Effect of Interferon (IFN) on Refractory Idiopathic Thrombocytopenic Purpura: Administration of 6 Million Units of Recombinant IFN alpha-2b

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Five patients (six courses) with refractory idiopathic thrombocytopenic purpura (ITP) were given 6 million units of recombinant interferon (IFN) alpha-2b in 12 doses to achieve an improved response rate compared to previous studies using 3 million units. From the initial IFN administration, the platelet count increased from a pre-treatment level of 20.7 ± 17.7 × 10^3/μl (mean ± SD) and reached its first peak in weeks 2 or 3 of therapy (p<0.05). In week 5, the platelet count made its second and maximum peak (66.5 ± 57.9 × 10^3/μl; p < 0.05). A relatively good response of the platelet count (an increase to >50 × 10^3/μl) was observed in three patients (four courses) out of five. These responses were not much faster or more improved than in previous reports, and a dose of 6 million units may be too large to treat some ITP patients. The platelet-associated IgG level showed a tendency to be reduced with IFN therapy. The mechanism for the increase of the platelet count may be the modification of platelet autoantibody production.

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Key words: platelet count, platelet-associated IgG, megakaryocyte, platelet kinetics, lymphocyte subset analysis

Introduction

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder of unknown etiology that is characterized by thrombocytopenia. The conventional methods of treatment include steroids (1–3), splenectomy (4, 5), intravenous immunoglobulin therapy (6–8), and immunosuppressive drugs (9), and these are effective in 50% of patients. If these standard treatments fail, advanced methods such as danazol therapy (10, 11), slow infusion of vinca alkaloid (12, 13), administration of ascorbic acid (14), and cyclosporine therapy (15) are tried, but some patients will still have refractory ITP. Recently, the efficacy of interferon (IFN) alpha-2b for HIV-1-associated thrombocytopenia has been reported (16, 17). In 1989, Proctor et al. demonstrated that a short course of IFN alpha-2b caused a significant increase in the platelet count in 11 of 13 patients with severe steroid-resistant ITP (18, 19). Other investigators have performed further trials (20, 21) but could not show a better efficacy rate than Proctor et al. Proctor and Jackson summarized earlier reports by classifying patient responses to five categories, demonstrating that the positive response rate was 69% (22). In earlier studies, most patients received an IFN dose of 3 million units; only 19% had a complete response (22). To increase the response rate, we gave 12 doses of 6 million units of IFN and observed changes in these hematological parameters; platelet count, platelet-associated IgG (PAIgG) level, alterations of megakaryocytes (MgKs), platelet kinetics, and immunophenotypes of lymphocytes.

Materials and Methods

Patients

The subjects were five patients (1 male and 4 females), with refractory ITP (mean age: 49.4), who were being treated in the Second Department of Internal Medicine,
Table 1. Profiles of the Patients in this Study

<table>
<thead>
<tr>
<th>Sex/Case No.</th>
<th>Age (yr)</th>
<th>PLT (x10^3/μl)</th>
<th>Previous Treatment</th>
<th>Prior Splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/41</td>
<td>50</td>
<td>PSL, DNZ, CEP</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>2a M/46</td>
<td>21</td>
<td>PSL, DNZ, CEP, COH, Vit C, NTP, CyA, VASI, IVlgG</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>2b M/46</td>
<td>4</td>
<td>PSL, DNZ, CEP, COH, Vit C, NTP, CyA, VASI, IVlgG, IFN</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>F/59</td>
<td>31</td>
<td>PSL, COH, Vit C, NTP, CyA, VASI, IVlgG</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4 F/64</td>
<td>3</td>
<td>PSL, DNZ, Vit C, NTP, VASI</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5 F/37</td>
<td>14</td>
<td>PSL, NTP, VASI, IVlgG</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

PLT: platelet, PSL: prednisolone, DNZ: danazol, CEP: cepharnthin, COH: colchicine, Vit C: ascorbic acid, NTP: neurotropin, CyA: cyclosporine A, VASI: vinca-alkaloid slow infusion, IVlgG: intravenous IgG with splenectomy. Case 2b was administered two courses of interferon (IFN) alpha-2b at 3 months because the platelet count decreased after the first course.

Chiba University School of Medicine and its allied hospitals between April and September 1991 and gave their consent to IFN therapy (Table 1). None of the patients had clinical evidence of viral infection and, in particular, none had any serologic evidence of HIV or hepatitis B infection.

All patients had refractory ITP, i.e., therapy-resistant ITP despite administration of various drugs, including steroids, for more than six months. Before IFN was administered, all patients had been given various treatments, as shown in Table 1. However, these treatments had failed to increase the platelet count and relieve bleeding. The history of ITP was as follows: 1 year for case 1, 3 years for case 2, 9 years for case 3, 10 years for case 4, and 28 years for case 5. None of the patients had antibodies against IFN alpha-2b before treatment.

Administration of IFN

The IFN therapy schedule consisted of recombinant IFN alpha-2b (6 x 10^6 U, Schering-Plough) given subcutaneously three times per week for four weeks, for a total of 12 doses. The patients who had been on other therapies before this study, including prednisolone, continued those therapies at the same dose, and no additional drugs were administered throughout the study. Three cases (case 1, 2b, and 5) were inpatients; the others were treated as outpatients.

Platelet response criteria

The platelet count was observed from the initial IFN administration for eight weeks and the platelet response to IFN was classified based on the criteria determined by the ITP Research Group of the Japan Ministry of Health and Welfare as follows: [1] complete response (CR), a platelet count >100 x 10^3/μl which did not decrease without maintenance therapy; [2] partial response (PR), a platelet count of 50 x 10^3/μl and 100 x 10^3/μl; [3] improvement (I), a platelet count between 50 x 10^3/μl and 100 x 10^3/μl; [4] slight improvement (SI), a platelet count <50 x 10^3/μl with an increase of >20 x 10^3/μl above the pre-treatment level; [5] no change (NC), an increase or decrease of the platelet count within 20 x 10^3/μl; and [6] worse (W), a decrease of the platelet count by >20 x 10^3/μl compared with the pre-treatment level or an increase in the bleeding tendency at any time.

Peripheral blood examination

Until 2 months after the start of IFN administration, the platelet count was determined and the PAIgG level was estimated using an enzyme-linked immunosorbent assay, ELISA (the normal range: 9.0–25.0 ng/107 cells).

Bone marrow examination

Before and after IFN therapy, bone marrow aspiration was performed. The MgKs count (Fuchs-Rosenthal method) and the proportion of apparently “nonbudding” MgKs that were smooth in contour were determined from the bone marrow smears (23). The proportion of such MgKs was determined by counting 100 MgKs per smear.

Platelet kinetic studies

Autologous platelets were labeled based on the method established by Dewanjee et al (24). The indium-111 tropolone-labeled platelets were injected into the patient and blood samples were collected every day for one week after administration. Platelet mean life span was determined as the duration until the radioactivity was decreased by 50%. Spleen and liver radioactivity was recorded in anterior and posterior images to calculate the spleen to liver ratio (S/L ratio).

Lymphocyte subset analysis

Heparinized peripheral whole blood samples were obtained at pretreatment and in week 5. CD4 (Leu3), CD8 (Leu2) (Becton Dickinson Co., Monoclonal Center, Mountain View, CA) were used as monoclonal antibodies to perform lymphocyte subset analysis.

Results

Platelet responses and clinical signs

In all cases, IFN therapy was well tolerated in compliance with the schedule. The responses were: two PR (cases 1 and 2a), two I (cases 2b and 3), one SI (case 4), and one W response (case 5) (Table 2). The platelet-increasing response was transient in five cases (1, 2a, 2b, 3, and 4) and was not found in case 5. A decrease in the platelet count compared with the pre-treatment level during IFN therapy was observed in cases 1, 3,
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Table 2. Responses to IFN Therapy

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Maximal Platelet Counts (×10^3/μl)</th>
<th>Time to Maximal Response (day)</th>
<th>Duration of Response * (day)</th>
<th>Steroid Treatment During IFN</th>
<th>Type of Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>158</td>
<td>35</td>
<td>21</td>
<td>Yes</td>
<td>PR</td>
</tr>
<tr>
<td>2a</td>
<td>113</td>
<td>7</td>
<td>35</td>
<td>Yes</td>
<td>PR</td>
</tr>
<tr>
<td>2b</td>
<td>54</td>
<td>21</td>
<td>21</td>
<td>Yes</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>35</td>
<td>35</td>
<td>No</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>35</td>
<td>7</td>
<td>Yes</td>
<td>SI</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Yes</td>
<td>W</td>
</tr>
</tbody>
</table>

PR: partial response, I: improvement, SI: slight improvement, W: worse, *: the duration for which the platelet count increased 20 × 10^3/μl or more.

and 5. After a temporary decrease in the first week of the platelet count in cases 1 and 3 (to 16 × 10^3/μl and 13 × 10^3/μl, respectively), platelet numbers began to increase rapidly. In all cases, there was a clinical impression of improvement of bleeding during therapy, except that in case 5 the platelet count decreased slightly from the pre-treatment level of 14 × 10^3/μl to reach 4 × 10^3/μl in week 4 and then petechiae on the legs became more severe.

Hematological parameters

The platelet count increased from 20.7 ± 17.7 (mean ± SD) × 10^3/μl at pre-treatment and showed significant increases as follows: 49.0 ± 36.6 × 10^3/μl (p < 0.05) in week 2; 49.2 ± 29.8 × 10^3/μl (p < 0.05) in week 3; and 66.5 ± 57.9 × 10^3/μl (p < 0.05) in week 5. In the other weeks, no statistically significant changes of platelet count were found (n = 6; Fig. 1).

The mean level of PAIgG had a tendency to be decreased in association with IFN therapy, although no significant difference was found (Table 3).

The MgKs count in week 5 was reduced compared to the pre-treatment count (p < 0.1). The mean proportion of “nonbudding” MgKs was increased slightly, but no significant difference was found (Table 4).

The mean values of platelet survival were prolonged slightly, although no significant difference was found (Table 4). The S/L ratio was reduced from 0.83 at pre-treatment to 0.5 in week 5 in case 1, and from 5.67 to 3.91 in case 4.

Lymphocyte subset analysis showed that the mean values of the CD4^+/CD8^+ ratio had a tendency to be decreased, but no significant difference was found (Table 4).

Side effects

Fever, malaise, and appetite loss were observed in four cases (cases 1, 3, 4, and 5) and depression was

Table 3. PAIgG Level

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Week 2</th>
<th>Week 5</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAIgG (ng/10^7 cells)</td>
<td>331.0 ± 345.3</td>
<td>202.1 ± 206.2</td>
<td>169.0 ± 148.6</td>
<td>358.0 ± 267.6</td>
</tr>
</tbody>
</table>

PAIgG: platelet-associated IgG. (n = 6)
observed in one case (case 1). These disappeared after IFN administration was completed. No abnormal laboratory findings requiring therapy to be stopped were observed in any case.

**Discussion**

From the initial administration of IFN at 6 million units, the platelet count began to increase, and reached its first peak in weeks 2 or 3 of therapy (p<0.05). However, the platelet count decreased slightly in week 4, probably because of the suppressive effect of IFN. In week 5, the platelet count increased again to reach its second and maximum peak (p<0.05), which probably represented a rebound phenomenon. Four relatively good responses (better than SI) were obtained (cases 1, 2a, 2b, and 3) and the proportion of such results was similar to the studies using 3 million units (22). Maximum platelet counts were obtained in week 5, which was not much sooner than the 37.7 or 12.7 days previously reported (19, 20). In case 5, the platelet count fell and bleeding became more severe. In this context, a recent report has indicated that IFN administered to a patient with ITP may cause life-threatening bleeding (25).

Therefore, a dose of 6 million units may be too large and individualized doses might be necessary. As suggested by other investigators (19, 26), the granulocyte count may be a useful parameter for determining the suitable dose of IFN; in this study, the responding patients (cases 1, 2a, 2b, 3, and 4) showed a significant decrease in the absolute number of granulocytes in comparison with the non-responding patient (case 5).

It is of interest to note that the mean PAIgG level had a tendency to be reduced in association with IFN therapy. In previous reports, some investigators observed a relationship between an increase of platelet count and a decrease of PAIgG level (27), but others did not (19). After IFN therapy, the mean life span of subjects’ platelets was prolonged slightly and the S/L ratio (autologous indium-111 platelet sequestration) in the non-splenectomized patients (cases 1 and 4) was decreased. These observations may indicate that IFN modulates anti-platelet antibody production and suppresses the destruction of peripheral platelets by the reticuloendothelial system such as splenic macrophages.

It has been demonstrated that abnormal cellular immunity in active ITP is caused by a defect of T lymphocytes, and involvement of B lymphocytes is not apparent (28). Our data on lymphocyte subsets indicated that the mean values of the CD4+/CD8+ ratio had a tendency to be decreased slightly; no significant changes were found. It is possible that IFN may affect B cells via T cell modulation, but it cannot be denied that we are seeing a direct effect of IFN on B cell activity (22). It is necessary to accumulate further experience using IFN for ITP.

Bone marrow examination showed that IFN administration reduced the MgKs count (p<0.1). There was a tendency for the proportion of “budding” MgKs to be reduced. We cannot absolutely say that IFN had only a platelet-increasing effect. These observations may indicate that IFN causes immaturity of the platelet-producing system as a side effect, although there is a possibility that IFN prematurely releases platelets from the bone marrow (23).

There was no difference in response between patients who used a steroid simultaneously with IFN therapy and a patient who did not.

In conclusion, a dose of 6 million units of IFN seems to be too large for some patients with ITP. Maintenance therapy with low doses such as 1.5 million units for a long period of time might show a better response.

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**References**


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**Table 4.** Bone Marrow Examination, Platelet Kinetics, and Lymphocyte Subset Analysis

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Week 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow Examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgK Count (/μl)</td>
<td>117.1 ± 93.2 (n = 5)*</td>
<td>64.2 ± 32.9 (n = 5)*</td>
</tr>
<tr>
<td>&quot;nonbudding&quot; MgK (%)</td>
<td>78.8 ± 4.2 (n = 5)</td>
<td>84.5 ± 9.5 (n = 5)</td>
</tr>
<tr>
<td>Platelet Kinetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Survival (day)</td>
<td>1.77 ± 1.25 (n = 4)</td>
<td>2.81 ± 1.98 (n = 4)</td>
</tr>
<tr>
<td>Lymphocyte Subset Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ Cell (%)</td>
<td>39.4 ± 13.5 (n = 5)</td>
<td>36.6 ± 11.8 (n = 5)</td>
</tr>
<tr>
<td>CD8+ Cell (%)</td>
<td>35.6 ± 10.9 (n = 5)</td>
<td>37.2 ± 11.3 (n = 5)</td>
</tr>
<tr>
<td>CD4+/CD8+</td>
<td>1.27 ± 0.80 (n = 5)</td>
<td>1.12 ± 0.67 (n = 5)</td>
</tr>
</tbody>
</table>

MgK: megakaryocyte, CD4+/CD8+: the ratio of CD4+ to CD8+. *p < 0.1.
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