Complement in Endotoxin Shock: Effect of Decomplementation by Aggregated Gamma Globulin.* (29537)

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The immediate onset of canine endotoxin shock is associated with a decline in the titer of complement and a rise in plasma histamine suggesting that an immune mechanism is involved(1). Nevertheless, the precise mechanism responsible for the depletion of complement has not been delineated. Evidence at hand cannot exclude a non-immune mechanism such as inactivation of an "endotoxin labile" component(s) of the complement system. In addition, endotoxin preparations that produce shock have been poorly defined chemically.

The present report is concerned with studies to determine if the *in vivo* reduction of complement could modify the subsequent reaction of the dog to an injection of endotoxin. The decrease in serum complement was achieved by intravenous administration of heat aggregated human gamma globulin (2).

Materials and methods. Animals: A total of 20 adult mongrel dogs of both sexes were used in 2 experimental groups. The dogs were anesthetized with sodium pentobarbital (30 mg/kg).

Complement titration: Complement levels were measured in 50% hemolytic units ($C'H_{50}$) by the method of Kabat and Mayer (3). All determinations on a single animal were carried out simultaneously.

Method of decomplementation. Human gamma globulin prepared from Cohn Fraction II was aggregated by heating for 30 minutes at 56°C.‡ The method of heat aggregation and Na_2SO_4 fractionation was that described by Christian(2).

Endotoxin: Each experimental group received a standardized dose of 1.0 mg/kg of endotoxin prepared from a stock culture of *Escherichia coli* type 077 H18 K+. The method of preparation has been previously described(4).

Observations on shock: The femoral arterial pressure was continuously monitored before and after intravenous administration of aggregated globulin and endotoxin. Femoral arterial blood was collected from control and treated animals for complement titration.

Experimental groups: Ten dogs in Group I received endotoxin only. Blood for complement determinations was obtained immediately before and 1 minute, 10 minutes, 1 hour and 4 hours after endotoxin administration. Survival was recorded in hours and considered permanent if the animal was alive 72 hours post-endotoxin.

Ten dogs in Group II received aggregated human gamma globulin (AHGG) in 0.15 N NaCl (5 mg protein/ml) in a dose of 20 mg/kg followed 2 hours later by 1.0 mg/kg endotoxin. Blood samples were obtained for complement titration immediately before and 1 minute, 1 hour and 2 hours after globulin administration. Additional samples were then taken after endotoxin injection as in Group I.

Results. In the endotoxin control group, 8 of the animals died within 72 hours establishing an LD_{80} for 1 mg/kg of endotoxin. The average survival time post-endotoxin was 30.2 hours. Comparative data for the control group and AHGG group are shown in Table I. One minute after the endotoxin the average C′ level was reduced to 68% of the control value.

In the Group II of 10 dogs given AHGG, followed 2 hours later by endotoxin, none of 10 dogs survived beyond 36 hours. As noted in Table I the AHGG animals died faster than the endotoxin-control group. The average survival time ranging from 1.5 to 36 hours, with an average of 12.2 hours. Administration of AHGG was followed promptly

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TABLE I. Serum Hemolytic Complement Titers, Systemic Blood Pressure and Survival Rates in Dogs Receiving Endotoxin: Group I Control Endotoxin; Group II Pre-treatment with Heat Aggregated Human Gamma Globulin.

Measurement	Group	Control period	Post-AHGG 1 min	Post-AHGG 1 hr	Post-AHGG 2 hr	Post-endotoxin 1 min	Post-endotoxin 10 min	Post-endotoxin 1 hr	Post-endotoxin 4 hr	Post-endotoxin 72 hr
% $C'H_{50}$	I	100			100	58	56	49	62	—
	II	100	65	62	59	46	44	46	48	—
Blood pressure, mm Hg	I	165/110			165/110	90/60	70/45	110/65	165/100	—
	II	200/130	100/70	130/80	135/85	100/55	80/45	115/65	130/70	—
No. survivors	I	10			10	10	10	10	10	2
	II	10	10	9	9	9	9	8	4	0

by the appearance of hypotension, which continued until endotoxin was given.

Within 1 minute following AHGG administration complement fell to 65% of the control level and declined further to 59% in 2 hours, just prior to the injection of endotoxin. One minute after endotoxin the average level of complement was 46%.

Discussion. Complement inactivation by AHGG probably occurs through a mechanism similar to the fixation of complement by an immunologic reaction (antigen-antibody)(5). In addition AHGG produces inflammatory changes in the skin after intracutaneous injection, and anaphylactic-like manifestations have been observed in guinea pigs following intravenous administration(2).

Despite a significant reduction in the average hemolytic complement activity in dogs, pretreatment with AHGG enhanced, rather than decreased, the lethal action of endotoxin. Dogs pre-treated with AHGG showed an average survival of 12.2 hours post-endotoxin compared to 30.2 hours in the group given only endotoxin. There were no permanent survivors (beyond 72 hours) in the AHGG treated group, one dog dying within minutes after AHGG injection. In other studies in this laboratory additive lethal effect was not observed in dogs given normal human gamma globulin (FII).

The basis for this enhancement of endotoxin shock could be due to one or a combination of several mechanisms. 1) A nonspecific hypotensive effect could be related to the macromolecular size of AHGG, similar to that exerted by peptone and a wide variety of colloidal substances. 2) Blockade of the reticuloendothelial system by AHGG could have resulted in impaired clearing of endotoxin from the blood stream. 3) AHGG could have an inherent property that provoked anaphylaxis. The immediate reduction in hemolytic complement and the sustained hypotension by AHGG support this possibility.

The similarities between the initial phase of endotoxin shock and systemic anaphylactic shock in the dog have been the subject of several studies in this laboratory. Christian and Thurber(6) have shown that decomplementation with AHGG in guinea pigs does not protect against passive systemic anaphylaxis although passive cutaneous anaphylaxis (PCA) is modified significantly. Endotoxin enhances systemic anaphylaxis in mice(7). The results of the present study have demonstrated a similar enhancing effect in the dog.

The question as to whether complement is a crucial factor for induction of lethal canine endotoxin shock was not answered by these experiments. Further studies require a more precise chemical delineation of endotoxin and a more satisfactory *in vivo* method for decomplementation. Since we have observed *in vitro* that AHGG does inactivate canine complement considerably, it would be desirable to ascertain if endotoxin acts directly upon a specific component or components of complement.

Summary. It has been postulated that the initial hemodynamic alterations in canine endotoxin shock are due to an immune mechanism (antigen-antibody) involving complement. Partial decomplementation of dogs following intravenous injection of heat-aggravated human globulin, enhanced rather than decreased the lethal effect of endotoxin. The results of the present experiments sug-

gested that because of the macromolecular size of the heated globulin anaphylaxis was produced, and in this manner the anaphylactic action of endotoxin was enhanced.

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Relation of Blood Group Genotypes to Therapeutic Effectiveness of Bone Marrow Transplants Following X-Irradiation.* (29538)

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The purpose of this study was to determine if the genetic relationship of donor and host blood group genotypes has any effect upon survival of lethally X-irradiated chicks receiving bone marrow transplants and also to investigate the presence and persistence of donor type red blood cells in the circulatory system of the graft recipients during the post-irradiation period.

A number of investigators have established the therapeutic effect of bone marrow transplants following lethal X-irradiation in mice (1-4) and rats(5,6). Similar studies with guinea pigs(1,7) have reported survivors subsequent to lethal X-irradiation and bone marrow transplanting. Autotransfusion of bone marrow in protecting dogs from X-irradiation has been reported by Alpen and Baum(8).

In work with isologous, homologous, and heterologous bone marrow transplantation, Van Bekkum and Vos(6) concluded that the quantity of hematopoietic cells necessary for recovery of lethally irradiated rats and mice depends upon the genetic relationship between donor and host. This was further demonstrated by Urso *et al*(4) in work with rats and mice. Makinodan(9) and Soska *et al*(2) reported that homologous bone marrow injections in lethally irradiated mice merely postponed death. Uphoff and Law(3) confirmed the importance of genetic relationship in work with 2 inbred strains of mice where a known single gene difference existed. The persistence of graft-donor type cells in the blood of lethally irradiated recipients has been studied by a number of investigators. Using agglutinating antisera, Van Bekkum and Vos(6) found a mixed population of donor and host cells in the recipients over an extended interval of time. This has also been demonstrated by other workers using agglutinating techniques(4,10), and by Nowell *et al*(11) using the alkaline-phosphatase test. Gengozian *et al*(12) showed by immunological tests that the thymus glands of lethally irradiated mice protected with rat bone marrow were repopulated by rat type cells, and that this repopulation was complete by 30 days after treatment.

Materials and methods. Chicks of known parentage were produced for this study from a partially inbred line of Single Comb White Leghorns (Texas Line 24). Artificial inseminations were made such that the resulting progeny would express varying degrees of blood group heterozygosity with respect to the 5 blood group systems known to be segregating within the parental stock. This was

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