The Effect of Foreign Protein on the Blood and Reticuloendothelial System of the Hamster

By Gilbert H. Friedell, Joseph D. Sherman and Perry G. Rigby

In previous studies on the anemia of malignancy, a three-way relationship was noted between tumor growth, splenomegaly, and the development of anemia in the tumor-bearing hamster. It was further shown that the anemia and splenomegaly were in fact related to the progressive increase in the amount of necrotic tissue within the growing tumor. In subsequent studies it was found that anemia and splenomegaly could be produced by the daily injection of a sterile, cell-free saline extract of the necrotic portion of the tumor. Extracts of hamster tumor were also found to affect erythrocytes in vitro, resulting in hemolysis or agglutination. Microscopic examination of the enlarged spleens in these experimental animals revealed reticuloendothelial cell hyperplasia, plasmacytosis and hemosiderosis as well as extramedullary hematopoiesis.

The increased hemosiderin indicated that abnormal splenic hemolysis was present, an interpretation confirmed by studies with radioactive chromium, while the plasma cell proliferation suggested the presence of an antibody response to some antigen. It was thought that the antigenic stimulus in these animals might have been furnished by altered or "foreign" protein resulting from the necrosis of tumor tissue.

A foreign protein was therefore injected into normal hamsters in an attempt to reproduce the hematologic and pathologic findings seen in the tumor-bearing animals. In addition, since the anti-hamster serum erythroagglutination reaction was shown to be positive in a high proportion of tumor-bearing hamsters, the effect of foreign protein on the development of this erythrocyte reaction was also studied.

Materials and Methods

Golden hamsters of both sexes, 8 to 10 weeks old, were used in these experiments. They were maintained on Purina Laboratory Chow and water ad lib. A dosage of 0.5 cc. of human blood plasma was chosen, since it was found that 1.0 cc. of human plasma alone or combined with Freund's adjuvant resulted in cachexia, diarrhea, weight loss, serosanguinous ascites, and death in 3-7 days. Erythroagglutination reactions were positive in these animals. All of the animals were therefore given daily intraperitoneal injections of 0.5 cc. of human blood plasma until the date of sacrifice when complete autopsies were performed. Animals were weighed at the start of the experiment and again when they were sacrificed. Hemoglobin values were determined using standard hematologic technic. Agar gel diffusion studies were made on Ouchterlony plates prepared with Noble Agar (Difco), 2.0

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per cent saline, and 0.02 per cent merthiolate contained in clear plastic disposable petri dishes. These tests were performed on undiluted serum at 28 C.

Fourteen animals were sacrificed after 14 days, 15 after 23 days, 15 after 32 days, 10 after 52 days, 5 after 58 days, 10 after 61 days and 7 after 68 days. Tissues were fixed in Carnoy's fluid and in 10 per cent neutral formalin. Spleen volumes were calculated from measurements in three dimensions. Microscopic sections of the spleen, liver and bone marrow were prepared and stained with hematoxylin and eosin, methyl-green pyronine and Giemsa stains.

Microscopic sections of the spleens were examined with particular reference to the number and size of malpighian corpuscles, presence of a perifollicular "halo" of immature lymphocytes, degree of congestion, number of neutrophils and phagocytes, degree of hyperplasia and/or anaplasia of reticuloendothelial cells.

Blood samples from the animals to be tested for erythroagglutination were obtained by cardiac puncture. The erythrocytes were washed three times with normal saline, then a 5 per cent suspension in saline was prepared. Two drops of this suspension were mixed with two drops of antihamster serum (prepared by a method similar to that reported by Betts et al.), in clean glass test tubes (10 x 75 mm.), then centrifuged at 1500 rpm, resuspended and recentrifuged. The contents of the tube were then shaken, flicked sharply and examined grossly and microscopically for agglutination. Reactions were read as 0, + to ++++. Doubtful results were recorded as negative (0). Saline controls were run concomitantly.

Saline suspensions of washed red cells from normal hamsters, from hamsters injected with human plasma, and from hamsters injected with human plasma plus Freund's adjuvant were mixed with 1) blood serum collected from normal hamsters, 2) blood serum from hamsters injected with human plasma, 3) Freund's adjuvant, and 4) fresh normal human plasma. One set of tubes was incubated for 5 minutes and another for 2 hours at room temperature. The tubes were then centrifuged for 2 minutes at 1500 rpm, and read microscopically as above. Washed human red cells were similarly treated with serum from hamsters injected with human plasma or with human plasma plus Freund's adjuvant and examined as above.

RESULTS

All of the experimental animals gained weight and appeared healthy when they were sacrificed. The normal hemoglobin value for hamsters of the age used in this experiment is 16.2 ± 1.3 Gm. The normal spleen volume in hamsters at this age is 240-270 mm³.

Hematologic Data

The animals sacrificed on day 14 were not anemic, and the average spleen volume was within the normal range. Of the 15 animals sacrificed on day 23, 3 were found to have hemoglobins of 11.6, 12.6 and 13.3 Gm. and all had enlarged spleens. The remaining hamsters were not anemic. The hamsters sacrificed on day 32 were not anemic but 5 animals had spleen volumes over 350 mm³. At the end of 52 days, 3 of the 8 hemoglobin values in this group were abnormally low, 13.1, 12.3 and 12.3 Gm., while the remaining 5 animals had hemoglobin values that ranged from 14.0 to 16.5 Gm. The average spleen volume for the 10 animals in this group was 484 mm³.

The five animals sacrificed on day 58 and the 10 sacrificed on day 61 will be considered together. None of the hamsters were anemic and the average spleen volume was 456 mm³. In the last experimental group sacrificed on day 68, none of the 7 animals were anemic although the hemoglobin values were
somewhat low and varied from 14.6 to 15.9 Gm. The average spleen volume was 449 mm$^3$.

*Morphologic Data*

**Splenic histology**

NORMAL MORPHOLOGY. In hamsters of the age and weight used in this study, only a few Marshalko-type plasma cells are normally seen in the red pulp, and immature pyroninophilic cells resembling plasma cell precursors are rather scarce. Malpighian corpuscles resemble those in man and are not normally outlined by a "halo" of immature lymphoid cells. Congestion of slight degree is a constant finding in spleens from normal hamsters if the animals have been sacrificed by an overdose of Nembutal (sodium pentobarbital, Abbott). A small amount of hemosiderin can generally be found in the red pulp. Extramedullary hematopoiesis is only rarely seen in the normal spleen of a hamster 15 weeks of age or older, and when it is present it is usually represented by a few scattered foci of erythropoiesis and occasionally a rare megakaryocyte. Granulopoiesis is almost never seen in the normal hamster spleen of this age.

Like other small animals and unlike man, who possesses an abundance of yellow marrow that can revert to active hematopoiesis under stress, the bone marrow of the hamster is almost all "red" marrow with relatively little reserve capacity with which to meet hematopoietic challenges. Thus the presence of extramedullary erythropoietic activity in the hamster is a fairly sensitive indicator of some degree of increased need for red blood cells.

EXPERIMENTAL GROUPS. Reticuloendothelial cell hyperplasia, plasmacytosis, hemosiderosis and extramedullary erythropoiesis were seen in spleens from all the animals in the experimental groups. Reticuloendothelial cell hyperplasia was present in some animals at 14 and 23 days but was found in all animals in the 32-day and subsequent groups. Proliferation of plasma cells followed a similar pattern. Splenic hemosiderosis became progressively more prominent in the 6 experimental groups and was most marked in the oldest group. Erythropoiesis appeared in the 14-day group and increased slightly in subsequent groups.

In some hamsters in the 32-day group there was a "halo" of immature-appearing, but generally not pyroninophilic cells around most of the malpighian follicles. This "halo" was somewhat similar in appearance to that reported to be characteristic of human cases of hypersplenism$^{10,11}$ and also somewhat resembled the early changes produced by Germuth in rabbits by the injection of bovine albumin.$^{12}$ A similar type of perifollicular "halo," although variable in degree, was present in 6 of 10 animals in the 52-day group. The perifollicular "halo" was noted in only 4 of the 15 animals in the 58- to 61-day group and in all 4 it was of minimal degree. In the 68-day group of 7 animals, the presence of the "halo" around malpighian follicles was noted only once.

**Hepatic Histology**

NORMAL MORPHOLOGY. In sections of liver from normal hamsters of comparable age to those used in this experiment, there is no extramedullary hem-
Table 1.—Anti-Hamster Serum Test Results in 20 Hamsters Injected Intraperitoneally Daily with 0.5 cc. of Whole Human Plasma

<table>
<thead>
<tr>
<th>Erythroagglutination</th>
<th>Day 0</th>
<th>1*</th>
<th>2*</th>
<th>5</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>10</td>
<td>8</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Only ten animals were studied on these days.

Table 2.—Incubation Studies: Summary of Agglutination Reactions for Control and Experimental Hamster Erythrocytes Incubated with Various Sera

<table>
<thead>
<tr>
<th>Condition of Red Cell</th>
<th>Serum</th>
<th>Human Plasma Injected</th>
<th>Human Plasma and Freund's Injected</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human plasma injected</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Human plasma and Freund's injected</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

atopoiesis. There are occasional lymphocytes seen in portal areas but neither plasma cells nor plasma cell precursors are present in the liver.

**EXPERIMENTAL GROUP.** The livers from animals in the 14- and 23-day group were generally unremarkable, save for the presence of rare plasma cells and plasma cell precursors in the portal areas. In the 32-day group, plasma cells and pyroninophilic plasma cell precursors were somewhat more numerous in the portal areas. Extramedullary hematopoiesis was not seen. In the 58- to 61-day group there was a still further small increase in the number of plasma cell precursors.

**BONE MARROW.** The bone marrow sections from all groups showed hyperplasia of the myeloid, erythroid and megakaryocytic elements.

**Immunologic Data**

The antihamster serum erythroagglutination reaction became positive in 15 of 18 hamsters during the 2-week period of daily intraperitoneal injections of human plasma. Two animals died following cardiac puncture. These data are summarized in table 1. This reaction remained positive for as long as 7 days after the last injection had been given. None of the 15 animals with positive tests were anemic, all appeared healthy and no splenomegaly was evident at autopsy.

The various in vitro combinations of erythrocytes and blood sera from normal hamsters, or hamsters injected with either human plasma or human plasma plus Freund's adjuvant did not result in any agglutination. Similarly, combinations of human red cells with plasma from hamsters injected with human plasma or human plasma plus Freund's adjuvant did not result in agglutination. The mixture of normal red cells and fresh human plasma, however, caused agglutination. The addition of erythrocytes directly to Freund's adjuvant did not result in agglutination (tables 2 and 3).

The agar diffusion study showed precipitates between the antihamster
Table 3.—In Vitro Studies: Summary of Agglutination Reactions for Hamster Erythrocytes Combined with Human Plasma, Freund's Adjuvant and Saline

<table>
<thead>
<tr>
<th>Agglutination Reaction</th>
<th>Human Plasma</th>
<th>Saline</th>
<th>Freund's Adjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed RBC's</td>
<td>+++ to +++++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Whole blood</td>
<td>+++ to +++++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

serum and human plasma as well as with normal hamster serum or hamsters injected with human plasma. The reaction of identity observed between anti-hamster serum and various hamster sera, however, was not seen between human plasma and hamster serum from an animal injected with human plasma, indicating a reaction of nonidentity. No precipitate was observed between human plasma and serum from a hamster injected with human plasma. (figs. 1 and 2).

DISCUSSION

Hamsters repeatedly injected with human plasma developed splenomegaly, reticuloendothelial cell hyperplasia, plasmacytosis, hemosiderosis, extramedullary erythropoiesis and a positive antihamster erythroagglutination reaction. Hemoglobins were generally in the low normal range in the latter half of the experimental period and some animals were mildly anemic in the middle of the experiment. These results furnish the picture of compensated erythropoiesis in response to an increased splenic erythrocyte destruction which appears to be related to, if not the direct result of, an immunologic response on the part of the spleen to repeated injections of foreign protein.

The changes in splenic morphology produced in our hamsters by the repeated injection of human plasma are comparable to those observed by Rich et al. in the acute splenic tumors developing in rabbits injected with bovine serum albumin. It is of interest that repeated injections of a 50 per cent glucose solution and of a nonantigenic polysaccharide failed to produce either splenomegaly or the morphologic changes noted in the acute splenic tumor. The moderate splenic hemosiderosis present in animals injected with human plasma represents morphologic evidence for increased splenic hemolysis. The degree of hemosiderosis was comparable to that observed in hamsters injected with sterile cell-free saline extracts of necrotic tumor tissue.

The presence of increased numbers of plasma cells and plasma cell precursors implies antibody production and suggests a response to the injected foreign protein. In addition to this morphologic evidence of an antigen-antibody reaction, the demonstration of a positive erythrocyte agglutination test also suggests that an immunologic mechanism was operative in producing red cell destruction.

The production of a positive erythroagglutination test in the hamster by the injection of foreign plasma is highly suggestive of an immunologic response. The human plasma presumably acts as an antigen to provoke the response of the reticuloendothelial system, but whether this antigenic reaction is due to the plasma itself or to some erythrocyte-human plasma combination...
is not known. The latter possibility is suggested by our findings that human plasma agglutinates hamster red cells in vitro. However, although the plasma may affect enough red cells to provide an antigenic stimulus, it is highly unlikely that there is enough human plasma in the circulating hamster blood to account for the red cell alteration detected by the antihamster erythroagglutination test. The agar diffusion studies showing a reaction of nonidentity between antihamster serum and human plasma/serum from a hamster injected with human plasma, as well as no reaction between the latter substances, also support this interpretation. Thus, serum from hamsters injected with human plasma had no agglutinating effect on normal hamster red cells when they were incubated in vitro, and erythroagglutination reactions remained positive for as long as 1 week after the last injection of human plasma.
Fig. 2.—Agar gel diffusion plate (K1), showing lines of identity and nonidentity. Center, absorbed antihamster serum; 12 o'clock, human plasma; 2 o'clock, serum from a hamster injected with human plasma; 4 o'clock, unabsorbed antihamster serum; 6 o'clock, Freund's adjuvant; 8 o'clock, normal hamster serum; 10 o'clock, unabsorbed antihamster serum (see text).

Motulsky et al. pointed out that the "rate of red cell destruction limited to the spleen (splenic hemolysis) does not appreciably exceed the production capacity of normally responsive bone marrow." They feel that "either destruction of blood in other areas of the body . . . or an associated impairment in erythropoiesis" must be present in addition to splenic hemolysis in order to produce a significant degree of anemia. In the experiment reported here there did not appear to be any other important site of blood destruction, and any impairment in erythropoiesis was not evident.

Billingham and Hildemann pointed out that there are no grounds for postulating any deficiency in the immunologic machinery of the hamster, despite the fact that there is a high degree of compatibility of skin homografts exchanged between members of the same Syrian hamster colony or
that tumors survive transplantation to other adult hamsters of the same origin as the tumor donor, or even into hamsters of unrelated stocks. The current study would support this view, for the hamster reticuloendothelial system is capable of responding to foreign protein as other animals do and one would not appear to be justified in attributing to reticuloendothelial inactivity the apparent immunologic tolerance on the part of the hamster.

**SUMMARY**

The injection of human plasma into hamsters over a period of 14 to 68 days induced splenomegaly, accompanied histologically by reticuloendothelial hyperplasia, plasmacytosis, increased erythropoiesis and hemosiderosis. These findings suggest both an antigen-antibody response and an abnormal increase in splenic hemolysis. The demonstration of positive erythroagglutination reactions when red cells from plasma-injected hamsters are tested also suggests an immunologic response by these animals. Mild anemia was present in some animals during the course of this study but at the termination of the experiment anemia was not present and the animals were apparently in a compensated state. It is clear that the reticuloendothelial system of the hamster is capable of responding to foreign protein. Apparent immunologic tolerance in this animal should not be attributed to inactivity of the reticuloendothelial system.

**SUMMARIO IN INTERLINGUA**

Le injection de plasma human ad in hamsters durante periodos de 14 a 68 dies induceva splenomegalia, accompaniate—histologicamente—de hyperplasia reticuloendothelial, de plasmocytosis, de augmentate activitate erythropoietic, e de hemosiderosis. Iste constatationes suggere tanto un responsa de antigeno-anticorpore como etiam un augmento anormal del hemolyse splenic. Le demonstration de positive reactiones erythroagglutinatori quando erythrocytos ab hamsters portante injectiones de plasma es testate etiam suggere un responsa immunologic del parte de iste animales. Leve grados de anemia eseva presente in certe animales durante le curso del studio, sed a su termination nulle anemia esseva constatate, e le animales se trovava apparentemente in un stato compensate. Il es clar que le systema reticuloendothelial del hamster es capace a responder a proteina alien. Le apparente tolerantia immunologic in iste animal non deberea esser attribuite a un inactivitate del systema reticuloendothelial.

**REFERENCES**

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