Circulating Rat Platelets in Lethally X-radiated Mice Given Rat Bone Marrow

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By the use of immunohematological technics it has been established that erythropoietic bone marrow cells of the rat implant, proliferate, and function hematopoietically when infused into lethally irradiated animals (6, 7, 9). Furthermore, a cytochemical technic has been used to show that granulopoietic elements of the rat likewise implant and proliferate in irradiated, marrow-injected mice (7, 8). Ford et al. (2) have observed rat chromosomes in the bone marrow of irradiated mice given injections of rat bone marrow. These same workers also identified a distinctive chromosome in dividing cells of bone marrow of irradiated CBA mice given injections of a cell suspension of spleen from mice heterozygous for an unequal reciprocal translocation. The present experiments were designed to determine whether platelet precursors of rat bone marrow would also implant and proliferate in lethally irradiated mice.

MATERIALS AND METHODS

Preparation of antisera.—Platelets were obtained from the whole blood of C3H × 101F1 mice and Sprague-Dawley rats by a modification of a method described by Cronkite et al. (1). For the antimouse platelet serum preparation, pooled mouse platelets were washed 3 times in a NaCl- Sequestrene1 solution (1 per cent Sequestrene in 0.7 per cent NaCl). A 1 per cent suspension of the washed platelets was emulsified with an adjuvant composed of Bayol F, Falba, and killed TB organisms (8). Aliquots of this emulsion were injected subcutaneously into two Sprague-Dawley rats. Thirty days later the rats were bled, and the sera obtained were inactivated by heat (56° C. for 30 minutes). Merthiolate (1:10,000) was added before the sera were stored at 2° C.

Similarly, antirat platelet sera were obtained from C3H × 101F1 mice given injections of Sprague-Dawley platelets, except that the donor-recipient ratio was 1:4.

Agglutination test method.—Platelets were obtained as described from the blood of one mouse or one rat. One drop of the platelet suspension (usually about a 0.05 per cent suspension) was placed on a glass microscope slide. Fifteen μl. of the antisera was added directly to the suspension and the slide tilted back and forth several times to insure good mixing. A cover slip was placed over the mixture and the preparation examined microscopically for agglutination of the platelets. Degrees of agglutination were judged by the number of free platelets and the number and size of the clumps. Preparations were graded as 0, +, + + , or + + + . When it occurred, agglutination was immediate, although, upon standing for several minutes, the clumps usually became larger. Beyond 5 minutes, no further increase in clump size was observed. Concomitant controls, in which normal rat and mouse sera were used, were run on all platelet suspensions.

Irradiation and bone marrow injection.—Male or female C3H × 101F1 mice 9–4 months old were exposed to a single dose (950 r) of whole-body x-rays. The mice were irradiated in groups of twelve in a revolving lucite cage. Radiation factors were as follows: 250 kv at 15 ma; rate in air about 160 r/min; target-skin distance, 60.0 cm.; inherent filtration, 1.0 mm. Al; added filtration, 1.0 mm. Al; and a HVL of 0.5 mm. of Cu.

Within 2 hours after irradiation, each mouse received by tail vein the combined bone marrow from one femur and one humerus of a Sprague-Dawley rat in 0.5 ml. of 0.9 per cent NaCl. Two mice of each irradiated group served as uninjected controls. The mice were caged individually or in two's.

Tests performed.—At various intervals after irradiation, platelets were obtained and tested as previously described against rat antimouse platelet and mouse antirat platelet sera and against normal mouse and rat sera. Peripheral erythrocytes were also tested with species-specific antisera according to a method described by Makinodan (7). Gomori (4) and Wachstein (10) reported that
rat granulocytes are alkaline phosphatase-positive, while mouse granulocytes are alkaline phosphatase-negative. Blood smears were obtained from each mouse, therefore, to test granulocytes for the presence of alkaline phosphatase (5). Each mouse was autopsied, and hematoxylin and eosin sections were prepared from the bone marrow and spleen in all but two animals.

RESULTS (Table 1)

Platelets.—Rat platelets were present 7 days after treatment in the blood of irradiated mice given injections of rat bone marrow. Residual kinodan (7). On the 20th and 30th days after irradiation, the six mice tested had rat platelets only, although only 24 and 37 per cent, respectively, of the erythrocytes were those of a rat. At points 60 days and beyond, the RBC and platelets paralleled each other in species type, with one exception. Mouse 22, 92 days after irradiation, contained a preponderance of mouse RBC but no mouse platelets.

Alkaline phosphatase.—The presence of alkaline phosphatase was detected in granulocytic elements of the blood of treated mice at all postirradiation intervals where rat RBC and platelets were de-

mouse platelets were also present in the same mice at this time. Some mice in the 11- and 14-day groups showed evidence of mouse platelets; but in five of the six animals in these groups, there was a preponderance of rat platelets. At 20 and 30 days after irradiation, all circulating platelets were of rat origin. The blood of treated mice tested at several periods thereafter up to 114 days contained only rat platelets, with two exceptions. An 82-day animal had mouse platelets only, and one 60-day mouse had a mixture of mouse and rat platelets.

Erythrocytes.—The appearance rate of rat erythrocytes and the disappearance rate of mouse erythrocytes in the circulation of x-radiated mice given injections of rat bone marrow followed a pattern similar to that previously reported by Ma-tected. In one mouse, 82 days after irradiation only mouse peripheral blood elements were detected.

Bone marrow and spleen histology.—The sections of bone marrow and spleen showed a nearly normal amount of blood cell-forming tissue in all the animals examined from day 7 through 114. Erythropoietic, granulopoietic, and megakaryocytic cell formation was present in the red pulp of the spleen and in the bone marrow of all animals. The relative amounts of each varied considerably, however, from animal to animal, as seen in the histologic sections. The white pulp of the spleen did not regenerate normally in any of the mice. The granulo-cytic cells were often intensely eosinophilic.
There was no evidence of hemorrhage in any of the mice at autopsy.

DISCUSSION

The results of these experiments show that rat platelets appear and persist in the peripheral blood of lethally x-radiated mice given injections of rat bone marrow. Since it is generally accepted that platelets are derived from megakaryocytes, this observation implies that megakaryocyte precursors of the rat implant and proliferate rat platelets in irradiated mice given injections of rat bone marrow. It was observed that, in all but one of the mice tested between 14 and 30 days after irradiation, 100 per cent of the circulating platelets were those of a rat. At 30 days, however, only 37 per cent of the erythrocytes were rat erythrocytes. The 100 per cent rat erythrocyte level is not attained until the 60th day (7). The results of the present studies show that the appearance rate of foreign platelets is more rapid than that of the foreign erythrocytes and, conversely, the disappearance rate of mouse platelets is greater than that of the mouse erythrocytes. The latter observation is probably related to the life span of the two formed elements of mouse origin, because the life span of circulating platelets is shorter than that of the erythrocytes. On this basis, one would expect to find residual mouse erythrocytes after mouse platelets were no longer present. It may be, however, that some mouse platelets continue to be produced after irradiation, because megakaryocytes persist in the bone marrow of mice for several days after irradiation.

The absence of hemorrhage in all mice autopsied suggests that the rat platelets were functioning in the mouse at least in the capacity to prevent hemorrhage.

Mouse platelets were present in the blood of two mice out of eight at later periods, viz., 60 and 82 days after irradiation. The progressive increase in the proportion of rat platelets between 7 and 20 days, together with the persistence of 100 per cent rat platelets exclusively in twelve of fourteen mice between 20 and 114 days, suggests that regression of rat platelet precursors and reestablishment of mouse platelet precursors occurred in these two animals.

SUMMARY

Circulating platelets of lethally irradiated mice given injections of rat bone marrow were tested with species-specific antiplatelet sera. Rat platelets were detected in the peripheral blood of treated mice at various intervals from 7 to 114 days after treatment. The 100 per cent rat platelet level was attained in some mice by the 14th day and in all mice by the 30th day. The results indicate that platelet precursor implants and produce functional platelets in irradiated mice given injections of rat bone marrow.

REFERENCES

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