THE ROLE OF THE RETICULOENDOTHELIAL SYSTEM IN MOUSE ANAPHYLAXIS AS TESTED WITH HOMOLOGOUS ANTIBODY-ANTIGEN COMPLEXES*

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The influence of the reticuloendothelial system (RES)[1] on hypersensitivity, immunity, and resistance has been studied by a number of authors. Microbial infection (1–4), tumor growth (5, 6), antibody formation (7, 8), delayed hypersensitivity (9), and "stress" (10) have all received attention. A review of the earlier literature can be found in Jaffé (11).

Our interest in the possible participation of the RES in anaphylaxis stemmed from Germuth and McKinnon's demonstration of the crucial role of antigen-antibody (AgAb) complexes in guinea pig anaphylaxis (12). The present study is concerned with the role of the RES in the disposition of intravenously injected AgAb complexes.

Much of the early work on the RES and anaphylaxis was done before techniques necessary for defining RES physiology were available. The importance of AgAb, complexes in anaphylaxis was also unknown at the time. Nevertheless, Peterson *et al.* (13) and Moldovan and his colleagues (14, 15) implicated the RES in anaphylaxis in the dog and guinea pig. Peterson *et al.* (13) injected saccharated iron oxide intravenously into sensitized dogs and observed an inhibition of the changes in hepatic lymph characteristic of anaphylaxis. They surmised that the injection of colloid effected an "endothelial blockade" which inhibited the transfer of soluble serum components and erythrocytes across the capillary wall during anaphylaxis.

Moldovan and coworkers (14, 15) demonstrated that intravenous or intraperitoneal injections of China ink would protect sensitized guinea pigs from a lethal injection of antigen. Inhibition of anaphylaxis in the guinea pig by intraperitoneal injection of

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[1] No precise, uniformly acceptable definition exists for the reticuloendothelial system; it is used here in the limited sense as the system of cells, capable of phagocytosis, which removes colloidal and particulate matter from the circulation.

Congo red and other electronegative dyes was reported by Nicolaeff and Goldberg (16) and Gordon and Robson (17). Bier (18) was unable to confirm these results, however. Rice (19) obtained results indicating that several intraperitoneal injections of India ink enhanced anaphylactic sensitivity in the guinea pig.

The initial part of this study was undertaken to determine how rapidly AgAb complex was removed from the circulation after intravenous injection. We then studied the effect of RES "blockade" on passive homologous anaphylaxis.

Methods and Materials

Passive homologous anaphylaxis was produced with mixtures of antigen, bovine plasma albumin, and mouse antibody in the region of antigen excess as previously described by Treadwell *et al.* (20). Intravenous injection of this material will cause rapidly progressive shock with death in 20 minutes.

Blockade of the RES is accomplished by the injection of an amount of colloid or particles sufficient to inhibit further uptake of intravenously injected test doses of a substance such as carbon. Blockade is never complete since whatever the initial dose given, the test dose will be cleared, although much more slowly. Reticuloendothelial function and blockade are discussed in detail in a number of papers (21–24). Important factors in blockade are the dose and type of material used, route of injection, and time between blockade and testing.

Carbon particles, thorotrast, zymosan, and a suspension of *Bordetella pertussis* were used as blockading agents at doses and time intervals which have previously been shown, with the exception of *B. pertussis*, to significantly inhibit the phagocytic capacity of the mouse RES. The blockading agents were assumed to act primarily as inert particles. The possibility that the endotoxic properties of zymosan and of *B. pertussis* also contributed to blockade was not excluded.

Carbon particles approximately 250 A in diameter were obtained from Gunther Wagner, in Hanover, Germany (Lot no. C 11/1431*a*). This material was centrifuged at 3000 R.P.M. for 20 minutes to remove any large aggregates. The carbon concentration of the centrifuged suspension was 100 mg./ml. For some experiments this was diluted with saline and gelatin to a final concentration of 60 mg./ml. in 0.5 per cent gelatin.

Thorotrast (Testagar and Co., Detroit) is a 24 to 26 per cent stabilized suspension of ThO_2 in 25 per cent aqueous dextrin with a preservative.

The *B. pertussis* suspension was phase 1 pertussis vaccine (Cutter Laboratories, Berkeley) containing 600 million organisms per ml.

Zymosan (Standard Brands, Inc., Stamford, Connecticut), an inhomogeneous extract of yeast wall, was prepared in a saline suspension containing 10 mg./ml.

The mice used were of a *Salmonella*-resistant Webster Swiss strain from 5 to 8 weeks old. In any experiment the mice used were of the same age and sex.

Intravenous injections were made into a tail vein using a 27 gauge needle. A modification of the quantitative method of scoring mouse anaphylaxis developed by Sobey and Adams (25) was used which allowed statistical analysis. This method utilized the severity of shock in surviving animals as well as the incidence of fatalities as a measure of anaphylaxis.

Preparation of I^{131}-labeled Antigen-Antibody Complexes.—Bovine plasma albumin was labeled with I^{131} using a modification of McFarlane's method (26). Ten to fifty microcuries of carrier-free I^{131} was added to an iodinating solution containing 8.3 mg. KI and 2.14 mg. KIO_3 in 10 ml. of distilled water. Iodine was released by adding 0.1 ml. of 1 N HCl. Either 5 or 10 ml. of the iodinating solution was then added dropwise to 10 ml. of 5 per cent bovine

TABLE I

Antigen-Antibody Complex Clearance

Mouse No.	Half-Time	
	Normal	Sensitized*
	min.	*min.*
1	10.0	6.5
2	12.5	8.7
3	23.5	2.0
4	2.5‡	8.5
5	6.5	3.5
6	10.5	6.5
7	13.7	9.5
8	14.5	6.5
9	9.5	
10	14.0	
11	14.0	
Average	11.9	6.5

I^{131}-labeled antigen-antibody complexes were injected intravenously and their disappearance followed by counting 0.005 ml. samples of tail blood taken at increasing intervals thereafter. The difference between normal and sensitized mice is significant at the 0.1 per cent level.

* 15 mg. BPA intraperitoneal 7 days apart. Clearance done 3 weeks later.

‡ Died during first 30 minutes.

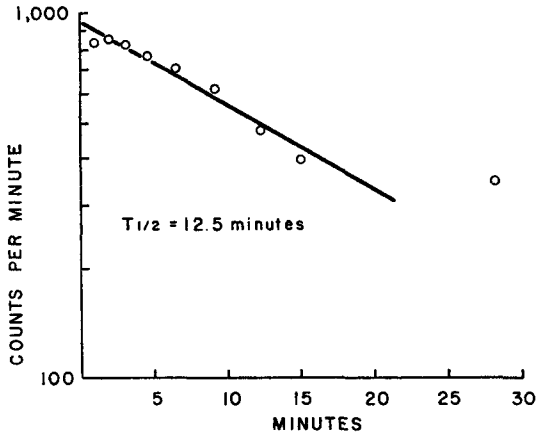

FIG. 1. A representative curve of AgAb complex clearance showing disappearance of the complexes following intravenous injection of 0.1 ml. 0.005 ml. samples of tail blood were counted at various times thereafter.

TABLE II

The Effect of Various R-E Blocking Agents on Anaphylaxis in the Mouse

Substance	Route and dose	Time between injection and challenge	Deaths	Control deaths	Significance*
		hrs.			
Carbon	12 mg.i.v.	2	3/10	10/10	0.01
	18 mg.i.v.	5	0/10	10/10	0.001
	18 mg.i.v.	5	0/9	4/8	0.01
	6 mg.i.v.	5	0/10	10/10	0.001
	12 mg.i.p.	12	1/10	7/10	0.025
Thorotrast	0.25 ml.i.v.	3	1/10	10/10	0.001
Zymosan	1.0 mg.i.v.	12	0/10	7/10	0.025
Pertussis	0.1 ml.i.v.	12	3/10	7/8	0.025

* Mann Whitney U Test using a modification (20) of Sobey's scoring method.

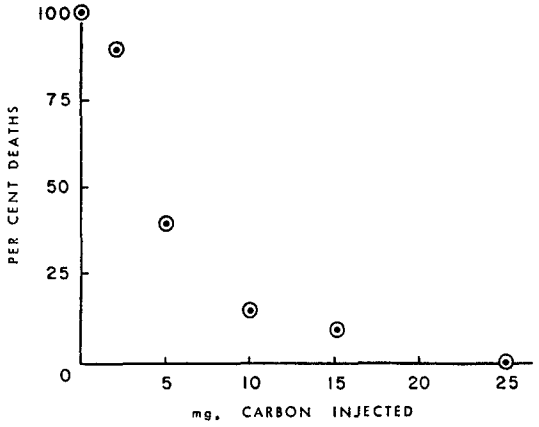

FIG. 2. Relationship between the dose of carbon injected intravenously and the subsequent deaths in terms of percentage after the injection of AgAb complex. Challenge was made 6 hours following the carbon injection with an AgAb complex preparation which killed all the controls.

plasma albumin in carbonate-bicarbonate buffer. The pH of the resulting solution was adjusted from 9.3 to 7.5. It was then run through an amberlite column to remove non-protein-bound iodine and stored at 4°C. The extent of iodination was less than 1 molecule of iodine per molecule of albumin. Complexes were made using mouse antiserum and bovine plasma albumin in antigen excess. The complexes were injected intravenously in volumes of 0.1 ml. Their disappearance from the circulation was determined by taking 0.005 ml. blood samples from the tail at timed intervals. These samples were hemolyzed in 1 ml. of distilled water in counting bottles. Counting was done in well-type scintillation counters for a period of time sufficient to obtain between 1600 and 6400 counts. The resulting counts per minute were corrected for background and plotted on semilogarithmic paper against the time the samples were taken. Half-times were determined from these plots.

RESULTS

Antigen-Antibody Complex Clearance.—The half-times for clearance from the circulation of labeled AgAb complexes were determined on 19 mice, both normal and sensitized (Fig. 1 and Table I). Previously sensitized mice cleared the complex more rapidly than normal mice. The two groups were different at the 0.1 per cent level of significance.

Antigen-antibody complexes are protein macromolecules with a molecular weight of 300,000 and greater (27). The above data indicate that these complexes are cleared at about the same rate as colloidal suspensions. These considerations led us to the tentative conclusion that the RES participates in AgAb complex uptake, as recently shown by Benacerraf and his coworkers (28).

Reticuloendothelial Blockade.—Table II gives the results of 8 experiments concerning RES blockade and passive homologous anaphylaxis in the mouse.

A single experiment was carried out on 20 mice actively sensitized with 2 intraperitoneal injections of 5 mg. alum-precipitated bovine plasma albumin 7 days apart. They were challenged 21 days later. Four hours before challenge half the mice were injected intravenously with 12 mg. of carbon. Eight of 10 controls died, whereas 3/10 animals receiving carbon died, a difference significant at the 5 per cent level.

The protection afforded by carbon is proportional to the amount of carbon injected as can be seen from the dose-response curve (Fig. 2). Graded amounts of the stock carbon suspension (100 mg./ml.) were injected intravenously 6 hours before challenge with an AgAb complex mixture which killed all the controls within ½ hour.

Time Course of the Blockade Effect.—The experiments of Parker and Finney (29) with carbon blockade have indicated that there is a latent period following the injection of a blockading dose of carbon (12 mg. per mouse) during which time the test dose is cleared at the normal rate. Inhibition of clearance begins about 3½ hours after blockade. Accordingly, the protective effect of carbon during the first 6 hours following blockade was tested.

Twelve mg. of carbon was injected intravenously into 130 mice which were challenged with AgAb complex 1 to 6 hours later. Two AgAb complex mixtures, *A* and *B*, were employed in testing the mice. The antibody nitrogen injected per mouse was about 23 μg. with mixture *A* and 13.8 μg. with mixture *B*.

It was expected that the slightly weaker challenge material would disclose differences in susceptibility which the stronger complex would mask. It can be seen (Fig. 3) that with the lower dose of AgAb complex there is a progressive increase with time in the prevention of anaphylactic death, following carbon injection. Using a stronger challenge material a latent period becomes evident during which there is no inhibition of anaphylaxis for at least 3 hours after the injection of carbon.

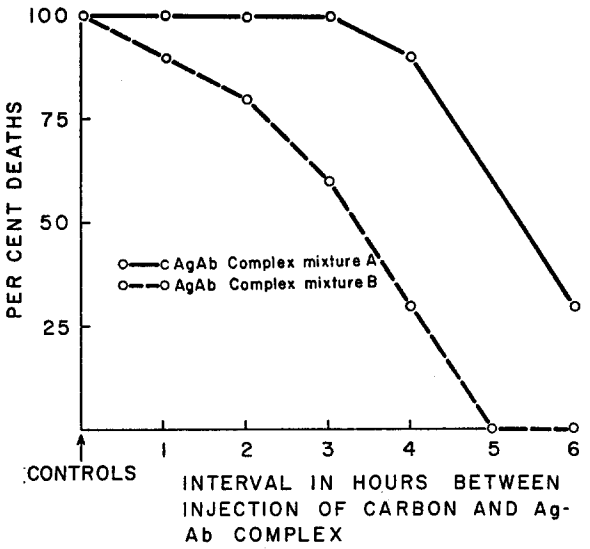

FIG. 3. The time lag or "latent period" following the injection of 12 mg. carbon per mouse. At hourly intervals after the intravenous injection of 12 mg. carbon two different doses of complex were injected. Utilizing complex A, about 23 μg. AbN was injected; with complex B, 13.8 μg. AbN was injected.

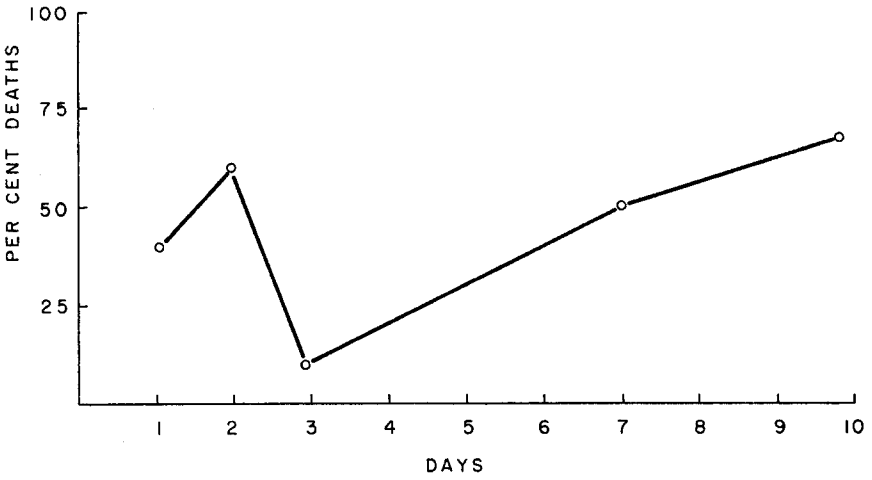

FIG. 4. The time course of protection following injection of 15 mg. carbon per mouse. The dose of AgAb complex killed 15/15 controls.

Fifty-seven mice were given intravenous injections of 15 mg. carbon per mouse and subsequently challenged at intervals from 26 to 234 hours after the blockade with a dose of complex which killed 15/15 controls.

It can be seen (Fig. 4) that the protective effect of carbon injection did not

decrease in a simple manner with time. The observed protection at 10 days cannot be explained on the basis of our present knowledge of RES physiology.

The Effect of Fresh Mouse Serum and Guinea Pig Complement on the Carbon-Induced Resistance to Passive Homologous Anaphylaxis.—Germuth (30) has suggested that the protection against anaphylaxis afforded by intravenous injection of colloidal material might be due to the removal from the serum, by adsorption to the colloid, of a factor (possibly complement) necessary for anaphylaxis. Such an effect has been shown *in vitro* (31, 32). The following experiment was undertaken to explore this possibility.

TABLE III

Effect of Fresh Mouse Serum and Saline on Mice Injected with Carbon and Subsequently Challenged with AgAb Complex

GR	0 hrs.	5 hrs.	6 hrs.	deaths
I	12 mg. carbon	0.5 ml. fresh mouse serum	0.4 ml. complex	1/16
II	12 mg. carbon	0.5 ml. saline	0.4 ml. complex	0/13
III	—	0.5 ml. fresh mouse serum	0.4 ml. complex	14/16
IV	—	0.5 ml saline	0.4 ml complex	13/13

TABLE IV

Effect of Fresh Mouse Serum, Guinea Pig Complement, and Saline on Mice Injected wth Carbon and Subsequently Challenged with AgAb Complex

GR	0 hrs.	5 hrs.	6 hrs.	Deaths
I	12 mg. carbon	0.5 ml. fresh mouse serum	0.4 ml. complex	1/5
II	12 mg. carbon	0.5 ml. guinea pig complement	0.4 ml. complex	0/5
III	12 mg. carbon	0.5 ml. saline	0.4 ml. complex	0/5
IV	—	0.5 ml. fresh mouse serum	0.4 ml. complex	5/5
V	—	—	0.4 ml. complex	5/5

Forty-four mice were injected with 12 mg. of carbon intravenously and 5 hours later, by which time the carbon was cleared from the circulation, both the treated and control mice were given fresh mouse serum, guinea pig complement, or saline. The serum was gathered from mice exsanguinated immediately preceding the experiment. The guinea pig complement diluted 1:4, contained about 30 CH_{50} units per ½ ml., which was the dose injected intravenously. One hour after receiving the serum, complement, or saline, the mice were challenged with AgAb complex.

The results are presented in Tables III and IV. Injection into the blockaded mouse of homologous serum or heterologous complement did not alter the carbon-induced resistance to subsequent challenge with AgAb complex. It is therefore unlikely that adsorption of serum components by injected carbon is the important factor in the protection observed. This conclusion can also be deduced from the data in Fig. 3. The observed latent period is not explicable on the

basis of adsorption from the serum of factors necessary for anaphylaxis because such an effect would be seen immediately following the colloid injection, not 3 hours later by which time the colloid has essentially disappeared from the blood.

Histopathology of Anaphylaxis Following RES Blockade.—Mouse tissues were examined histologically at various times after challenge with AgAb complex, during moderate and severe shock and after death. Mice receiving 12 or 18 mg. of carbon intravenously were challenged with a lethal dose of complex and autopsied at a time when all controls were dead. The mice given AgAb complex alone showed severe congestion throughout the gastrointestinal tract. Many of the liver sinusoids were packed with erythrocytes. Widespread congestion and interstitial hemorrhage were evident in the renal medulla. The changes observed, especially those in the kidney, were minimal in the mice pretreated with carbon and were commensurate with the mild shock these mice exhibited.

DISCUSSION

The experiments presented in Fig. 1 and Table I, indicate that AgAb complexes disappear rapidly from the circulation and are therefore most likely taken up by the RES, as has been recently shown by Benacerraf *et al.* (28), using isotopic and phagocytic competition techniques with heterologous AgAb complexes in the mouse. These authors demonstrated the presence of complexes in the Küpffer cells of the liver using fluorescein dye technique, and presumably, these RE cells play a major role in the rapid removal of anaphylactogenic complexes.

In anaphylaxis the fate of the complexes in the first few minutes is of prime importance because death occurs rapidly. The studies on the metabolic half-life of AgAb complexes over a period of days are not of significance in the consideration of immediate anaphylactic shock (33).

The intravenous injection of colloidal materials consistently protects mice against severe signs of passive homologous anaphylaxis. The extent of protection is dependent on both the dose of substance administered and the time interval between colloid and complex injection. The nature of the protection is uncertain. That blockade is at least partly involved is indicated by the close correlation between our data and those of Parker and Finney (29) over the first 6 hours following blockade with 12 mg. of carbon per mouse. There is a latent period, between 4 and 5 hours in both cases, before maximal effect of blockade is evident. This suggests that phagocytosis governs both the removal of carbon and AgAb complex.

This hypothesis may be further extended to explain the protection afforded by carbon. If the RE cells are "blockaded", that is saturated with material and less capable of removing further colloid, they will not remove AgAb complexes as rapidly as will normal RE cells. This means that the AgAb

complexes will remain in the circulation for a longer time and in higher concentrations than is normally the case. From this it may be inferred that the circulating blood is not the site of action of the AgAb complexes. If this were the case blockade would enhance, not inhibit, anaphylaxis.

These experiments do not reveal the nature of the events which occur subsequent to removal of AgAb complexes by RE cells and which culminate in severe visceral congestion and death. Moldovan and his coworkers (15) suggested that the injection of China ink into guinea pigs or rabbits caused the RE cells to secrete an amine-like substance, which they called reticuline M. This substance could be passively transferred to a sensitized guinea pig and cause an inhibition of bronchiolar spasm following subsequent anaphylactic challenge. Experiments are being carried out to explore the possibility that such a substance is of importance in mouse anaphylaxis. The importance of mediator substances such as 5-hydroxytryptamine and other toxic amines, mast cell disruption, and proteolytic enzyme systems must also be considered.

Although resistance to anaphylaxis has been correlated with RES blockade in this discussion, it is clear from our results that blockade alone is unable to account for the results obtained. The data of Parker and Finney (29) indicated that clearance had returned to normal values 6 days after blockade. The anaphylaxis-protecting effect was still evident at 10 days.

SUMMARY

Blockade of the RES was accomplished by the intravenous injection of carbon particles, thorotrast, zymosan, or a suspension of *Bordetella pertussis*. The blockade resulted in a decrease in sensitivity to anaphylaxis produced by the intravenous injection of soluble antigen-antibody complexes consisting of an optimal shocking mixture of bovine plasma albumin and mouse antibody to this antigen. The decrease in sensitivity to anaphylaxis was dependent on the dose of blockading agent and on the time between blockade and challenge with complex. The loss of sensitivity to anaphylaxis could not be restored by the administration of fresh serum from normal mice nor by guinea pig complement. Antigen-antibody complexes were rapidly removed from the blood with an average half-time of 11.9 minutes in normal mice. Complexes were cleared at significantly more rapid rates in mice previously sensitized to antigen.

Although not all the results can be explained on the basis of blockade the facts indicate that the RES does play an important but as yet undefined role in passive homologous anaphylaxis in the mouse.

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