The Effect of Leukocyte Antiserum on the Generalized Shwartzman Reaction

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SINCE THE DEMONSTRATION that nitrogen mustard prevents the generalized Shwartzman reaction, there has been considerable controversy regarding the role of the leukocyte.[1] Thomas and Good considered that the presence of circulating neutrophils was required for the production of the lesions. Horn and Spicer[2] expanded the hypothesis of Thomas and coworkers[3,4] by suggesting that the leukocyte provides a high molecular weight acid mucopolysaccharide which combines with and precipitates fibrinogen to produce the thrombotic lesions. More recently, Horn and Collins have proposed that preparation for the Shwartzman reaction is actually mediated by a granulocytic response to bacterial endotoxin.[5,6] According to them, granulocytes release a clot-promoting substance as they are trapped and destroyed in the pulmonary capillaries following an injection of endotoxin. In support of this theory, they have produced the Shwartzman reaction with a single injection of endotoxin when nitrogen mustard-treated rabbits were given transfusions of peritoneal granulocytes intravenously or leukocyte granules intra-aortically.

In contrast to this, several other experiments suggest that leukocytes are not essential to the generalized Shwartzman reaction. Although lysates of peritoneal granulocytes possess slight procoagulant activity when substituted for "platelet extract" in the "partial thromboplastin time," they do not contain enough procoagulant activity to provoke the lesions.[7] The experiments of Wendt *et al* also exclude a direct role for the leukocyte.[8] They obtained a high incidence of the Shwartzman reaction 4–5 days after pretreatment with nitrogen mustard—ie, during the time interval of severe leukopenia. Their experiment demonstrates that prevention of the Shwartzman reaction 3 days after nitrogen mustard therapy is due to some activity other than the development of peripheral leukopenia.

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In the studies to be reported, rabbits were made leukopenic with an antileukocyte serum. The purpose of the experiment was to determine whether or not antibody-induced leukopenia prevents the generalized Shwartzman reaction. The role of the leukocyte could not be evaluated because the antigen-antibody complex itself prepared for the Shwartzman reaction. Further studies revealed that the activity of the leukocyte antiserum was due to stimulation of α-adrenergic receptor sites rather than release of leukocyte granules into the circulation.

Materials and Methods

Female albino New Zealand rabbits weighing approximately 1.5 kg were used in all experiments. The generalized Shwartzman reaction was produced with two intravenous injections of 0.2 mg *E. coli* lipopolysaccharide (Difco Laboratories) spaced 22 hr apart.

Leukocyte antiserum was prepared in 2 goats from washed rabbit peritoneal granulocytes. The peritoneal exudates were collected by the method of Ponder and MacLeod in which the cells are harvested 18 hr after an intraperitioneal injection of 300 ml of isotonic saline.[9] The granulocytes were incorporated in complete Freund's adjuvant (Difco Laboratories) and injected subcutaneously at weekly intervals for 1 month. The goats were exsanguinated 1 week after the last injection. The pooled serum was incubated at 56° C for 30 min and then absorbed with packed rabbit red cells. Aliquots of sera were stored frozen until used. Rabbits were actively immunized with crystallized bovine serum albumin (BSA—Armour Pharmaceutical Co.) at four weekly intervals according to the method of Lee.[10]

In experiments with ellagic acid (K & K Laboratories), ε-aminocaproic acid (Lederle Laboratories), and norepinephrine (Winthrop Laboratories), the protocol reported previously by McKay, Müller-Berghaus, and Cruse was followed.[11] Two injection of 1.0 ml leukocyte antiserum spaced 2 hours apart were substituted for each of the above three agents. Lysates of rabbit peritoneal granulocytes were also substituted for norepinephrine in this protocol. The lysates were prepared as described by McKay, Margaretten, and Phillips in a previous experiment.[7] The lysates were reconstituted in 100 ml normal saline and infused slowly over a 4½-hr period.

White blood cell and platelet counts were performed in duplicate at selected time intervals.[12] Histologic sections of the kidneys were examined for fibrin thrombi with hematoxylin-eosin and phosphotungstic acid-hematoxylin stains.

Results

The intravenous injection of antileukocyte serum produced selective leukopenia without thrombocytopenia. The leukocyte antibody induced neutropenia for only 2 hr. Table 1 shows the average white blood cell,

Table 1. White Cell, Neutrophil, and Platelet Counts in Rabbits Injected with Leukocyte Antiserum

Time	Total WBC	PMN	Platelet
0	5500	1770	413,000
30 min	642	40	250,000
2½ hr	1250	73	233,000
4½ hr	1350	98	317,000

neutrophil, and platelet counts in a group of 6 normal rabbits given two injections of 1.0 ml of leukocyte antiserum spaced 2 hr apart. The average preinjection white cell count was 5500. Of this number, 1770 were neutrophils. Thirty min after the first injection, the white cell count dropped to 642, of which 40 were neutrophils. Thirty min after the second injection the count was 1250 with 73 neutrophils, and 2½hr after the second injection (4½ hr after the first injection), the count was 1350 with 98 polymorphonuclear leukocytes. During the same 4½ hr period, the average platelet count was reduced from 413,000 to 250,000, 233,000 and 317,000. Therefore, two injections of specific antiserum induce severe granulocytopenia during a 4-hr period.

In the first experiment using leukocyte antiserum, 11 rabbits were given injections of the serum 30 min before and again 2 hr after the second, or provoking, dose of endotoxin. The animals were sacrificed 4 hr after the second injection of endotoxin. Glomerular capillary thrombi were present in 100% of the animals. Of 9 rabbits given a single preparing dose of endotoxin followed by two injections of antiserum at 22 and 24 hr none developed the lesions. Therefore, antibody-induced leukopenia does not prevent provocation of the Shwartzman reaction by endotoxin and the antiserum itself does not provoke the lesions in rabbits prepared 22 hr earlier with endotoxin.

In the next experiment, the effect of the antiserum was tested during the stage of preparation. The serum was injected 30 min before the first dose of endotoxin and again 2 hr later. The second dose of endotoxin was given 22 hr after the first. The results are shown in Table 2 (Group I). Glomerular capillary thrombi were present in 7 of the 10

Table 2. Incidence of the Shwartzman Reaction with Various Methods of Preparation

Group	Protocol	No. rabbits	GSR
I	LAS (preparation) 2 doses Endotoxin	10	7 (4 RCN)
II	1 dose LAS 5 min 1 dose endotoxin	13	11
III	1 dose LAS 22 hr 1 dose endotoxin	8	0
IV	2 doses endotoxin (5 min apart)	8	0
V	2 dose endotoxin (22 hr apart)	10	10
VI	BSA—anti-BSA 5 min 1 dose endotoxin	5	4

LAS, Luekocyte antiserum; RCN, Renal cortical necrosis; BSA, Bovine serum albumin.

rabbits but, more important, 4 of the 7 had well-developed renal cortical necrosis at necropsy. Since the animals were sacrificed 4 hr after the second, or provoking, dose of endotoxin, the presence of infarct necrosis indicates that fibrin deposition occurred in relation to the first as well as to the second injection of endotoxin. This was confirmed in the next experiment (Table 2, Group II). Thirteen rabbits were given one injection of leukocyte antiserum 5 min prior to one injection of endotoxin. When sacrificed 4 hr later, 11 of the 13 animals had glomerular capillary thrombi. If the timing was changed so that the antiserum was given 22 hr before the injection of endotoxin, thrombotic lesions were not present in the glomeruli (Table 2, Group III). This experiment indicates that leukocyte antiserum does not prepare for the Shwartzman reaction in the classical way. If two doses of endotoxin were spaced 5 min apart, none of 8 rabbits had glomerular thrombi (Table 2, Group IV). In contrast to this, 100% of a control series of 10 rabbits developed thrombotic lesions typical of the Shwartzman reaction when the two injections of endotoxin were spaced 22 hr apart (Table 2, Group V).

The question arises whether the activity of leukocyte antiserum is due to the presence of circulating antigen-antibody complexes or to the release of some leukocyte product into the circulation. Five rabbits were immunized to bovine serum albumin (BSA) [10] and then each received an intravenous injection of 50 mg BSA followed in 5 min by an intravenous injection of 0.2 mg endotoxin. Four of the 5 rabbits developed glomerular thrombotic lesions (Table 2, Group VI).

In the final experiment, leukocyte antiserum was substituted for ellagic acid, ε-aminocaproic acid, or norepinephrine in the experimental protocol reported by McKay, Müller-Berghaus, and Cruse.[11] When norepinephrine was replaced by two injections of leukocyte antiserum spaced 2 hr apart, 5 of 7 rabbits were positive for glomerular capillary thrombi (Table 3, Group I); substitution for ellagic acid or ε-aminocaproic acid gave negative results (Table 3, Groups II and III). In comparison, when lysates of peritoneal granulocytes were substituted for norepinephrine, none of 5 rabbits developed thrombotic lesions (Table 3, Group IV). These lysates contained the total content of the leukocyte cytoplasm and nucleus including the "leukocyte-specific granules." The 5 rabbits were infused with lysates from 700, 700, 1,250, 412, and 412 million leukocytes, respectively. In the control experiment, 4 of 7 rabbits infused with ellagic acid, ε-aminocaproic acid, and norepinephrine were positive for glomerular thrombi (Table 3, Group V).

Discussion

Thomas and Good prevented the Shwartzman reaction with nitrogen

Table 3. Incidence of Shwartzman Reaction when Leukocyte Antiserum or Lysed Granulocytes are Substituted for Norepinephrine, Ellagic Acid, or ε-aminocaproic Acid

Group	Protocol	No. rabbits	GSR
I	Leukocyte antiserum Ellagic acid ε-aminocaproic acid	7	5
II	Norepinephrine Leukocyte antiserum ε-aminocaproic acid	8	0
III	Norepinephrine Ellagic acid leukocyte antiserum	8	0
IV	Lysed granulocytes Ellagic acid ε-aminocaproic acid	5	0
V	Norepinephrine Ellagic acid ε-aminocaproic acid	7	4

mustard when the total number of circulating neutrophils was reduced below 400/mm^3.[1] Two injections of leukocyte antiserum provide this requirement during a 4 hr period. Unfortunately, antibody-induced neutropenia does not permit an evaluation of the role of the leukocyte, since one injection of endotoxin will elicit thrombotic renal lesions in the presence of circulating antigen-antibody complexes. The studies of Wendt *et al* are more pertinent concerning the role of the leukocyte.[8] They produced the generalized Shwartzman reaction with two doses of endotoxin in rabbits made leukopenic with nitrogen mustard 4–5 days previously. Their experiment demonstrates that circulating leukocytes are not required in the pathogenesis of the lesions.

It is well known that antigen-antibody complexes can trigger the intrinsic blood clotting mechanism to elicit the generalized Shwartzman reaction.[10] Lee's experiment was based on the observation by Robbins and Stetson that soluble complexes shorten the clotting time in vitro.[13] For this reason, it was necessary to determine whether the activity of leukocyte antiserum was due to the presence of circulating complexes or to a specific product released by the neutrophil. The intravenous injection of BSA into immunized rabbits produced the same result as did the antileukocyte serum. This experiment suggests that the activity of the serum is due to the formation of soluble complexes.

McKay, Müller-Berghaus, and Cruse were able to substitute pharmacologic agents for endotoxin in the generalized Shwartzman reaction.[11] We substituted the leukocyte antiserum for ellagic acid (activation of Hageman Factor), ε-aminocaproic acid (inhibition of fibrinolysis), or

norepinephrine (stimulation of α-adrenergic receptor sites), according to their protocol. When norepinephrine was replaced by the antiserum, 70% of the rabbits developed thrombotic glomerular lesions. This experiment indicates that either antigen-antibody complexes or some leukocyte product provides stimulation of the α-adrenergic receptor sites. The question was resolved with the failure to elicit thrombotic lesions when norepinephrine was replaced by an infusion of lysed rabbit peritoneal granulocytes.

Stimulation of the α-adrenergic receptor site should be added to the long list of biologic effects mediated by antigen-antibody complexes, Brunson and Davis,[14] as well as McKay,[15] have demonstrated multiple thrombotic lesions in a wide variety of clinical hypersensitivity diseases. Since stimulation of α-adrenergic receptor sites is essential for the formation and localization of fibrin thrombi in the Shwartzman reaction,[11,16] this effect may be as important as activation of the intrinsic prothrombin activator system for the production of thrombotic lesions in the hypersensitivity diseases of human. In this regard, Arhelger *et al* produced the Shwartzman reaction with a single dose of endotoxin in rabbits in which an antikidney serum had been previously or simultaneously injected.[17,18] More recently Katz, Unanue, and Dixon have reported the occurrence of diffuse thrombotic lesions in rats injected with a variety of anticonnective tissue antibodies.[19] These experiments indicate that stimulation of α-adrenergic receptor sites is a general effect common to immunologic injury rather than a specific activity of soluble, circulating complexes *per se*. It is of interest that immunologic injury prepares for the Shwartzman reaction immediately before, or simultaneously with, a provoking dose of endotoxin since this property is not shared by endotoxin itself.

Summary

Antibody-induced neutropenia does not prevent the generalized Shwartzman reaction. In fact, the lesions can be produced when a single injection of leukocyte antiserum is followed by a single injection of endotoxin. Additional studies indicate that this property depends on the formation of antigen-antibody complexes and is not related to the release of leukocyte products into the circulation. In addition to their ability to activate the intrinsic clotting system, antigen-antibody complexes stimulate α-adrenergic receptor sites.

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