Passive Anaphylaxis in Mice with γG Antibodies. II. Release of Histamine with Heterologous γG Antibody Preparations* (32627)

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Recent experiments comparing the ability of antibodies obtained in different animal species to sensitize mouse skin for the passive cutaneous anaphylactic (PCA) reaction have demonstrated that heterologous antibodies differ widely in their capacity for sensitization of mouse tissues.[3] As compared with homologous antibodies, rat antibodies were found to be quite effective, and guinea pig antibodies completely ineffective. The production of PCA reactions with rabbit antibodies required the use of relatively large doses of antibody and antigen,[3] similar to those necessary to produce PCA reactions in rat skin with rabbit antibodies (1,2). These latter reactions were claimed to be independent from mast cell participation and histamine release (3,4) and to depend primarily on the formation of antigen–antibody precipitates (3).

Pepsin-digested rabbit antibodies, although retaining their precipitating abilities, are totally ineffective in sensitizing the tissues of guinea pigs for anaphylaxis (5). On the other hand, pepsin-digested rabbit antibodies were claimed to be able to sensitize mice to passive systemic anaphylaxis (6).

It has been previously demonstrated that histamine release is a typical aspect of mouse anaphylactic reactions produced by homologous antibodies, as tested by *"in vivo"* (7) or *"in vitro"* methods (8).

The present studies were undertaken to investigate the occurrence of histamine release during anaphylactic reactions induced in mice by heterologous antibodies. In addition, the ability of pepsin-digested rabbit antihapten antibodies to produce *"in vivo"* and *"in vitro"* anaphylactic reactions in mouse tissues was investigated.

Material and Methods. Animals. Randombred adult female Swiss Webster mice, obtained from a local dealer, were used throughout the studies. Adult Hartley strain guinea pigs, of both sexes, were immunized to obtain immune sera, and also used as recipients for PCA tests. Adult Long Evans rats and New Zealand albino rabbits, were also immunized to obtain immune sera.

Antigens. Five times crystallized hen's egg albumin (Ov, Pentex Inc., Kankakee, Ill., lot F61), bovine serum albumin (BSA, Armour, Kankakee, Ill., lot A69908), bovine gamma globulin (BGG, Armour, lot X-30604), and guinea pig serum albumin (GPA) obtained in the laboratory by salt fractionation were coupled to twice recrystallized dinitrophenyl sulfonic acid (Eastman Kodak) according to methods previously described (9). The following dinitrophenylated (DNP) proteins were used: $DNP_{13}Ov$, $DNP_{30}GPA$, DNP_{37} BSA, and $DNP_{54}BGG$. The numbers refer to the mean number of DNP groups per molecules of carrier protein.

Antisera and antibody preparations. Mouse, rat, and rabbit antisera were obtained as pools from several animals after hyperimmunization with $DNP_{54}BGG$ in complete Freund's adjuvant. Guinea pig antisera to $DNP_{30}GPA$ were fractionated in their γG1 and γG2 fractions as previously described (10). Anti-DNP_{45} BGG antibodies from mice, rats, and rabbits were specifically purified from antigen–antibody precipitates as described in (11,12). Preparations of purified rabbit antibodies were digested by incubation with two times crystallized pepsin (Worthington Biochem. Corp., Freehold, New Jersey) according to Nisonoff *et al.* (13).

Tests for antibodies. Hemagglutination and

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hemolysis of DNP-coated sheep red blood cells (SRBC, Certified Blood Donor Service, Jamaica, New York), prepared as described in (14), as well as PCA tests in guinea pigs and mice, were done as previously described (10,14,15).[3]

The anaphylactic release of histamine from mouse peritoneal cell suspensions, containing mast cells, was induced after passive *"in vitro"* incubation with antibody dilutions and subsequent exposure to the specific antigen. The technic used was essentially that described by Prouvost-Danon *et al.* (8). Shortly, aliquots of pooled cell suspensions collected from normal mice were incubated with antibody for 10 min at 37°C, and then exposed to adequate (8)[4] concentrations of $DNP_{37}BSA$ for further 10 min. The magnitude of the histamine release induced was determined assaying the amounts of histamine present in the supernatants and in the cell pellets of the different aliquots after centrifugation. The results were expressed as percentages of the total histamine released, after deduction of the control release (range 5–17%). Histamine was assayed biologically in atropinized guinea pig ileum preparations.

In some experiments the occurrence of histamine release *"in vivo"* was also investigated by collecting small blood samples from mice undergoing passive anaphylactic reactions, and transferring them immediately to isolated organ baths containing guinea pig ileum preparations, as described in (7).

Results. Normal peritoneal cell suspensions from Swiss Webster mice were incubated with anti-DNP antibodies from mice, rats, rabbits, and guinea pigs. Essentially similar results were obtained whether whole, unfractionated antisera dilutions, or specifically purified antibodies were used. The data shown in Table I summarize some representative results, where mast cells were sensitized by purified anti-$DNP_{54}BGG$ antibodies from mouse, rat, and rabbit, and also by γG1 and γG2 fractions of guinea pig anti-$DNP_{30}GPA$ antisera. Mouse and rat antibodies proved to be effective in similar dose ranges. Approximately 50% of total histamine was released after sensitization with 2 μg AbN/ml and challenged with adequate concentrations of $DNP_{37}BSA$.[4] Rather elevated concentrations of rabbit antibodies were required to induce significant levels of histamine release, whereas no sensitizing activity could be detected in the guinea pig γG1 or γG2 antibody fractions.

TABLE I. Passive *"in Vitro"* Sensitization of Mouse Mast Cells by Purified Anti-DNP Antibodies from Mice, Rats, Rabbits, and Guinea Pigs.

Origin	Antibodies (μg N/ml)	Challenge (μg/ml $DNP_{37}BSA$)	Histamine release (%)
Mouse	2	0.1	38[a]
		1	54
		10	31
	0.4	0.02	21
		0.2	43
	0.08	0.004	0
		0.04	14
Rat	20	1	40
		10	54
	2	1	54
		10	14
Rabbit	20	10	34
		100	0
	2	1	5
		10	0
Guinea pig			
γ1	40	10	0
		100	0
γ2	50	10	0
		100	0

[a] After deduction of control release (5–17%).

Sensitization by incubation with Ab for 10 min at 37°C.

Purified anti-$DNP_{54}BGG$ antibodies from mouse, rat, and rabbit; γ2 and γ1 globulin fractions from guinea pig anti-DNP-Gp albumin.

Essentially similar results were obtained from *"in vivo"* experiments where the anaphylactic release of histamine was detected in the blood of mice undergoing passive systemic anaphylaxis (7). Normal mice were passively sensitized by intravenous injection of homologous or heterologous antibodies. Purified anti-$DNP_{54}BGG$ antibodies from mouse, rat, and rabbit (50, 100, and 100 μg AbN per

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TABLE II. Serological and Biological Properties of Native and Pepsin-Digested Rabbit Anti-$DNP_{64}BGG$ Antibodies.

Experimental tests	Antibody preparations	
	Native	Pepsin-digested
	(threshold concentration, μg AbN)	
Passive hemagglutination	1.7[a]	5.8[a]
PCA in guinea pigs	0.02[b]	negative with 11.8
PCA in mice	2.1[b]	negative with 62.5
Sensitization of mouse mast cells	effective[c]	not effective[c]

[a] μg AbN/ml for threshold agglutination of DNP-coated cells.

[b] μg AbN/skin site for threshold PCA reaction; in mice, 0.03 ml/site, 2 hours sensitization period; in guinea pigs, 0.1 ml/site, 4 hours sensitization period.

[c] Anaphylactic release of histamine after incubation with 20 μg AbN/ml and challenge with 10 μg/ml of $DNP_{37}BSA$; release with native Ab: 34%; release with pepsin-digested Ab: 0%.

mouse, respectively) and γ1 and γ2 guinea-pig anti-$DNP_{30}GPA$ (40 and 50 μg AbN per mouse, respectively, were used for sensitization. At 30 min after sensitization the animals were challenged intravenously with 1 mg $DNP_{13}Ov$; blood samples of 50 μl were collected by orbital puncture at 1 and 4 min after challenge and tested for the presence of histamine in a guinea pig ileum preparation, equilibrated by repeated stimulation with standard doses of 0.003 μg histamine per ml. Concentrations of histamine on the order of 0.3 μg per ml of blood, or more, were consistently detected in the blood of mice undergoing shock after sensitization with mouse or rat antibodies, both in the 1- and 4-min blood samples. Very small amounts of histamine, on the threshold of sensitivity of the method, were detected during shock induced by rabbit antibodies, while no histamine was detected in mice that were sensitized with guinea pig γ1 or γ2 antibodies.

Properties of pepsin-digested rabbit antibodies. Purified preparations of anti-$DNP_{54}BGG$ rabbit antibodies were subjected to pepsin digestion. Dilutions of the pepsin-digested antibody preparations were then compared with dilutions of the native antibody in several different test systems. The summary of these experiments is shown in Table II. The digested antibody preparations were still able to precipitate $DNP_{37}BSA$, as revealed by double diffusion agar tests, and to agglutinate DNP-coated SRBC in concentrations about threefold higher than the native antibody preparation. It is clear, however, that even very high concentrations of pepsin-digested antibodies were unable to sensitize guinea pig or mouse tissues for passive anaphylactic reactions. No PCA reactions could be obtained in mice with up to 62.5 μg AbN per skin site, or in guinea pigs with up to 12 μg AbN per skin site. Also the capacity for sensitization of mouse mast cells was abolished in the pepsin-digested preparation: no release of histamine could be induced after incubation with 20 μg AbN/ml, while significant release was induced by the same concentration of native antibodies.

Discussion. Recent studies on the mechanisms of anaphylactic reactions have suggested that different immunopharmacological pathways may be involved in passive sensitization by homologous and heterologous antibodies. It is generally admitted that sensitization induced by homologous antibodies depends on fixation of antibodies to tissue receptors—possibly on tissue mast cells. Subsequent interaction with antigen molecules, through formation of antigen-antibody complexes of relatively simple structure, would activate processes that finally result in the mobilization of pharmacological mediators, such as histamine (16-19). On the other hand, it is not clear if the reactions induced after sensitization with heterologous antibodies depend on the same mechanisms, particularly when very high doses of heterologous antibodies are required for sensitization, e.g., sensitization of rat tissues by rabbit γG anti-

bodies (1, 2). It has been suggested, for instance, that the formation of antigen–antibody precipitates is necessary to elicit the reaction (3).

The present results, confirming previous *"in vivo"* findings (20),[3] demonstrate that heterologous antibodies differ widely in their capacity for sensitization of mouse mast cells for the anaphylactic release of histamine *"in vitro."* In addition, it is shown that preparations of pepsin-digested rabbit antibodies within the dose range tested were totally unable to sensitize mouse tissues for anaphylactic reactions, as have been previously demonstrated for sensitization of guinea pig tissues (5).

The preparations of rat anti-DNP antibodies used were quite effective in sensitizing mouse mast cells (Table I). They were devoid of any reaginic activity for rat tissues.[3] It seems, therefore, that similarly to what is known to occur in the sensitization of guinea pig tissues by rabbit γG antibodies, rat γG antibodies are much more effective in sensitizing the tissues of one heterologous species (the mouse) than in sensitizing homologous tissues.

Rabbit antibodies, although much less effective than mouse or rat antibodies, were clearly able to sensitize mouse mast cells for the anaphylactic histamine release (Table I). When, however, these antibodies were digested with pepsin, they lost their sensitizing properties completely, although retaining largely their precipitating and hemagglutinating properties (Table II). It seems, thus, that the integrity of the Fc portion of the antibody molecule is necessary for the elicitation of PCA and *"in vitro"* anaphylactic reactions in mice (Table II) as well as for PCA in guinea pigs (5). It has been previously reported by Kind and Goodman (6) that pepsin-digested rabbit antibodies were able to sensitize mice to passive systemic anaphylaxis. It is conceivable, however, that the severe and lethal reactions observed after systemic sensitization, although being caused by antigen-antibody aggregation in the circulation, would be distinct from genuine anaphylactic mechanisms. The present data demonstrate that antigen–antibody aggregation, by itself, is not sufficient to initiate anaphylactic reactions in mouse tissues. Very recent observations of Casey and Tokuda (21) have shown that pepsin-digestion of rabbit antibodies, although reducing significantly its capacity to sensitize mouse skin, is not able to destroy it completely. Significant reactions were obtained with doses of antibody as high as 35–70 μg AbN/skin site, while in the present experiments 62.5 μg AbN/site were completely ineffective. The reason for those differences are unknown.

Similar to previous *"in vitro"* data, guinea pig antibodies proved to be remarkably ineffective in inducing anaphylactic sensitization of mouse tissues (Table I). It was reported (22) in studies using fluorescein labeled antibodies, that guinea pig γ1 antibodies are able to bind to mouse mast cells. If such binding occurred in our experiments, it seems that it is functionally inactive because guinea pig γ1 and γ2 did not sensitize mast cells for histamine liberation.

The present results suggest that the reactions induced in mouse tissues by homologous antibodies, rat or rabbit antibodies, are mediated by similar mechanisms, although larger concentrations of rabbit antibodies are required for sensitization. It is possible that the varying effectiveness of antibodies from different species in sensitizing mouse tissues occur as a function of the extent to which the molecular configuration of the Fc portion of these immunoglobulins are able to interact with receptors on tissue mast cells.[4]

Summary. Anaphylactic release of histamine was induced in mouse mast cells after sensitization with mouse, rat, or rabbit antibodies. As compared with homologous antibodies, rat antibodies were quite effective, whereas, rabbit antibodies were poorly effective. Guinea pig antibodies (γG1 and γG2) were ineffective. Pepsin-digested preparations of rabbit antibodies were unable to sensitize mouse mast cells, or mouse skin, for the PCA reaction.

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Serum Tocopherol Levels of Normal Preschool Children and Children with Protein-Calorie Malnutrition in South India* (32628)

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Majaj (1) has drawn attention to the possibility that vitamin E responsive anemias may occur in association with protein–calorie malnutrition. This has created considerable interest in the investigation of the causes of anemias associated with protein–calorie malnutrition which occur in various parts of the world. Data have been reported on the concentration of α-tocopherol in the serum of normal children above 5 and below 2 years of age (2,3,4). However, no data on the α-tocopherol content of the serum of normal preschool children between 2 and 5 years of age has been reported (preschool years show the highest incidence of protein-calorie malnutrition). For this reason α-tocopherol has been determined in normal South Indian children between 2 and 5 years of age and in children with kwashiorkor. Two groups of healthy preschool children have been studied, a group from a typical South Indian village and a group from an orphanage. The α-tocopherol in the serum of these healthy children has been compared to that found in patients with kwashiorkor, most of whom come from similar villages. The anemias occurring in patients with kwashiorkor are also under investigation.

Materials and Methods. Forty-two children aged 1–4 years with the clinical signs of protein–calorie malnutrition (5), admitted to the Nutrition Research Unit of the Christian Medical College and Hospital, Vellore, took part in the study.

The patients were fed a diet which provided 3 gm protein per kg per day from skimmed milk, and 150 cal/kg/day from rice, sugar, and coconut oil (6). Calories from oil formed 15% of the daily intake. The patients were given 10,000 IU of vitamin A in 1 cc peanut oil daily as part of their therapy. This diet provided about 3 mg α-tocopherol/day, calcu-

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