patients and that \(\gamma\)-IFN was the possible mediator of hematopoietic suppression. The \(\gamma\)-IFN level of our patient's serum on admission was under 2 IU (international unit)/ml, which was lower than the detectable limit in the cytopathic effect assay (7). However, 1.5 NU/ml of anti-\(\gamma\)-IFN Ab diminished the suppressive effect of the patient's serum. Therefore, even a low concentration of \(\gamma\)-IFN is considered to be enough to suppress hematopoiesis.

Successful cases of bolus mPSL therapy for severe posthepatitic AA have been only rarely reported and BMT has been the first choice of treatment for most cases of AA (1). However, as described above, bolus mPSL therapy was effective in our case. Bolus mPSL therapy should be tried in cases for which BMT is not feasible for whatever reason.

**REFERENCES**

4) Gordon MY. Circulating inhibitors of granulopoiesis in patients with aplastic anemia. Br J Haematol 39: 491,
Tohda et al

1978.

