Hematologic Effects of Splenic Implants

By Achmad Tarnuzi and R. Kennedy Smiley

It is well known that after splenectomy in normal animals and man the number of circulating white blood cells and platelets increases greatly. In many diseases accompanied by splenomegaly, there is a decrease in the leukocyte and platelet count. These observations have indicated that the spleen has a function in the maintenance of the white cell and platelet levels in the peripheral blood. The mechanism by which the spleen exerts an effect on peripheral blood elements has not been established. One hypothesis suggests that the regulatory mechanism is a manifestation of a direct effect of the spleen—i.e., splenic sequestration of white cells and platelets. The second hypothesis suggests the effect is indirect—i.e., due to production by the spleen of a humoral substance which influences production and/or delivery of white cells and platelets from the bone marrow.

In 1951, experiments reported by Palmer suggested that the normal rat spleen produces a humoral substance which suppresses postsplenectomy leukocytosis. This conclusion was based in part on the observation that subcutaneous or intraperitoneal reimplantation of approximately 10 per cent of the spleen of splenectomized rats partially inhibited the leukocytosis which follows splenectomy.

The purpose of the present work was to reexamine the effects of splenectomy in rats on the circulating formed elements of the blood and to see if the effects of splenectomy are influenced by intraperitoneal reimplantation of approximately 10 per cent of the spleen either as a free implant or as an implant enclosed in a Millipore diffusion chamber.

Materials and Methods

One hundred and twenty-six male albino rats of the Sprague Dawley strain weighing 300-400 Gm. were used in the study. They were housed in individual wire cages and fed Purina Chow and water ad libitum. The animals were divided into groups as shown in Table 1.

All operative procedures were done under ether anesthesia. Splenectomy and partial omentectomy were performed through a longitudinal incision about 1½ inches long in the midline of the abdomen which began just below the costal margin. The splenic pedicle was ligated with three silk ligatures. In the sham-operated (omentectomized) group, the omentum was tied off a few mm. from its gastric attachment and excised. To prepare the splenic implants, the spleen was weighed when removed and a portion which approximated 10 per cent of the spleen by weight was removed from the middle portion.

From the Departments of Medicine and Pathology, University of Ottawa and the Ottawa General Hospital, Ottawa, Ontario, Canada.

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Achmad Tarnuzi, M.D.: Colombo Plan Fellow in Hematology, Department of Pathology, University of Gadjah Mada, Jogjakarta, Indonesia. R. Kennedy Smiley, M.D.: Professor of Medicine, University of Ottawa, Ottawa, Ontario, Canada.
Table 1.—Distribution of Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment Number</th>
<th>Total Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>I  Normal</td>
<td>6 5 2 6 3 5 5</td>
<td>32</td>
</tr>
<tr>
<td>II Splenectomized</td>
<td>6 3 3</td>
<td>12</td>
</tr>
<tr>
<td>III Sham operated</td>
<td>6 5</td>
<td>11</td>
</tr>
<tr>
<td>(partial omentectomy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Splenectomized</td>
<td>6 6 4 5 5 7</td>
<td>33</td>
</tr>
<tr>
<td>+ 10% spleen implant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V  Splenectomized</td>
<td>4 10 10 24</td>
<td>24</td>
</tr>
<tr>
<td>+ 10% spleen in chamber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Splenectomized</td>
<td>4 6 4 14</td>
<td>14</td>
</tr>
<tr>
<td>+ empty chamber</td>
<td></td>
<td></td>
</tr>
</tbody>
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The fragment was kept moist with sterile saline, weighed, and then placed loosely intraperitoneally in the left upper quadrant near the original spleen site.

All blood counts were taken directly from freely flowing tail vein blood obtained by cutting off the tip of the tail without anesthesia. Leukocyte counts were done with National Bureau of Standards (N.B.S.) certified white cell diluting pipettes and chambers.

Fig. 1.—Mean WBC’s in unoperated, partially omentectomized and splenectomized rats.
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Platelet counts were done by the method of Brecher and Cronkite using N.B.S. certified red cell diluting pipettes and chambers. The volume of packed red cells was determined by the microhematocrit method. All hematologic examinations were done the day prior to the operation on the test and control groups and were repeated on days 4 and 7. The determinations were then done weekly for 6 weeks. At the end of this time, the animals which had spleen implants were sacrificed and the spleen fragments recovered. The fragments were recognizable easily in all cases and after removal were fixed in Bouin's solution and sectioned for histologic examination.

The diffusion chambers were made from acrylic plastic in the form of a ring measuring 17 mm. in diameter and 5 mm. in height, with a thickness of 1.5 mm. Millipore membranes of 0.45 micra average pore size were glued on the acrylic ring with an acrylic cement (Tensol No. 6, Canus Equipment Ltd., Ottawa). A thin film of cement was applied to the edge of the ring and the membrane laid flat on the ring. The ring was inverted and pressed against the membrane. A final seal was obtained by running a second film of cement around the outer edge. The acrylic discs and membranes were sterilized with ethylene oxide in a Petri dish which was then sealed until the time of the operative procedure. When the portion of the spleen to be reimplemented was obtained as described, it was placed inside the chamber, moistened with three drops of sterile saline, and sealed with a second membrane. The chamber was then slipped into the peritoneal cavity in the left upper quadrant. In group 6 (Table 1) animals, three drops of sterile normal saline only were placed in the chamber.

Aseptic technic was observed during all operative procedures. Rats received tetracycline (35 mg./day) by mouth for 3 days postoperatively.

Fig. 2.—Mean platelet counts in unoperated, partially omentectomized and splenectomized rats.
Fig. 3.—Total WBC’s (upper), mononuclear cells (middle) and neutrophils (lower) in splenectomized and unoperated rats.

RESULTS

Effects of Splenectomy on the Circulating Blood Elements

Splenectomy resulted in leukocytosis and thrombocytosis which persisted for the 6 weeks following operation that counts were done. Leukocyte and platelet levels in the splenectomized animals were elevated at all points in time at which observations were made. This effect of splenectomy is specific and does not occur after partial omentectomy (Figs. 1 and 2).

At no point in time did the volume of packed red cells differ between the unoperated, splenectomized, and partially omentectomized group. These observations are not presented.

The leukocytosis which follows splenectomy is due to an increase in the
Effects of Free Splenic Implant Postsplenectomy

The data on the effects of intraperitoneal implantation of approximately 10 per cent of the spleen on the course of leukocyte and platelet levels in splenectomized rats is illustrated in Figures 4 and 5. The total leukocyte count in the “implant” group is lower \((p < .01)\) than in the splenectomized group at 14 and 21 days after implantation. At all points after 21 days, the leukocyte counts in the implant group do not differ from the counts in the group subjected to splenectomy only.

The free splenic implant suppresses the thrombocytosis which follows splenectomy in the rat. This is shown by the significant differences in mean platelet counts \((p < .01)\) between the splenectomized group and the “spleenectomized plus implant” group at all points up to 35 days after splenectomy. By day 42, the thrombocytosis which follows splenectomy has subsided progressively so that the splenectomized and implant groups do not differ significantly. Both groups still have platelet counts which are higher
Fig. 5.—Platelet counts in unoperated, splenectomized and splenectomized plus 10 per cent implant groups.

(p = <.01) than the unoperated group. It should be noted that the splenic implant does not prevent postsplenectomy thrombocytosis completely; the group carrying the implants have platelet counts which are higher (p = <.01) than those in the unoperated group at all points except days 4 and 21.

Effects of Millipore Chamber

The changes in total leukocyte count in splenectomized rats who had either an empty Millipore diffusion chamber or a Millipore diffusion chamber containing a splenic implant are shown in Figure 6. The data for the “splenectomized only” and the “splenectomized plus implant” groups previously presented are shown for comparison. The insertion of the chamber alone in splenectomized rats results in leukocyte counts which are much higher at all points in time than those in rats subjected to splenectomy only. The same effect is seen in the group in which a splenic implant is enclosed in the chamber. To determine if the splenic implant has any effect when enclosed in the chamber, it is necessary to compare the leukocyte counts in the splenectomized rats bearing an empty chamber with the leukocyte counts in the group of splenectomized rats bearing a spleen implant in the chamber. When this is done, a significant difference (p = <.01)
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Splectomy

empty chamber

Splenectomy.

spleen implant

2 3 WEEKS

Fig. 6.—WBC’s in splenectomized rats with chamber containing 10 per cent splenic implant.

suggestive of a suppressive effect of the splenic implant on postsplenectomy leukocytosis is present only on day 14.

Figure 7 illustrates the effect of the splenic implant enclosed in a Millipore diffusion chamber on the platelet count of splenectomized rats. The data presented previously on platelet counts in the “splenectomized only” and the “splenectomized plus implant” groups are redrawn in Figure 7 for comparison. The consistent effect of the splenic implant enclosed in the Millipore chamber in preventing thrombocytosis after splenectomy is seen. At all points from days 4 to 42 inclusive, the mean platelet count is lower (p < .01) in the group of rats bearing a splenic implant enclosed in a Millipore chamber than in the splenectomized rats bearing an empty chamber.

In summary, the suppressive effect on postsplenectomy thrombocytosis of a 10 per cent splenic implant during the 6 weeks after splenectomy is similar whether the implant is free or enclosed in a Millipore diffusion chamber.

Appearance of Splenic Implants

In all but 2 rats, free splenic implants were recognized easily at the end of 6 weeks. The fragments resembled normal splenic tissue (Fig. 8) and appeared to be about the same size as when implanted. They adhered to the
Splenectomy only
+ empty chamber
- Splenectomy + spleen implant

Fig. 7.—Platelet counts in splenectomized rats with empty Millipore chamber and chamber containing 10 per cent splenic implant.

The chambers were found loose in the peritoneal cavity. When the Millipore membrane was cut away, the chamber contained mucoid material and the implants differed strikingly from the free implants. The fragments appeared pale in contrast to the reddish color of the free implants. Microscopically, a large portion of the implants had degenerated (Fig. 10). The remaining cells (Fig. 11) appeared to be lymphocytes, probably residual Malpighian corpuscles.

DISCUSSION

The results of the present experiments do not provide convincing evidence that postsplenectomy leukocytosis is influenced by splenic implants. Although mean leukocyte counts were lower in the splenectomized animals bearing an implant at 14 and 21 days after splenectomy, the effect was not persistent. When the implant was enclosed in a Millipore diffusion chamber, evidence of a suppressive effect on postsplenectomy leukocytosis was seen only on
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Fig. 8.—Free splenic implant after 6 weeks intraperitoneally (I.P.). HPS × 21.

Fig. 9.—Free splenic implant after 6 weeks I.P. HPS × 380.
day 14. Palmer's experiments on the effect of implantation of splenic fragments included observations on days 8 and 28 postsplenectomy and a suppressive effect of the implant was found at both times. Our experiments do not confirm the persistence of the suppressive effect beyond the third week after implantation, and the magnitude of the differences between the splenectomized group and either implant group before 21 days is not impressive.

The suppressive effect of the splenic implant on circulating platelet levels
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is more marked and persistent than on leukocyte levels. Platelet levels are suppressed whether the implant is free or enclosed in a Millipore chamber with an average pore size of 0.45 μ. These results suggest the effect is mediated by a humoral substance released from the implants.

The degenerative changes seen in the splenic implants after 6 weeks in the diffusion chamber appear similar to those described and illustrated by Osoba and Miller in thymic implants enclosed in Millipore chambers.

By 6 weeks postsplenectomy, the platelet levels in the splenectomized rats gradually decrease to the levels found in the groups bearing free implants or implants in chambers; the platelet levels in these three groups remain higher than in the unoperated or partially omentectomized controls. The sequence of events suggests that the degenerating implant (and the free implant) serves as a source of a humoral substance which prevents thrombocytosis during a 5 to 6 week period after splenectomy. By the end of 6 weeks, production of the substance elsewhere in the body masks any effect of the implant, although still inadequate to restore the platelet count to the same level as in intact animals.

Previous work has shown that platelet life span in normal and splenectomized rats is similar. If prolongation of platelet lifespan is not a factor in postsplenectomy thrombocytosis then decreased sequestration or increased thrombopoiesis must be the explanation. Matter et al. concluded that "the spleen exerts its effects on the level of circulating platelets through alteration in the rate of thrombopoiesis." Presumably the humoral substance suggested by the present experiments is produced normally by the rat spleen and has a suppressive effect on thrombopoiesis. After removal of the spleen, marked thrombocytosis results which gradually decreases as the postulated humoral substance is produced elsewhere or other control mechanisms become established.

SUMMARY

1. Splenectomy in the rat results in a marked elevation in the number of circulating leukocytes and platelets.

2. Transient suppression of the leukocytosis may result from the presence of a 10 per cent splenic implant, either free in the peritoneal cavity or enclosed in a Millipore diffusion chamber.

3. Marked and persistent suppression of postsplenectomy thrombocytosis results from the presence of a 10 per cent splenic implant, whether free or enclosed in a 0.45 μ Millipore diffusion chamber.

4. The results provide indirect evidence of a humoral substance produced by rat spleen which influences platelet levels; presumably this substance acts by suppressing thrombopoiesis.

SUMMARIO IN INTERLINGUA

1. Splenectomia in le ratto resulta in un elevation marcate del numero de circulante leucocytos e plachettas.

2. Un suppression transiente del leucocytosis resulta possibilemente ab le
presentia de un implantation splenic de 10 pro cento le qual pote esser libere in le cavitate peritonee o includite in un camera diffusional Millipore.

3. Un marcate e persistente suppression de thrombocytosis post splenectomia resulta ab le presentia de un implantation splenic de 10 pro cento, sin reguardo a si illo es libere o includite in un camera de diffusion Millipore de 0,45μ.

4. Le resultatos provide evidentia indirecte de un substantia humoral producite per le splen de rattos influentiante le nivellos del plachettas. Presumite-mente iste substantia age per supprimer le thrombopoiese.

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REFERENCES

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ACHMAD TARNUZI and R. KENNEDY SMILEY