Brief Definitive Report

(NZW × BXSB)F₁ MOUSE
A New Animal Model of Idiopathic Thrombocytopenic Purpura

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There has recently been an increase in data indicating that autoimmune mechanisms are involved in the etiopathogenesis of idiopathic thrombocytopenic purpura (ITP) (1, 2). Although antibodies that react with platelets are found in most patients with ITP, the pathogenetic nature of the antibodies remains to be clarified. The discovery of an animal model for ITP has therefore been long-awaited. Here we have found that (NZW × BXSB)F₁ (W/B F₁) mice, which develop lupus nephritis with myocardial infarction (3), show thrombocytopenia with age, and that this is due to the presence of both platelet-associated antibodies (PAA) and circulating antiplatelet antibodies.

Recently, we have demonstrated that allogeneic bone marrow transplantation (ABMT) has curative effects on autoimmune diseases in (NZB × NZW)F₁, BXSB, MRL/Mp-lpr/lpr (MRL/lpr), and NOD mice (4–6). These results prompted us to examine whether ABMT can be used to treat ITP. In the present study, we provide evidence that the transplantation of bone marrow from BALB/c mice to W/B F₁ mice does indeed have preventative and curative effects on ITP.

Materials and Methods

Mice. Mice of the inbred strain BALB/c nu/nu, BALB/c, C57B/6, C3H/HeN, BXSB, NZW were raised under specific pathogen-free conditions in our animal facility. W/B F₁ males were obtained from the Nippon Shinyaku Research Laboratories, Kyoto, Japan.

Staining Procedure and Data Analysis. Platelet-rich plasma was obtained as described previously (7). The platelets were suspended in 1% paraformaldehyde solution for 5 min. After

This work was supported in part by a grant from the Japanese Ministry of Health and Welfare, a grant from the Naito Foundation, A grant from the Mitsubishi Foundation, a grant-in-aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, a grant from Suzuken Memorial Foundation, the Science Research Promotion Fund of the Japan Private School Promotion Foundation (1987), and grants AG-03592, AG-05628, AG-05633, and AI-19495 from the U.S. National Institutes of Health. Address correspondence to Dr. Susumu Ikehara, 1st Department of Pathology, Fumizono-cho, Moriguchi City, Osaka 570, Japan.

J. Exp. Med. © The Rockefeller University Press · 0022-4007/88/06/2017/06 $2.00 2017
Volume 167 June 1988 2017-2022
TABLE I

Counts of Platelets and Megakaryocytes in (NZW × BXSB)F1 (W/B F1) Mice

| Mouse     | Age    | Sex | Number examined | Platelet (×10⁴/µl) Mean ± SD | Megakaryocyte (/mm²) Mean ± SD |
|-----------|--------|-----|-----------------|------------------------------|--------------------------------|
| BALB/c    | 2-4 mo | ♂   | 15              | 54.0 ± 10.8                  | 132.5 ± 19.1                   |
| NZW       | 4 mo   | ♂   | 1              | 46.5                         | 128                            |
| BXSB      | 8 mo   | ♂   | 14             | 57.9 ± 12.0                  | 127.2 ± 11.1                   |
| W/B F1    | 1-2.5 mo | ♂ | 19             | 52.5 ± 10.7                  | 118.0 ± 12.6                   |
| W/B F1    | 4.5-7 mo | ♂ | 19             | 15.0 ± 5.9*                  | 150.8 ± 10.0*                  |

*p < 0.001 vs. data in young (1-2.5 mo) W/B F1 mice.

Transplantation of Bone Marrow Cells. 3-5-mo-old W/B F1 males were exposed to 9.5 Gy from a ⁶⁰Co source and then reconstituted by intravenous injection of 1.4 × 10⁶ bone marrow cells from BALB/c nu/nu mice, as previously described (4). The mice were killed 5.5 mo after ABMT.

Platelet and Megakaryocyte Count. Platelet counts in the peripheral blood were made in heparin blood sample on a hemocytometer. For megakaryocyte count, femur and tibia were obtained at sacrifice, and sections stained with hematoxylin and eosin. The number of megakaryocytes per square millimeter was counted under a microscope.

Results

Male W/B F1 mice at the age of 1-2.5 mo showed platelet counts similar to those of BALB/c, NZW, and BXSB mice (Table I). W/B F1 mice at the age of >4.5 mo, however, showed a marked reduction in platelet count. In contrast, bone marrow megakaryocyte counts increased in these mice with age, though they retained their normal shape.

To elucidate the cause of thrombocytopenia in peripheral blood of W/B F1 mice, we first examined PAA on the platelets. As shown in Fig. 1, PAA were found in the platelets of 4.5-mo-old W/B F1 mice with thrombocytopenia (10.5 × 10⁹/µl). BALB/c platelets treated with anti-H-2d serum were used as a positive control.

The next step was to examine whether circulating antiplatelet antibodies are present in the plasma of aged W/B F1 mice. As shown in Table II, circulating antiplatelet antibodies were found in the plasma of W/B F1 mice that were >5 mo old.

The characterization of the antiplatelet antibodies revealed that both PAA and circulating antiplatelet antibodies belong to the IgG and IgM classes, but not IgA (Table III).

Since we know that ABMT has curative effects on autoimmune diseases (4-6), W/B F1 mice at the age of 3-5 mo were lethally (9.5 Gy) irradiated and then recon-
Platelet-associated antibodies in W/B F1 mice. Aliquots of the platelets (2 × 10⁶) were labeled with FITC-anti-mouse Ig and analyzed on a FACS analyzer. As a positive control, BALB/c platelets treated with anti-H-2k antisera were used. Platelet counts (Plt. count: x 10⁻⁵/ml) for each mouse are shown.

Discussion
In the present study we have demonstrated that W/B F1 mice develop thrombocytopenia with age, and have found the presence of both PAA and circulating antiplatelet antibodies in such mice.

Several mechanisms that would explain the cause of thrombocytopenia in patients with ITP have been proposed. One is the presence of antiplatelet antibodies, which results in platelet destruction by complement-mediated lysis (8) or sequestration by the reticuloendothelial system (1). It has been reported that the antibodies in humans belong mainly to the IgG class (9). In W/B F1 mice, we have found the presence of IgG and IgM (but not IgA) antibodies both in the plasma and on the platelets (Table III). Since W/B F1 mice show high levels of circulating immune complexes
(CICs) from the age of 2.5 mo (3), it is conceivable that CICs are involved in the development of thrombocytopenia, CICs being found to affect platelets by activating complement or platelet-aggregating factor (1, 2). However, murine platelets have

### Table II

*Circulating Antiplatelet Antibodies in Old (NZW × BXSB) F1 (W/B F1) Mice*

| 1st antibody (plasma from) | Age (mo) | 2nd antibody (anti-Ig)* | Percent positive (Mean ± SD) |
|---------------------------|----------|-------------------------|-------------------------------|
|                          |          |                         |                               |
| -                         | -        | -                      | 0.1                           |
| -                         | -        | +                      | 14.0 ± 3.1                    |
| Anti-H-2d                 | -        | +                      | 34.9 ± 1.8                    |
| BALB/c                    | 1.5 to 2.0| +                      | 26.0 ± 0.3                    |
| (W/B) F1                  | 1.0      | +                      | 17.8                          |
|                           | 1.5      | +                      | 16.5                          |
|                           | 1.5      | +                      | 21.7                          |
|                           | 1.5      | +                      | 24.9                          |
|                           | 2.0      | +                      | 16.8                          |
|                           | 2.0      | +                      | 22.9                          |
| (W/B) F1                  | 5.0      | +                      | 45.3                          |
|                           | 5.5      | +                      | 49.2                          |
|                           | 7.0      | +                      | 57.6                          |
|                           | 8.5      | +                      | 46.8                          |

Platelets were obtained from 2-mo-old BALB/c mice.
* FITC-labeled goat anti-mouse Ig.
† p < 0.001 vs. data in young (1-2.0 mo) W/B F1 mice.

### Table III

*Characterization of Antiplatelet Antibodies in Old (NZW × BXSB) F1 (W/B F1) Mice*

| Source of platelet | Platelet-associated antibodies | Circulating antibodies |
|--------------------|--------------------------------|------------------------|
|                    | 1st antibody | 2nd antibody | Percent positive | 1st antibody (plasma from) | 2nd antibody | Percent positive |
| BALB/c             | -            | -            | 2.1             | -                          | Anti-Ig     | 9.9 ± 3.7       |
| BALB/c             | Anti-H-2d    | Anti-Ig*     | 49.9 ± 2.5      | Anti-H-2d                  | Anti-Ig     | 30.5 ± 0.2      |
| BALB/c             | -            | Anti-Ig      | 10.7 ± 0.7      | BALB/c                     | Anti-Ig     | 27.5 ± 8.3      |
| BXSB               | -            | Anti-Ig      | 23.6 ± 1.2      | C57BL/6                    | Anti-Ig     | 22.7 ± 2.0      |
|                   | Anti-Ig      |              |                 | BXSB                       | Anti-Ig     | 24.8 ± 3.5      |
| W/B F1 (1.0 mo)    | -            | Anti-Ig      | 23.1 ± 4.2      | W/B F1                     | Anti-Ig     | 12.9 ± 1.3      |
| W/B F1 (4.5 mo)    | -            | Anti-Ig      | 44.8 ± 0.8      | W/B F1                     | Anti-Ig     | 32.8 ± 4.3      |
|                   | IgG          |              | 41.7            | (7.0 mo)                   | IgG         | 33.7 ± 3.0      |
|                   | IgA          |              | 18.8            |                             | IgA         | 20.9 ± 2.3      |
|                   | IgM          |              | 30.5            |                             | IgM         | 35.9 ± 0.4      |

Platelets used in circulating antibody assay were obtained from 4-wk-old BALB/c mice.
* FITC-labeled goat anti-mouse Ig.
† Platelet counts were 5.5 × 10⁵/µl.
§ Platelet counts were 13.0 × 10⁵/µl.
†† FITC-labeled goat anti-mouse IgG.
no Fc receptors (2), and the sera from BXSB (Table III) and MRL/lpr (data not shown) mice, which show high CIC levels, did not bind to the platelets of BALB/c mice. It is therefore unlikely that antibodies bound to platelets and circulating antplatelet antibodies exist as a form of CIC.

Hang et al. (10) investigated the etiopathogenesis of autoimmune diseases in these W/B F1 mice by reciprocal transfer experiments of spleen cells between males that exhibit early-onset autoimmune disease and females with late-onset autoimmune diseases; the transfer of male lymphoid cells to female mice caused the development of accelerated lupus nephritis, hypertension, and myocardial infarction, whereas the transfer of female lymphoid cells to male mice delayed the onset. We have recently demonstrated that the transplantation of bone marrow cells from normal mice to autoimmune-prone mice has curative effects on autoimmune diseases, such as lupus nephritis, lupoid hepatitis, rheumatoid arthritis, and type I diabetes mellitus (4–6).

In the present study, W/B F1 mice after ABMT showed normal platelet counts and no evidence of the presence of circulating antiplatelet antibodies even at the age of 10.5 mo (Table IV). These results provide additional evidence that autoimmune mechanisms are involved in the development of thrombocytopenia in W/B F1 mice, although the exact mechanism by which it develops remains to be clarified.

We thus think that W/B F1 mice serve as a useful animal model of ITP not only for elucidating the mechanism of the development of antplatelet antibodies, but also for characterizing autoantibodies to platelets.

## Table IV
Effects of Bone Marrow Transplantation on Platelet Counts and Circulating AntiPlatelet Antibodies in Old (NZW x BXSB)F1 (W/B F1) Mice

| 1st antibody (plasma from) | Platelet count | 2nd antibody (Anti-Ig)* | Percent positive |
|----------------------------|----------------|------------------------|-----------------|
|                            | Age            | (Mean ± SD)            | (Mean ± SD)     |
| 2nd antibody (plasma from) | Age            | (Mean ± SD)            | (Mean ± SD)     |
|                            | Platelet count | (Mean ± SD)            | (Anti-Ig)*       |
|                            | Age            | (Mean ± SD)            | (Mean ± SD)     |
|                            | Platelet count | (Mean ± SD)            | (Anti-Ig)*       |
|                            | Age            | (Mean ± SD)            | (Mean ± SD)     |
|                            | Platelet count | (Mean ± SD)            | (Anti-Ig)*       |

Platelets were obtained from 2-mo-old BALB/c mice.

* FITC-labeled goat anti-mouse Ig.

† The W/B F1 mice at the age of 3–5 mo were exposed to 9.5 Gy from a 140Co source and then reconstituted with 1.4 x 107 bone marrow cells of BALB/c nu/nu mice. The mice were killed 5.5 mo after bone marrow transplantation.

‡ p < 0.01 vs. data in old (5–8.5 mo) W/B F1 mice without ABMT.

§ p < 0.02 vs. data in old (5–8.5 mo) W/B F1 mice without ABMT.
Summary
A decrease in thrombocyte count was observed in (NZW × BXSB)F₁ (W/B F₁) mice at the age of >5 mo, whereas megakaryocyte counts were found to increase in such mice. FACS analyses revealed the presence of both platelet-associated antibodies (PAA) and circulating antiplatelet antibodies. There is a correlation between the presence of these antibodies and the degree of thrombocytopenia. The transplantation of normal bone marrow cells from BALB/c nu/nu mice to W/B F₁ mice was found to have preventative and curative effects on thrombocytopenia; the mice showed normal platelet counts and no evidence of circulating antiplatelet antibodies. These results indicate that thrombocytopenia in W/B F₁ mice is due to the presence of antibodies to platelets. We therefore think that W/B F₁ mice serve as a useful animal model of idiopathic thrombocytopenic purpura (ITP) not only for elucidating the mechanism of the development of antiplatelet antibodies, but also for characterizing autoantibodies to platelets.

The authors thank Ms. K. Kitamura, Ms. K. Nomura, Mr. K. Kobayashi for their expert technical assistance, and Ms. S. Ohya for her help in the preparation of the manuscript.

Received for publication 25 January 1988 and in revised form 7 March 1988.

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