THE ROLE OF THE PLATELET IN THE GENERALIZED SHWARTZMAN REACTION*

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(Received for publication 14 November 1968)

The generalized Shwartzman reaction is produced in rabbits with two intravenous injections of bacterial endotoxin (1). Although the participation of the blood-clotting mechanism is well recognized (2), the role of the platelet has not been elucidated. Endotoxin will trigger coagulation in vitro and in vivo to release platelet factor 3 (3). Rodriguez-Erdmann infused purified platelet factor 3 to produce the Shwartzman reaction and suggested that this is the pathway by which endotoxin elicits the lesions (4). However, Muller-Berghaus et al. were unable to produce renal glomerular thrombi or cortical necrosis of the kidneys with the same preparation of purified platelet factor 3 or with a wide variety of agents that have platelet factor 3 activity (5). Levin and Cluff attempted to produce the Shwartzman reaction in thrombocytopenic rabbits (6). They were not able to evaluate the role of the platelet because the method used to induce thrombocytopenia, i.e., platelet antiserum, triggered enough intravascular clotting to result in renal cortical necrosis.

In the present study, rabbits were injected with platelet antiserum to produce thrombocytopenia. The Shwartzman reaction was prevented at this time with the anticoagulant heparin (7, 8). Thrombocytopenia persisted after the effect of heparin was no longer present, i.e., after 4 hr. At this time (4 hr), the animals were injected with endotoxin to elicit the generalized Shwartzman reaction. Profound thrombocytopenia prevented the development of renal cortical necrosis, indicating that a certain level of circulating platelets is required during the second injection of endotoxin. The platelet antiserum induced selective thrombocytopenia without having an effect on the level of circulating leukocytes. Thus, there can be no misinterpretation that the Shwartzman reaction was prevented as a result of granulocytopenia rather than thrombocytopenia.

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* Supported by Grant No. HE-11190-02 from the National Institutes of Health, United States Public Health Service.

Materials and Methods

Female albino New Zealand rabbits weighing 1.7–2.3 kg were prepared for the generalized Shwartzman reaction with *Escherichia coli*, lipopolysaccharide, 0.2 mg intravenously (Difco Laboratories, Detroit, Mich.). 16 hr after the first injection of endotoxin, each animal received a single intravenous injection of aqueous heparin, 3000 units/kg (Riker Laboratories, Northridge, Calif.). The rabbits were then divided into two groups of 10. The animals in group 1 received an intravenous injection of 0.5 cc of platelet antiserum immediately after

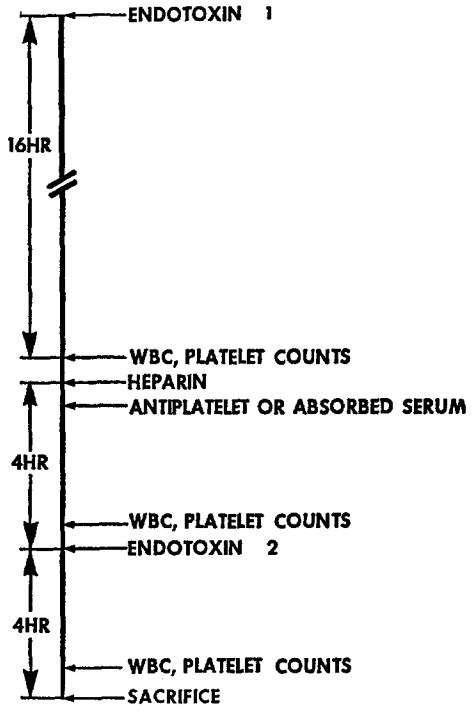

FIG. 1. Time relationship of various injections into rabbits of groups 1 and 2.

the injection of heparin. The rabbits of group 2 (controls) were injected with 0.5 cc serum from which the platelet antibody had previously been absorbed. The second injection of endotoxin was given 4 hr after the injection of either the antiplatelet or the absorbed serum. The rabbits were sacrificed 4 hr after the second injection of endotoxin. Histologic sections of the kidneys were examined for fibrin thrombi with hematoxylin-eosin and phosphotungstic acid–hematoxylin stains. White blood cell and platelet counts (9) were determined in duplicate in each animal prior to, as well as 4 and 8 hr after the injection of antiplatelet or absorbed serum. The time relationships of the various injections and laboratory determinations are shown in Fig. 1. Preliminary studies showed that almost complete thrombocytopenia (less than 10,000 platelets/mm^3) persisted for 8 hr after an injection of the platelet antiserum.

Platelet antiserum was prepared in a single goat from washed rabbit platelets. The platelets were incorporated in complete Freund's adjuvant (Difco), and injected subcutaneously

at weekly intervals for 1 month. The goat was exsanguinated 1 wk after the last injection of rabbit platelets. The serum was incubated at 56°C for 30 min and then absorbed with packed rabbit red cells. An aliquot of serum was removed and repeatedly absorbed with washed rabbit platelets. This serum was demonstrated not to produce thrombocytopenia and was subsequently injected into the rabbits of the control series (group 2). The sera were stored. frozen until used.

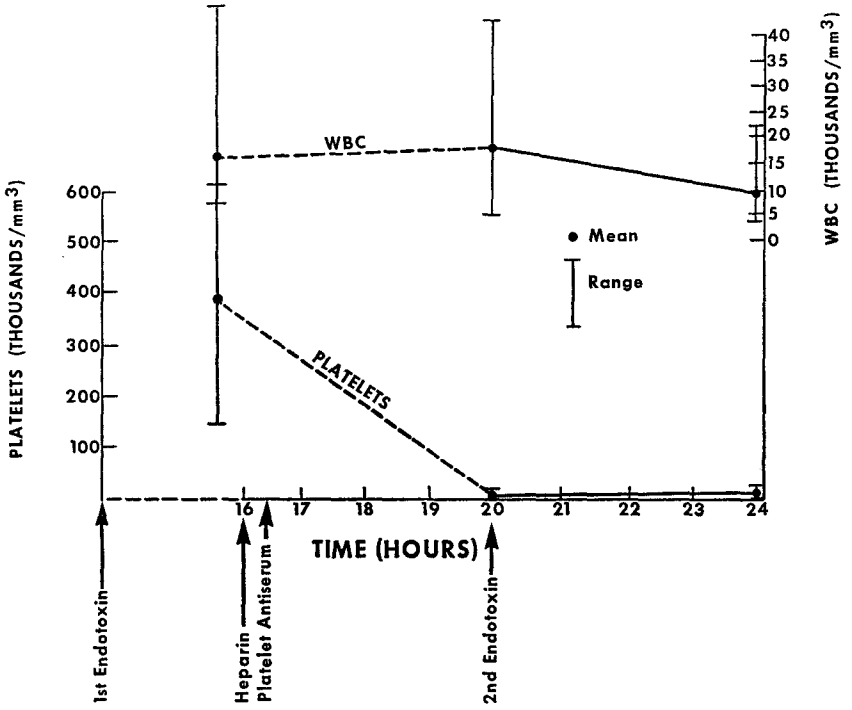

FIG. 2. Platelet and white cell counts in rabbits given antiplatelet serum and two doses of endotoxin.

RESULTS

Thrombocytopenia was protective against the Shwartzman reaction. None of the animals injected with the platelet antiserum (group 1) developed glomerular capillary thrombi. In comparison, fibrin thrombi characteristic of the Shwartzman reaction were present in the kidneys of six rabbits in the control series (group 2).

The platelet and white cell counts of the rabbits in group 1 are shown in Fig. 2. The platelet antiserum induced marked thrombocytopenia so that the average platelet count prior to the second injection of endotoxin was 6100/mm³. This compares with an average platelet count of 397,000/mm³ before the ani-

mals received the platelet antibody. The average white cell counts before and 4 hr after the injection of platelet antiserum were 16,200 and 17,200/mm³ respectively. Therefore, the administration of this serum produced the desired effect of selective thrombocytopenia without leukopenia.

The platelet and white cell counts of the rabbit in the control series (group 2) are shown in Fig. 3. The average platelet count prior to the second injection

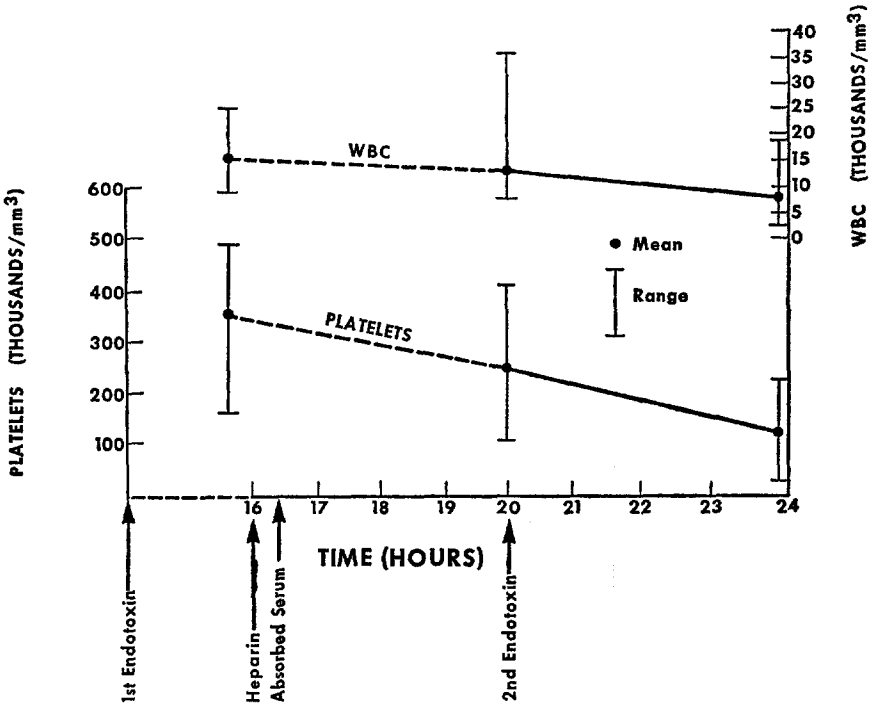

FIG. 3. Platelet and white cell counts in rabbits given absorbed serum and two doses of endotoxin.

of endotoxin was 248,000/mm³. This figure represents a slight reduction from the average count of 340,000 that was present prior to the injection of the absorbed serum. This implies that the absorbed goat serum retained slight antiplatelet activity. The lowest platelet count that was obtained before the second dose of endotoxin was 106,000/mm³. This rabbit subsequently developed glomerular capillary thrombi, indicating that the relative thrombocytopenia of 106,000 platelets was not sufficient to prevent the Shwartzman reaction. The average white cell counts before the injection of absorbed goat serum and 4 hr later were 15,400 and 12,500/mm³ respectively.

DISCUSSION

Renal cortical necrosis, the lesion which identifies the generalized Shwartzman reaction, is mediated by the localization of fibrin in the glomerular capillaries. The failure to elicit the lesion in thrombocytopenic rabbits indicates that endotoxin triggers intravascular coagulation by an effect on the intrinsic rather than the extrinsic clotting system. Although endotoxin will induce viscous metamorphosis of platelets, and platelets contribute to the formation of intrinsic prothrombin activator, this experiment does not determine how endotoxin activates the intrinsic system. At least seven factors besides platelet phospholipid contribute to the formation of plasma thromboplastin. These are: factors V, VIII, IX, X, XI, XII, and calcium (10). McKay and Muller-Berghaus were not able to elicit the Shwartzman reaction by substituting chemical substances and physiologic activities for endotoxin, unless activation of Hageman factor (factor XII) was also included (11). Their work suggests that endotoxin activates Hageman factor to trigger the intrinsic clotting system.

Levin and Cluff tried to evaluate the role of the platelet by producing thrombocytopenia with an injection of platelet antiserum 1 hr before the provoking dose of endotoxin (6). In this circumstance, thrombocytopenia did not prevent the generalized Shwartzman reaction. In fact, Levin and Cluff showed that platelet antiserum itself will elicit the lesions in suitably prepared rabbits. Evidently, the antiserum triggers intravascular clotting when platelets are destroyed by the antibody. In the present experiment, aqueous heparin was used to prevent that amount of intravascular coagulation which ordinarily is induced by the injection of platelet antiserum. The second dose of endotoxin was given 4 hr after heparin. At this time, the rabbits were still thrombocytopenic although aqueous heparin is effective as an anticoagulant for only 4 hr. Since none of the thrombocytopenic rabbits developed the Shwartzman reaction, it can be assumed that platelets are required during the second stage of "provocation," i.e., at the time when the second injection of endotoxin triggers intravascular clotting. The lesions were not prevented in the control series (group 2), which received the absorbed serum. The control experiment was designed to rule out the possibility that the Shwartzman reaction was prevented by the earlier injection of heparin or by a nonspecific effect of the goat serum.

SUMMARY

Rabbits were injected with an antiplatelet serum to produce selective thrombocytopenia without inducing a significant alteration of their leukocyte counts. Thrombocytopenic levels persisted for 8 hr after the injection of platelet antiserum. During this time, the generalized Shwartzman reaction could not be provoked with the second injection of endotoxin. Since platelet phospholipid

is required for the formation of plasma thromboplastin, the results indicate that platelets are essential to the evolution of the generalized Shwartzman reaction and endotoxin triggers the intrinsic rather than the extrinsic clotting system to elicit the lesions.

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