Heritable Severe Combined Anemia and Thrombocytopenia in the Mouse:
Description of the Disease and Successful Therapy

By Luanne L. Peters, Eleanor C. McFarland-Starr, Bonnie G. Wood, and Jane E. Barker

A new autosomal recessive mouse mutation, scat (severe combined anemia and thrombocytopenia), causes intermittent episodes of severe bleeding in the homozygote. At birth, affected mice are pale with intradermal petechiae and bruises on exposed surfaces. Central nervous system (CNS) bleeding occurs in 22% of the mice. Gastrointestinal (GI) hemorrhaging and splenomegaly are noted in moribund mice at autopsy. Of the 291 mice studied, 113 mice survived the initial crisis and entered a spontaneous remission period lasting from day 16 to day 27. A second crisis period ensued, and all but 22 mice died by 45 days. Mice in crisis show significantly decreased platelets, erythrocytes, and leukocytes and increased reticulocytes when compared to normal littermates. During remission all parame-

E X P E R I M E N T A L L Y produced immune platelet destruction in laboratory animals has been used to study the effects of thrombocytopenia on megakaryopoiesis and thrombopoiesis.1-3 While such models permit analysis of in vivo platelet destruction and its consequences, they reveal little about its etiology. Recently, a new recessive mutation called scat (severe combined anemia and thrombocytopenia) occurred in the BALB/cBy mouse strain. In homozygous mice, presence of an antibody to a 110-Kd platelet protein coincides with a cyclical platelet insufficiency. The disease, including its immune component, is transferable through the hematopoietic stem cells. In this regard the scat mutation resembles known murine lupus models, including the NZB, BXSB, and MRL strains, which are also transferable through the stem cells.4-6 In these strains, antiplatelet antibodies have not been implicated in the pathogenesis of the respective autoimmune syndromes. In scat/scat mice, however, antiplatelet antibodies appear to be a primary cause of the symptoms produced by the gene defect.

The scat/scat mice share a number of disease symptoms and the presence of a platelet-specific antibody with patients suffering from some forms of autoimmune thrombocytopenic purpura (ITP). The current paper provides the first description of the defects in the mouse mutant, the transmission of the disease through the hematopoietic stem cells, and the development of one prophylactic measure.

MATERIALS AND METHODS

Animals. BALB/cBy (BALB)-scat/scat mice were discovered and maintained at The Jackson Laboratory. Female mutant mice were unable to survive pregnancy. As a consequence, the mutant mice were generated either by mating BALB-scat/ + parents or by transplanting ovaries from mutant females to congeneric C.B.Hbb female mice and mating the ovary recipients to BALB-scat/ + males. A difference at the albino locus permitted recognition of mice generated from the resident C.B.Hbb oocytes and the transplanted BALB oocytes. Progeny from the C.B.Hbb oocytes were brown agouti, while progeny from the BALB oocytes were albino. The extreme pallor, bruises, and intradermal petechiae at birth easily distinguished the scat/scat mice from their normal BALB littermates. Age-related BALB-+ / + mice generated from BALB-+ / + mating pairs were used as controls. Congenic C.B.Hbb mice were recipients in the transplantation studies. Erythrocytes and plasma from autoimmune NZB/BINJ-+ / + (NZB) mice were used as positive controls during immunoblotting.

The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Survival studies, gross pathology, and histopathology. The average life span of the mutant animals and the temporal progression of the disease state were determined by daily observations of 291 BALB-scat/scat mice for 60 days or until death occurred. The mice were classified as being in crisis or in remission based on overt symptoms. Mice in crisis were extremely pale, had bruised extremities, and occasionally had accumulation of blood in the dorsal cranial area. Additional scat/scat mice not included in this study were killed by cervical dislocation and autopsied as they became moribund. Comparisons were made between mutant mice and age-related BALB-+ / + mice killed at the same time. For histologic examination, tissues were fixed in Tellyznicksky's fixative (thymus, lung, lymph nodes, spleen, liver, kidney, and adrenal glands) or 10% nonbuffered formalin (femur), embedded in paraffin, and sectioned at 3 μm. Femurs were decalcified in Cal Ex (Scientific Products, McGaw Park, IL). Sections were stained with Mayer's hematoxylin and eosin (H and E), periodic acid-Schiff reagent (PAS), methyl green pyronin (MG/P), or Gomori's iron stain.

Tissues for plastic sections were fixed in 0.3% glutaraldehyde-0.5% paraformaldehyde in 0.05 mol/L PIPES buffer at pH 7.35.6 Sections were stained with benzidine dihydrochloride.

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745
Peripheral blood studies. Blood counts in mice are dependent upon the method and time of blood collection. For this reason, whole blood was withdrawn from the retro-orbital sinus between 1200 and 1400 hours. Erythrocytes were counted using a Coulter Counter Model ZBI. Leukocyte and platelets were counted by light microscopy using conventional manual methods. For differential leukocyte counts, blood smears were prepared and stained with Wright’s-Giemsa using an automatic HemaStainer (Geometric Data Corporation). Differential counts were performed on 100 cells to obtain the relative numbers (percent) of circulating lymphocytes and granulocytes. Absolute counts were calculated by multiplying the total white blood cell (WBC) count by the relative count. Reticulocyte percent was determined from direct counts of blood cells exposed to new methylene blue.

Serum bilirubin determinations. Serum total-bilirubin levels were determined by the Malloy-Evelyn method.

Urinary screening. Qualitative urinary hemoglobin and bilirubin levels were determined using Ames Multistix 10 SG test strips. Urine for microscopic analysis was obtained from the bladder using a 30-gauge needle and examined by light microscopy using a 40x objective for the presence of red blood cells (RBCs).

Indirect Coombs tests. RBCs from heparinized whole blood obtained from the thoracic cavity of mice anesthetized with avertin were washed four times and resuspended at 5% in normal saline (0.9% NaCl in H2O). The cells were incubated 1:1 with ficin (Cooper Biomedical) for 10 minutes at 37°C, washed in saline, and again resuspended to a 5% solution in saline. RBCs, autologous plasma, and 22% albumin (Ortho) in a 1:2:2 ratio were incubated for 30 minutes at 37°C, centrifuged for 5 minutes at 138g, and read for agglutination both macroscopically and microscopically.

Preparation of erythrocyte ghost membranes. Hemoglobin-free erythrocyte membrane ghosts were prepared by the method of Dodge et al. The amount of protein in the preparation was determined by the method of Lowry. The ghosts were dissolved in 4x Laemmli buffer and boiled for 5 minutes before storage at -20°C.

Preparation of solubilized platelet proteins. Platelets were obtained from adult (6 to 8 weeks) BALB-+/+ mice. The mice were anesthetized with avertin and blood from the thoracic cavity was collected in 3.8% sodium citrate in saline. Plasma was removed by centrifugation of the cells at 480g. The cells were washed two to three times in saline to remove residual plasma. A platelet-rich suspension in saline was obtained by a final centrifugation at 120g for 20 minutes at 4°C. Platelets in the supernatant were adjusted to 108/mL and collected by centrifugation at 11,000g in a Brinkman Eppendorf 5415 microcentrifuge. The pellets were washed and vigorously vortexed in distilled water twice. The final pellet was resuspended with 50 μL of distilled H2O, 50 μL of 10% sodium-dodecyl sulfate (SDS), and 50 μL of 4 x Laemmli buffer. The solubilized platelets were boiled before storage at -20°C and/or before electrophoresis on polyacrylamide gels.

Electrophoresis on denaturing polyacrylamide gels. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of red cell ghost proteins and of solubilized platelet proteins was performed by the method of Laemmli. Erythrocyte ghost proteins (125 μg) or 50 μL of solubilized platelet proteins were loaded in each sample well. Molecular weight standards (myosin [200,000 D], beta galactosidase [116,250 D], phosphorylase B [97,400 D], bovine serum albumin [BSA; 66,200 D], and ovalbumin [42,699 D]) were applied to one lane as markers. Electrophoresis was continued for 5 hours at a constant current of 40 mA or overnight at 8 mA. The proteins were either stained with Comassie blue or transferred to nitrocellulose for Western analyses.

Western blot analyses. Heparinized whole blood was obtained from the thoracic cavity under avertin anesthesia. Untreated plasma or plasma precipitated with antinose IgG (Sigma, St. Louis, MO; M8890) overnight at 4°C was tested for erythrocyte and platelet autoantibodies. A nitrocellulose filter with red-cell or platelet-membrane proteins was placed in buffered saline (20 mmol/L TRIS, 500 mmol/L NaCl, pH 7.5) for 20 minutes and blocked in 3% gelatin in buffered saline with 0.05% Tween-20 for 1 hour at room temperature. The filter was rinsed in buffered saline containing 0.05% Tween-20 and incubated for 2½ hours in serum diluted 1:50 to 1:100 in 1% gelatin in Tween-20 buffered saline. The filter was washed two times in Tween-20 buffered saline, incubated with goat antinose IgG (heavy and light chains) horseradish peroxidase conjugate (Bio-Rad, Richmond, CA) for 1 hour, washed and immersed in 0.3% horseradish peroxidase color development reagent (4-chloro-1-naphthol, Bio-Rad) in cold methanol with 0.02% H2O2 in buffered saline for 5 to 30 minutes.

Bone marrow transplantation. At least 2 x 106 spleen cells retrieved from mutant mice in crisis were transplanted intravenously (IV) into C.B.Hbb/+ mice with an LD100 dose (700 R) delivered from a 137Ca gamma irradiator at a dose rate of 220 rads/min. The difference at the beta globin locus, Hbb, allowed measurement of donor cell contribution to the host. Hemoglobin phenotype was determined on blood lysates by the technique of Whitney. Blood and plasma were obtained from moribund mice for the various analyses.

Splenectomy. Animals were splenectomized using standard techniques.

Statistical analyses. Numerical results were analyzed by Tukey’s test for significance of difference between mean values and analysis of variance (ANOVA) or the two-tailed student’s t test. All data expressed as percentages were transformed prior to statistical analysis. Survival studies were analyzed by the χ2 test.

RESULTS

Progeny testing. The scat mutation was inherited in a recessive fashion. Breeding of known heterozygotes, derived from matings of C.B.Hbb/+ females carrying scat/scat ovaries to BALB-+/- males, resulted in the appearance of affected newborns. Crosses between BALB-scat/+ and BALB-+-/+ mice never produced affected offspring. In heterozygous matings, approximately 18% of live offspring were affected, suggesting some death in utero occurred. The chromosomal location of the scat mutation has not yet been determined.

Survival studies and gross pathology. Newborn BALB-scat/scat mice were monitored daily for a period of 60 days or until death occurred (Fig 1). All the homozygous mice were pale at birth and showed bruising and petechiae. Twenty-two percent of the mice developed intracranial bleeding. Of the 291 mice studied, 178 (61.2%) died by 25 days without entering a remission stage. The remaining mice went into a spontaneous remission period in which all overt symptoms had completely disappeared by day 16. On average, this remission period lasted 11 days, ending at day 27. During the second crisis, 91 mice (31.3%) died while 22 (7.5%) recovered and entered an extended remission period free of all symptoms. These animals were not monitored more than 60 days. Of the mutant mice studied, 90% were dead by 50 days, with a mean survival time of 22 days. The mean life span of BALB-+-/+ mice is greater than 18 months.

Gross pathology at autopsy revealed extensive gastrointestinal (GI) and rectal bleeding. There was marked spleno-
HERITABLE ANEMIA AND THROMBOCYTOPENIA IN MICE

Chromatin and a small amount of cytoplasm (Fig 2D through F). Mitotic figures were frequent. Spleens of scat/scat mice in crisis showed histologically normal. Benzidine positive cells were absent or present only in small numbers in scat/scat crisis spleens, whereas a strong reaction was evident, as expected, in the red pulp areas of +/+ and scat/scat remission spleens. No proliferation of plasma cells or Mott cells could be found using MG/P or PAS staining and conventional morphological criteria in any of the sections studied.

Animals in crisis showed foci of mononuclear cells in the perivascular areas of the liver (Fig 2G). Mitotic figures were commonly seen in these foci. Whether these cells are derived from or are similar to those of the spleen is unknown at present. No other organs showed any cellular infiltrates such as those found in the liver. Liver sections from animals in remission did not differ histologically from +/+ animals.

Examination of femurs showed increased megakaryocytes in scat/scat mice in crisis when compared to either +/+ or scat/scat mice in remission (Fig 2H and I). The cellularity appeared normal in most cases, although moribund animals frequently showed increased fat deposition in the marrow.

Peripheral blood. The extreme pallor of the mice in crisis was indicative of a severe anemia. Anisocytotic and poikilocytic erythrocytes were noted in blood smears from crisis mice, and there were frequent burr cells, schistocytes, and acanthocytes, and markedly increased numbers of poly-

Fig 1. The survival of scat/scat mice as a function of age. Data is based on the daily observation of 291 animals.

Hemosiderin deposits were not observed in mutant animal tissues during crisis episodes using Gomori’s iron stain. Spleens of scat/scat mice in crisis showed increased mature megakaryocytes and a proliferation of large mononuclear cells that completely effaced the normal splenic architecture (Fig 2A through C). Lymphatic nodules and blood sinuses were conspicuously reduced or absent. The mononuclear cells were characterized by a large, pale nucleus with dispersed chromatophilic erythrocytes (Fig 2J through L). RBC counts from 17 mice in crisis at 20 to 26 days postnatally averaged 2.62 x 10^12/L ± 0.24 x 10^12/L (mean ± SEM). Counts from 16 normal-appearing littermates averaged 8.88 x 10^12/L ± 0.29 x 10^12/L. Hematocrits from 34 mice in crisis were 15.8% ± 1.3%, while those from 8 mice in remission were 41.4% ± 2.6% and were similar to the mean value of 42.6% ± 0.9% from 32 normal mice. The absolute reticulocyte count was increased from 0.81 x 10^12/L ± 0.08 x 10^12/L in normal mice (n = 12) to 1.97 x 10^12/L ± 0.15 x 10^12/L in scat/scat mice in crisis (n = 8).

The intradermal petechiae, bruising, and anemia noted at birth and during crisis periods were suggestive of a severe bleeding disorder. Bleeding times were noted to be increased in scat/scat mice during routine retro-orbital bleeding or when obtaining blood from tail veins. Clotting in both cases occurred almost immediately (less than 5 minutes) in BALB-+/+ mice, whereas BALB-scat/scat mice required cautery. In the case of retro-orbital bleeding, BALB-scat/scat mice usually bled to death. Subsequent to this observation, retro-orbital bleeding, when performed in BALB-scat/scat mice, was immediately followed by euthanasia. Prolongation of bleeding time suggested a platelet deficiency. The mutant mice were severely thrombocytopenic during crisis periods (Fig 3). During remission periods, complete (males) or near-complete (females) recovery occurred. On blood smears there was no evidence of platelets in crisis mice, although platelets were present and appeared normal in mice in remission (Fig 2K and L).

Total WBC counts indicated that crisis mice were severely leukopenic (Table 2). During remission periods, female WBC counts did not differ from normal controls, while male WBC counts improved but remained significantly (P < .05) less than that in normal males. Differential counts of blood smears showed an increase in the percentage of granulocytes and a decrease in the percentage of lymphocytes. Absolute counts based on these values indicated a severe lymphopenia that persisted in remission males but not females. Monocytes and eosinophils consistently represented 1% to 5% of the peripheral blood white cells and did not differ among the mice studied.

Serum bilirubin levels and qualitative urine screening. Serum bilirubin levels did not significantly differ between BALB-scat/scat mice in crisis and BALB-+/+ controls. For 2- to 4-week-old scat/scat mice in crisis, total serum bilirubin levels were 13.2 μmol/L ± 1.7 μmol/L (n = 9) compared to 10.1 μmol/L ± 1.7 μmol/L (n = 5) for +/+ mice of the same age. Qualitative urine screening showed no increase in urinary bilirubin in scat/scat crisis mice. Increased urine

Table 1. Spleen Weight

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>No. Mice</th>
<th>Weight of Spleen (% body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Normal</td>
<td>14</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>scat/scat</td>
<td>Crisis</td>
<td>33</td>
<td>3.00 ± 0.30*</td>
</tr>
<tr>
<td>scat/scat</td>
<td>Remission</td>
<td>12</td>
<td>0.60 ± 0.14</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*P < .05 versus normal and remission animals.
abnormal and platelets are completely lacking in the scaf/scat crisis mouse.

**HERITABLE ANEMIA AND THROMBOCYTOPENIA IN MICE**

The scaf/scat mouse in remission... of plasma injection was limited to platelets. By indirect Coombs' tests were negative... No effect on erythrocyte morphology was detected in any of the recipients. Indirect Coombs' testing... anti-IgG no longer recognizes the 110-Kd platelet protein.

**Examination of serum for autoantibodies.** In six of nine trials, plasma from mice in crisis caused a diminution of platelet numbers when injected into the tail veins of BALB-+/+ mice (Fig 4). At 90 minutes postinjection, platelet counts ranged from 62% to 83% of baseline values. No effect was seen in any of six recipients of control (BALB-+/+) plasma. No effect on erythrocyte morphology was detected in any of the recipients. Indirect Coombs' tests were negative for mice in crisis (n = 6), further suggesting that the effect of plasma injection was limited to platelets. By indirect Coombs' testing, erythrocyte autoantibody was easily detected in aged NZB (n = 3) positive controls.

To determine whether the platelet inhibitory factor was an autoantibody, plasma from BALB-+/+, scaf/scat mice in crisis and in remission, and positive control plasma from the autoimmune NZB mice was reacted with erythrocyte membrane and/or platelet proteins affixed to nitrocellulose. All plasma samples were tested against autologous erythrocyte membrane proteins. In eight trials no circulating erythrocyte autoantibodies were detected in plasma from the mutant mice or from BALB-+/+ mice (Fig 5). Plasma from autoimmune NZB mice gave strong positive reactions to NZB, BALB-scat/scat and +/+ erythrocyte membrane proteins.

For detection of platelet-specific antibodies, it was necessary to react both BALB-+/+ and scaf/scat plasma against BALB-+/+ platelet proteins, since insufficient scaf/scat platelets were available for platelet membrane preparations and electrophoresis. Our assumption was that a platelet-specific antibody, if present in scaf/scat mice, would cross-react with normal platelet proteins. A specific BALB-+/+ platelet membrane protein gave a strong positive reaction with plasma from 12 of 14 scaf/scat mice in crisis (Fig 5). In each case the platelet protein had an apparent mol wt of 110 Kd. There was no reactivity with the 110-Kd protein when the filters were probed with plasma from BALB-+/+ mice (n = 11). Of five scaf/scat remission mice tested, one showed a weak positive reaction and four were negative. The probability that the antibody is an IgG moiety is enhanced by the observation that scaf/scat crisis plasma precipitated with anti-IgG no longer recognizes the 110-Kd platelet protein.

**Disease transmittance through spleen cell transplantation.** To determine whether the disease is intrinsic to hematopoietic progenitors, spleen cells from mice in crisis or from normal mice were transplanted into lethally irradiated +/+ C.B.Hbb' congenic hosts. All the recipients assumed the complete hemoglobin phenotype of the mutant mice 3 to 8 weeks after transplantation. The recipients of scaf/scat cells but not +/+ cells all displayed overt symptoms of the scat gene defect in an episodic manner within 8 weeks. Animals were autopsied as they became moribund (3 to 11 weeks post-transplantation). When compared to mice receiving +/+ cells (autopsied at 8 to 11 weeks post-transplantation), mice receiving scaf/scat cells had significantly increased spleen size and significantly decreased peripheral blood counts that exactly paralleled the changes seen in scaf/scat crisis versus +/+ animals (Table 3). In addition, the spleen and liver histology and RBC morphology were altered such that they took on the scaf/scat crisis appearance (Fig 6A through C). No change in spleen histology or erythrocyte morphology was seen in mice receiving +/+ cells.

Western analyses detected antibody to the 110-Kd platelet protein in 60% of the scaf/scat recipients (not shown). None of the mice receiving +/+ cells demonstrated any antiplatelet antibody.

The results indicated that the disease was transmitted by mutant spleen cells into irradiated +/+ congenic hosts.

**Effects of splenectomy on disease genesis.** The splenomegaly and autoimmune aspects of the disease in scaf/scat...
mice in crisis suggested that splenectomy might be a therapeutic measure. Splenectomy was performed in 14 mutant animals. All animals were in remission at the time of surgery as judged by overt symptoms and peripheral blood WBC and PLT counts. Twelve of the mice (86%) survived at least 60 days without any overt indications of disease. Five healthy mice with no sign of bruises or petechiae were killed at 60 days without any overt symptoms and peripheral blood WBC and PLT counts, normal hematocrits and platelet counts, normal WBC counts, normal differential counts and normal reticulocyte counts (Table 4). Of those mice remaining, all survived for longer than 10 months without ever showing scat symptoms.

The survival data (12 of 14 splenectomized mutant mice survived in remission for at least 60 days compared to 22 of 291 unperturbated mutant mice that had survived in remission for 60 days) indicated that splenectomy significantly (P < .005) improved survival of the mutant mice.

Screening of normal littermates. Unaffected offspring (scat/+ ) of scat/ + mating pairs were overtly normal; scat/+ (50%) pups could not be distinguished from +/+ (25%) pups at birth or subsequently. To determine if scat/+ littermates could be identified by other parameters, 17 normal-appearing littermates taken from litters in which at least one affected animal was produced were compared to age-related +/+ mice born of BALB—/+ mating pairs. The absolute spleen weight was also increased in scat/+ mice born of BALB—/+ mating pairs. The normal-appearing littermates did not differ from +/+ offspring of +/+ parents in WBC, platelet, reticulocyte, or differential counts or in erythrocyte morphology or hematocrit values (data not shown). Also, two populations of normal littermates representing scat/+ , and +/+ genotypes were not evident. In terms of spleen weights, however, offspring of scat/+ mating pairs had consistently and significantly (P < .05) increased spleen sizes compared to offspring of +/+ mating pairs. The spleen weight, as percent body weight, was 1.02 ± 0.03 for scat/+ mice and 0.73 ± 0.03 for +/+ mice. The absolute spleen weight was also significantly (P < .05) elevated in scat/+ versus +/+ mice (data not shown). Histologically no abnormalities in the spleens of scat/+ animals could be identified by light microscopy, indicating that the changes at this stage were subtle.

Western analysis for platelet antibody was negative in all 17 normal appearing offspring of scat/+ mating pairs.

The results presented here indicate that scat/scat mice suffer from an immune disorder. The presence of an antibody specific for a 110-Kd platelet protein in mice that are platelet deficient suggests cause and effect. The platelet insufficiency is manifested in low platelet counts and a lack of platelets on peripheral blood smears. (Manual platelet counts are falsely

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Table 2. WBC Counts in Normal and scat/scat Mice in Crisis and in Remission

<table>
<thead>
<tr>
<th>Group (No.)</th>
<th>WBC Count (×10⁹/L)</th>
<th>Granulocytes (%)</th>
<th>Absolute Granulocyte Count</th>
<th>Lymphocytes (%)</th>
<th>Absolute Lymphocyte Count (×10⁹/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/- (29)</td>
<td>13.8 ± 0.5</td>
<td>15.5 ± 1.3</td>
<td>2.2 ± 0.2</td>
<td>82.2 ± 1.4</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td>scat/scat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rem (5)</td>
<td>12.5 ± 2.1</td>
<td>15.2 ± 2.1</td>
<td>1.8 ± 0.3</td>
<td>84.2 ± 2.8</td>
<td>10.5 ± 1.9</td>
</tr>
<tr>
<td>Cr (13)</td>
<td>4.2 ± 4.0±</td>
<td>43.4 ± 4.0±</td>
<td>2.2 ± 0.8</td>
<td>53.8 ± 4.1±</td>
<td>2.3 ± 0.4±</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>+/- (25)</td>
<td>11.6 ± 0.3</td>
<td>18.7 ± 1.4</td>
<td>2.2 ± 0.2</td>
<td>79.1 ± 1.5</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>scat/scat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rem (8)</td>
<td>8.1 ± 0.6*</td>
<td>20.9 ± 1.6</td>
<td>1.7 ± 0.2</td>
<td>70.2 ± 3.2</td>
<td>5.7 ± 0.6*</td>
</tr>
<tr>
<td>Cr (8)</td>
<td>3.4 ± 0.5±</td>
<td>44.6 ± 7.7±</td>
<td>1.5 ± 0.2</td>
<td>50.6 ± 7.1±</td>
<td>2.0 ± 0.5±</td>
</tr>
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</table>

Values are mean ± SEM.
Abbreviations: Cr, crisis; Rem, remission.
*P < .05 versus +/-.
± P < .05 versus +/+ and scat/scat.

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Discussion

The results presented here indicate that scat/scat mice suffer from an immune disorder. The presence of an antibody specific for a 110-Kd platelet protein in mice that are platelet deficient suggests cause and effect. The platelet insufficiency is manifested in low platelet counts and a lack of platelets on peripheral blood smears. (Manual platelet counts are falsely
elevated by the presence of many microerythrocytes and unlysed erythrocytes with numerous membrane spicules.) The onset of the disease at birth is difficult to explain, since the immune system of the newborn is not yet fully developed. Recently we investigated the possibility that maternal antibodies are involved in the etiology of the defect. Plasma from pregnant scut/+ but not from virgin scut/+ mice is, in fact, positive for platelet antibody by Western blotting (paper in preparation). The etiology is suggestive of a platelet-limited transplacental-mediated disease in the scut/scut mice. The initial crisis in scut/scut newborn mice may be due to maternal antibody crossing the placenta.

The observation that spleen weights are increased in normal-appearing littermates of scut/scut mice further supports the hypothesis of a maternally mediated effect. Our Western blot results and plasma injection data clearly show that the antibody present in scut/scut crisis mice crossreacts with normal platelets. Hence, one could predict that the increased spleen size in normal littermates (apparently regardless of genotype) represented a response to an antibody that was present in utero and then cleared after birth. The second crisis period is not so easily reconciled, since the affected mice are frequently weanlings. This suggests antibody is produced in situ rather than transferred from the mother at this stage.

Autoantibodies to specific platelet membrane glycoproteins (GP) are present in some humans with autoimmune thrombocytopenic purpura. In many cases such antibodies appear to be directed against human GP Ib and GP IIb/IIIa. In the present study antibodies to the as yet unidentified 110-Kd platelet membrane protein were detected in the plasma of 12 of 14 scut/scut mice. Plasma from normal mice was consistently negative, and only one mouse classified as being in remission displayed reactivity. Initial tests showed that nonspecific protein binding occurs at plasma dilutions of 1:40 in all groups (data not shown). Hence all samples were diluted at least 1:50 in this study. Similar problems in obtaining low background using human material have been encountered with both serologic and immunoblot methods. In addition, as was the case with two scut/scut mice tested by immunoblotting and three tested by serum injections, circulating antiplatelet antibody is not always detectable in humans with ITP using either Western analysis or other methods.
Fig 6. Microscopic appearance of spleens and blood from a recipient of mutant cells. 2 x 10⁶ scat/scat Cr spleen cells were injected into a +/+ C.B.Hbb mouse. Recipient tissues were removed 8 weeks later. Photomicrographs (original magnifications) are of (A) spleen (x100); (B) spleen (x1000); and (C) peripheral blood (x1000).

Antibodies to erythrocytes have not been detected either by immunoblotting or by the indirect Coombs' test. During crisis episodes mice were not visibly jaundiced. Serum and urine bilirubin values did not differ from controls. In addition, no evidence of increased intravascular RBC destruction in kidney was seen using Gomori's iron stain. These observations suggest the anemia in the scat/scat mice in crisis is a consequence of bleeding due to the platelet insufficiency. The extreme pallor of newborn mice may result from excessive bleeding at birth. The development of misshapen erythrocytes as the mice become moribund is more difficult to understand. The scat/scat mice in crisis have grossly enlarged spleens. Unlike the human spleen, however, the mouse spleen functions as an adjunct hematopoietic organ throughout life.³⁸ Splenomegaly in scat/scat crisis mice could, therefore, be an indicator of compensatory erythropoiesis. However, if this were the case, splenectomy would be expected to worsen the anemia, not cure it. Furthermore, the histopathology of the spleen is not consistent with increased RBC proliferation but instead shows large mononuclear cells of unknown function. Based upon morphological characteristics, MG/P, PAS, and benzidine staining, these cells are neither erythrocyte precursors nor plasma cells. Misshapen erythrocytes may be a consequence of the altered role of the spleen in crisis mice. Further studies to characterize the spleen cell population in scat/scat mice are underway.

The primary cause of the murine disease is not clear. It is possible that the disease symptoms in the scat/scat mice are related to a spleen-limited neoplasm.³⁹,⁴⁰ It is noteworthy that such neoplasms in human beings are characterized by a lack of lymph node involvement, but liver involvement is often noted. Autoimmune complications of both platelets and erythrocytes occur secondary to myeloproliferative and lymphoproliferative disorders in human beings.⁴¹-⁴⁷ The destruction of splenic architecture and accumulation of large mononuclear cells in scat/scat mice during crisis are consistent with a neoplastic syndrome. Should such a disease exist in the mouse, one would predict the disease would be transferrable through hematopoietic "stem" cells. The fact that the syndrome is acquired by normal recipients of mutant spleen cells is consistent with the transfer of the following cell type: a spleen-derived neoplastic cell; an antibody-producing cell capable of proliferating in the host; or a cell recognizable as foreign by both host and donor.

The spleen appears to be critical to the genesis of the second crisis, since splenectomy during the initial remission halts disease progression. One can hypothesize that splenectomy cures the disease in scat/scat mice, as in human beings with ITP, by removing a site of antiplatelet antibody synthesis and/or of platelet destruction.⁴⁶,⁴⁷

There are a number of similarities between the murine disease and idiopathic thrombocytopenic purpura (ITP) in human beings. These include IgG-platelet antibody, mucosal bleeding, dermal bleeding (petechiae), increased numbers of megakaryocytes, and intracranial hemorrhaging.⁴⁸-⁵³ However, the resemblance is not absolute. ITP in human beings is

Table 4. Peripheral Blood Cells in Splenectomized scat/scat Mice

<table>
<thead>
<tr>
<th>WBC Count (x10⁹/L)</th>
<th>Platelet Count (x10¹²/L)</th>
<th>Granulocytes (%)</th>
<th>Lymphocytes (%)</th>
<th>Hematocrit (%)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.8 ± 2.9</td>
<td>1.24 ± 0.2</td>
<td>23.4 ± 3.9</td>
<td>74.4 ± 4.4</td>
<td>46.8 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. No difference was noted between males and females, and the results were pooled from five mice.
not associated with significant splenomegaly, misshapen erythrocytes, or leukemia. An important point is that both diseases are characterized by circulating antiplatelet antibodies and diminution of platelet number. This means that exploration of the murine disease should uncover common developmental pathways, consequences, and prophylactic measures for platelet insufficiency diseases.

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Heritable severe combined anemia and thrombocytopenia in the mouse: description of the disease and successful therapy

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