ANTIGEN-INDUCED RELEASE OF SLOW REACTING SUBSTANCE OF ANAPHYLAXIS (SRS-A^{RAT}) IN RATS PREPARED WITH HOMOLOGOUS ANTIBODY*

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It is now appreciated that different homologous immunoglobulins are capable of preparing the rat peritoneal cavity for the antigen-induced release of distinctly different chemical mediators of anaphylaxis (1). One antibody, the "mast cell–sensitizing" (2) or homocytotropic (3) antibody, appears early in the course of immunization and sensitizes rat mast cells in vivo (4) and in vitro (3, 5) for the release of histamine and serotonin upon subsequent interaction with specific antigen. A second homologous antibody associated with the IgG fraction of hyperimmune rat antisera (1) prepares the rat for the selective, antigen-induced release of slow reacting substance of anaphylaxis (SRS-A^{rat}).

In a series of preliminary experiments using heterologous, hyperimmune antisera (6), it appeared that, in the rat, the polymorphonuclear (PMN) leukocyte was a cellular prerequisite for the immunologic release of SRS-A^{rat}, whereas the peritoneal mast cell and circulating lymphocyte were not required. The present study confirms and extends this observation using homologous, hyperimmune antisera. The immunologic release of SRS-A^{rat} was suppressed by leukocyte depletion, and it was enhanced in the presence of an intraperitoneal exudate consisting predominantly of PMN leukocytes.

Inhibition of the antigen-induced release of SRS-A^{rat} by diethylcarbamazine is also described. This piperazine analogue blocks the immunologic pathway leading to the release of SRS-A^{rat} without interfering with the antigen-induced release of histamine. Thus, the reaction sequences initiated by interaction of specific antigen with distinctly different homologous immunoglobulins leading to the selective release of either SRS-A^{rat} or histamine involve different cellular elements and can be inhibited by different biological and chemical agents.

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Materials and Methods

Materials.—Male Sprague-Dawley rats weighing 200–450 g were used in all experiments; rats of comparable sizes were used in each experiment. Albino rabbits weighing 2–4 kg were used in the preparation of antisera against various rat cell types.

Bovine serum albumin (BSA) and hen egg albumin (Ea) were supplied by Pentex Inc., Kankakee, Ill. Dinitrophenyl bovine γ-globulin (DNP-BγG) and dinitrophenyl bovine serum albumin (DNP-BSA) were prepared as described (7); DNP-BγG and DNP-BSA contained 34 and 31 haptenic groups/mole of protein, respectively.

Nitrogen mustard (Mustargen) was obtained from Merck, Sharp & Dohme (Rahway, N. J.). Diethylcarbamazine citrate (Hetrazan, Lederle Laboratories, Pearl River, N. Y.) was generously supplied by Dr. H. G. Lockhard (Lederle Laboratories). The antihistamine, mepyramine maleate (Neo-Antergan, Merck, Sharp & Dohme), and a potent antiserotonin agent, methysergide (UML-491, Merck, Sharp & Dohme), were used in the bioassays.

A semipurified, nontoxic fraction of venom from the cobra (*Naja haje*) (8) was donated by Dr. R. A. Nelson. The *Bacillus pertussis* cultures were supplied by Dr. Irving Millman of Merck, Sharp & Dohme.

Immunization Procedures.—Hyperimmune rat antisera were obtained by injecting 10 mg of BSA or Ea emulsified in 1.0 ml of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) into the foot-pads of male Sprague-Dawley rats. 7 days later, the animals were injected intramuscularly in the thigh and at three sites subcutaneously with a total of 4–5 mg of antigen in 1.0 ml of complete Freund's adjuvant. On days 28–30, the rats were injected intradermally at two sites on a shaved area of their backs with 0.1 ml of a solution containing 100 μg of specific antigen in 0.15 M saline. The animals were exsanguinated 35–40 days after initial immunization, and the sera stored at −70°C.

Hyperimmune rat antiserum against DNP-BγG was obtained by injecting male Sprague-Dawley rats with an emulsion of 2.0 mg DNP-BγG in 1.0 ml of complete Freund's adjuvant; each rat received 0.1 ml in each foot-pad and 0.6 ml intraperitoneally. 7 days later, 1.0 mg of DNP-BγG in complete Freund's adjuvant was injected intramuscularly in the thigh and subcutaneously at three sites on the back. On day 27, the rats were injected intradermally at two sites with 0.1 ml of a solution containing 100 μg DNP-BγG in 0.15 M saline. This was repeated 1 wk later. The serum was obtained between days 35 and 45.

Antisera containing homocytotropic antibody were prepared as described in reference 2. Rats were injected intramuscularly with 1.0 mg Ea in 1.0 ml 0.15 M saline and intraperitoneally with 1.0 ml of a heat-killed *B. pertussis* culture containing 2×10^9 organisms/ml. Antisera were obtained 10–14 days later.

Rabbit antisera directed against rat mast cells were prepared as described in reference 9. Rabbit antisera directed against rat-thymic lymphocytes were generously supplied by Dr. C. B. Carpenter (10). Rabbit anti-rat PMN leukocyte antiserum was kindly supplied by Dr. C. G. Cochrane or was prepared as follows: 10 male Sprague-Dawley rats were each injected intraperitoneally with 20 ml of distilled water. 5 days later, they were injected intraperitoneally with 20 ml of 0.1% oyster glycogen (Baker Chemical Co., North Phillipsburg, N. J.) in 0.15 M saline, and 4 hr later, the peritoneal cells were recovered in 10 ml Tyrode's solution containing heparin, 50 μg/ml. The cells were washed three times in Tyrode's solution, pooled, and resuspended in 10 ml of buffer. The cell suspension containing about 1×10^8 cells/ml (>70% PMN leukocytes) was emulsified in 10 ml of complete Freund's adjuvant. 5.0 ml of this emulsion were injected into the toe-pads and foot-pads of albino rabbits; and 7 days later, an additional 5.0 ml were injected intramuscularly in the thigh and at three sites subcutaneously. On day 28, the rabbits were exsanguinated and the serum obtained was stored at −70°C.

Measurement of Antibody and Complement.—The specific antibody protein concentration of hyperimmune rat antisera was determined by precipitin analysis (11). Hyperimmune rat antisera against BSA or Ea contained 2.0–5.8 mg of specific antibody protein per milliliter. Rat antiserum against DNP-BγG contained 1.3 mg/ml of hapten-specific antibody protein (7).

Passive cutaneous anaphylaxis (1, 12) was carried out to determine the titer of homocytotropic antibody activity. 0.1 ml aliquots of serial dilutions of homologous antisera were injected intradermally into the shaved backs of male Sprague-Dawley rats weighing 225–250 g; 48 hr later, the animals were injected intravenously with 1.0 mg of specific antigen in 1.0 ml of 0.5% Evan's blue dye (Allied Chemical Co., Morristown, N. J.). 45 min later, the animals were sacrificed in ether, the skin reflected, transilluminated, and the size and intensity of the lesions were recorded. The serum pool used in experiments described below had a titer of 1:30 (1).

Whole serum complement determinations were carried out as described in reference 13.

Preparation of Rats for the Immunologic Release of SRS-A^{rat}.—Male Sprague-Dawley rats were injected intraperitoneally with hyperimmune rat antiserum diluted with 0.15 M saline so as to contain 1.0 mg of specific antibody protein. The pools of hyperimmune rat antisera used ranged in titer from 1.3–5.8 mg of specific antibody protein per milliliter. 2 hr later, the rats were challenged intraperitoneally with 2.0 mg of specific antigen in 5.0 ml Tyrode's solution containing heparin, 50 μg/ml. Exactly 5 min later, the animals were stunned, exsanguinated, and the abdominal wall was incised and reflected. The peritoneal fluid was recovered with siliconized Pasteur pipettes and centrifuged at 150 *g* for 4 min at 4°C; the supernatants were collected in iced polypropylene tubes. The cell button was resuspended in 3.0 ml of Tyrode's solution and boiled for 8 min to extract the residual cellular histamine. Histamine and SRS-A^{rat} were assayed on the isolated guinea pig ileum as described (1). Representative samples were assayed on the estrous rat uterus (14) for the presence of bradykinin and serotonin. Using the experimental model described for the immunologic release of SRS-A^{rat}, insignificant concentrations of bradykinin and serotonin were found.

In one series of experiments, rats were injected intraperitoneally with 20 ml of 0.1% oyster glycogen in 0.15 M saline 16–18 hr before being prepared for the antigen-induced release of of SRS-A^{rat} as described above. In the peritoneal exudate induced with glycogen, over 70% of the cells were PMN leukocytes, and the remainder were mononuclear cells (15).

In certain experiments, rats prepared for the immunologic release of SRS-A^{rat} by the intraperitoneal injection of hyperimmune rat antisera were challenged intravenously with specific antigen 2 hr later. 5.0 ml of Tyrode's solution containing heparin, 50 μg/ml, were injected intraperitoneally immediately after intravenous challenge with antigen, and the peritoneal fluid was recovered exactly 5 min later and treated as described.

Other experiments involved rats actively sensitized against BSA as previously described. 10–14 days after the last intradermal booster injections, these actively sensitized rats were challenged with 5.0 mg of specific antigen in 5.0 ml Tyrode's solution containing heparin, 50 μg/ml, administered intraperitoneally. Exactly 5 min later, the peritoneal fluid was recovered and treated in the usual fashion.

Preparation of Rats for the Antigen-Induced Release of Histamine.—Male Sprague-Dawley rats weighing 200–250 g were injected intraperitoneally with 5.0 ml Tyrode's solution containing a 1:10 dilution of rat antisera with homocytotropic antibody activity against Ea. 4 hr later, the animals were challenged with 2.0 mg Ea in 5.0 ml Tyrode's solution containing heparin, 50 μg/ml. Exactly 5 min later, the peritoneal fluid was recovered and treated as above. Homocytotropic antibody–mediated histamine release was associated with serotonin release in a ratio of 10–20:1.

RESULTS

Determination of the Cellular Prerequisites for the Antigen-Induced Release of SRS-A^{rat}.—

Effect of depletion of peritoneal mast cells on the subsequent antigen-induced release of SRS-A^{rat}: Preliminary experiments confirmed the observation (16) that the intraperitoneal injection of distilled water into rats produced disruption of the mesenteric mast cells as determined by microscopic examination of spreads of mesentery stained with toluidine blue. Morphologic examination and estimations of the total residual cellular histamine of the free peritoneal cells

TABLE I

The Antigen-Induced Release of SRS-A^{rat} in Rats Pretreated with Distilled Water or a Rabbit Anti-Rat Mast Cell Antiserum (Ra Anti-RMC)

Modifying procedure	No. of rats	Peripheral blood				Peritoneal cells	Mean SRS-A^{rat} release
		Total WBC* per mm^3	Differential			Total cellular histamine	
			Poly	Lymph	Mono		
						µg/rat	*units/rat*
A. Control	5	9,275	27.3	70.0	2.7	10.0	464
Distilled water	5	8,060	26.6	72.0	1.4	0.05	488
B. Control	2	8,450	24.0	72.5	3.5	6.6	578
Ra anti-RMC	5	10,100	21.2	74.2	4.6	0.08	512

* WBC, white blood cells.

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against DNP-BγG for the release of SRS-A^{rat}, and they were challenged intraperitoneally with DNP-BSA. Distilled water (20 ml intraperitoneally) was injected 5 days before Experiment A, and Ra anti-RMC (2.0 ml intraperitoneally) was injected 24 hr before Experiment B.

established that the free peritoneal mast cells were also depleted by this procedure (6). Rats pretreated with distilled water 5 days before being prepared for the antigen-induced release of SRS-A^{rat} with homologous, hyperimmune antiserum demonstrated no reduction in the release of SRS-A^{rat} when compared with untreated animals (Table I). The elimination of the peritoneal mast cell population by a specific rabbit anti-rat mast cell antiserum (9) also did not influence the subsequent immunologic release of SRS-A^{rat} (Table I), but did prevent the homocytotropic antibody–mediated release of histamine (Table II).

Effect of depletion of circulating leukocytes on the subsequent antigen-induced

release of SRS-A^{rat}: Rats pretreated with nitrogen mustard, 2.0 mg/kg intravenously, 5 days before an experiment developed a profound leukopenia, but the peritoneal mast cell population remained intact. When rats made leukopenic

TABLE II

The Antigen-Induced Release of Histamine in Rats Pretreated with a Rabbit Anti-Rat Mast Cell Antiserum (Ra Anti-RMC)

Modifying procedure	No. of rats	Peripheral blood				Peritoneal cells	Mean histamine release
		Total WBC per mm^3	Differential			Total cellular histamine	
			Poly	Lymph	Mono		
						µg/rat	*µg/rat*
Control	3	10,100	22	77	1	17.4	8.2
Ra anti-RMC	3	16,100	25	72	3	0.45	0.14

Each result represents a mean value for the rats in that group. Rats were prepared with antiserum containing homocytotropic antibody against Ea and were challenged intraperitoneally with Ea. Ra anti-RMC (2.0 ml intraperitoneally) was injected 24 hr before experiment.

TABLE III

The Antigen-Induced Release of SRS-A^{rat} in Rats Pretreated with Nitrogen Mustard or Rabbit Anti-Rat Polymorphonuclear Leukocyte Antiserum (Ra Anti-RPMN)

Modifying procedure	No. of rats	Peripheral blood				Peritoneal cells	Mean SRS-A^{rat} release	Suppression
		Total WBC per mm^3	Differential			Total cellular histamine		
			Poly	Lymph	Mono			
						µg/rat	*units/rat*	%
A. Control	3	11,666	24.7	73.0	2.3	15.3	594	—
Nitrogen mustard	3	2,200	23.7	73.3	3.0	14.7	146	75.5
B. Control	6	15,683	24.3	73.1	2.6	11.4	704	—
Ra anti-RPMN	6	4,800	0.7	91.2	8.1	0.013	129	81.8

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against Ea for the release of SRS-A^{rat}, and they were challenged intraperitoneally with Ea. Nitrogen mustard (2.0 mg/kg intravenously) was injected 5 days before Experiment A, and Ra anti-RPMN (3.0 ml intraperitoneally) was injected 18 hr before Experiment B.

with nitrogen mustard were then prepared for the antigen-induced release of SRS-A^{rat} with homologous hyperimmune antiserum, a 76% suppression of SRS-A^{rat} release was observed (Table III).

A marked neutropenia was effected in rats by the intraperitoneal injection of a rabbit anti- rat polymorphonuclear leukocyte antiserum (Ra anti-RPMN). Rats made neutropenic with this antiserum demonstrated more than 80% suppression of the antigen-induced release of SRS-A^{rat} (Table III). In contrast

TABLE IV

The Antigen-Induced Release of SRS-A^{rat} in Rats Pretreated with a Rabbit Anti-Rat Thymic Lymphocyte Antiserum (Ra Anti-RTL)

Modifying procedure	No. of rats	Peripheral blood				Peritoneal cells	Mean SRS-A^{rat} release
		Total WBC per mm^3	Differential			Total cellular histamine	
			Poly	Lymph	Mono		
						μg/rat	*units/rat*
Control	6	16,400	23.8	74.6	1.6	19.6	524
Ra anti-RTL	6	5,340	61.2	34.6	4.2	12.8	735

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against Ea for the release of SRS-A^{rat}, and they were challenged intraperitoneally with Ea. Ra anti-RTL (0.3 ml intravenously) was injected 4 hr before experiment.

TABLE V

The Antigen-Induced Release of SRS-A^{rat} in the Rat Peritoneal Cavity in the Presence of a Polymorphonuclear Leukocyte (PMN) Exudate

Modifying procedure	No. of rats	Peritoneal fluid		Mean SRS-A^{rat} release
		Total cells per mm^3	PMN	
			%	*units/rat*
Control	5	17.8×10^3	5.0	200
PMN exudate	5	50.7×10^3	75.0	450
Control	5	22.4×10^3	6.0	282
PMN exudate	3	62.3×10^3	89.0	1033

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against BSA for the release of SRS-A^{rat}, and they were challenged intraperitoneally with BSA. The PMN exudate was induced by the intraperitoneal injection of 0.1% oyster glycogen 18 hr before each experiment.

to the nitrogen mustard, the Ra anti-RPMN also depletes the rats of peritoneal mast cells, but previous experiments (Table I) had ruled out any significant role for the peritoneal mast cell in this reaction.

The leukopenia induced by nitrogen mustard involved lymphocytes as well as PMN leukocytes, whereas pretreatment with Ra anti-RPMN produced a

marked neutropenia with only a moderate reduction in the absolute lymphocyte counts. The possible role of the lymphocyte was further studied by experiments involving pretreatment of rats with a rabbit antiserum directed against rat

TABLE VI

The Antigen-Induced Release of SRS-A^{rat} in the Peritoneal Cavity of Actively Sensitized Rats in the Presence of a Polymorphonuclear Leukocyte (PMN) Exudate

Modifying procedure	No. of rats	Mean weight	Mean SRS-A^{rat} release
		g	*units/rat*
Control	3	435	377
PMN exudate	3	452	2174

Each result represents a mean value for the rats in that group. Rats actively sensitized against BSA were challenged intraperitoneally with BSA. The PMN exudate was induced by the intraperitoneal injection of 0.1% oyster glycogen 16 hr before the experiment.

TABLE VII

Changes in Whole Serum Complement (CH_{50}) in the Rat Produced by Different Modifying Procedures

Modifying procedures	Mean whole serum complement (CH_{50})		Reduction
	Control	Experimental	
			%
Antigen challenge	170	120	29.4
Rabbit anti-rat mast cell antiserum 2.0 ml intraperitoneally 24 hr before testing	234	203	13.3
Rabbit anti-rat polymorphonuclear leukocyte antiserum 3.0 ml intraperitoneally 24 hr before testing	234	161	31.2
Distilled water 20 ml intraperitoneally 5 days before testing	209	289	—
Nitrogen mustard 2.0 mg/kg intravenously 5 days before testing	209	274	—
Rabbit anti-rat thymic lymphocyte antiserum 0.3 ml intravenously 4 hr before testing	197	179	9.2
Nontoxic fraction of cobra venom (*Naja haje*) 0.5 ml and 0.25 ml intravenously 24 and 4 hr respectively before testing	172	0	100

Each result represents a mean value for six rats in each group. Experimental group of rats challenged with antigen were prepared for SRS-A^{rat} release by the intraperitoneal injection of hyperimmune antiserum against Ea. Control animals in this group received only antigen intraperitoneally. All other control groups represent normal rats of similar weight to experimental animals tested that day.

thymic lymphocytes (Ra anti-RTL). This antiserum effected a greater than 80% reduction in the absolute lymphocyte count without significant change in the circulating PMN leukocyte population or suppression of the subsequent antigen-induced release of SRS A^{rat} (Table IV).

Effect of an intraperitoneal PMN leukocyte exudate on the subsequent antigen-induced release of SRS-A^{rat}: A peritoneal exudate consisting predominantly of PMN leukocytes and representing a 40- to 50-fold absolute increase in this cell type was induced in rats by the intraperitoneal injection of glycogen 18 hr before preparation for the antigen-induced release of SRS-A^{rat}. As shown in Table V, the antigen-induced release of SRS-A^{rat} was enhanced twofold to fourfold in the presence of a peritoneal exudate. No attempt was made to determine

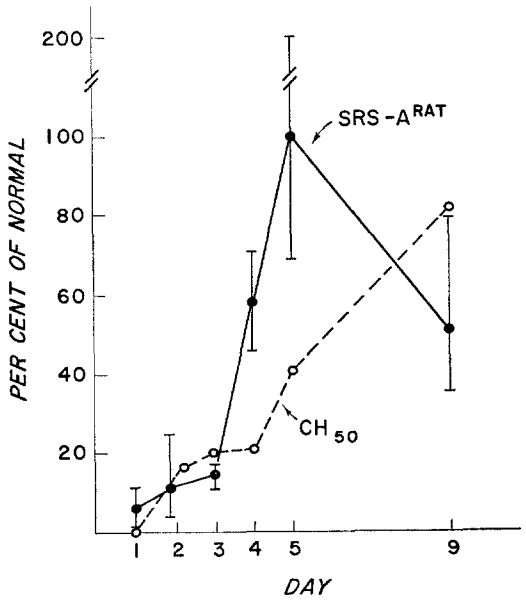

FIG. 1. Sequential changes in CH_{50} and SRS-A^{rat} release in rats pretreated with cobra venom fraction. Each point represents a mean value for three rats in that group. Rats were prepared with hyperimmune antiserum against Ea, and they were challenged intraperitoneally with Ea. Cobra venom factor (0.5 ml and 0.25 ml) was injected intravenously 24 and 4 hr respectively before the start of the experiment. Values were expressed as per cent of normal value for comparable group of untreated rats each day.

whether the use of greater concentrations of antiserum and antigen would lead to an even greater enhancement of SRS-A^{rat} release in the presence of a peritoneal exudate. When a peritoneal exudate was induced in actively sensitized rats prior to intraperitoneal challenge with specific antigen, a sevenfold enhancement in the release of SRS-A^{rat} was observed (Table VI).

Effect of pretreatment with a nontoxic fraction of cobra venom on the subsequent antigen-induced release of SRS-A^{rat}: The immunologic release of SRS-A^{rat}, using homologous hyperimmune antiserum to prepare the peritoneal cavity, was associated with a slight reduction in whole serum complement (Table VII).

Thus, attention was directed to the effect of decomplementation by pretreatment with a semipurified, nontoxic fraction of cobra venom (*Naja haje*) on the subsequent release of SRS-A^{rat}. Fig. 1 depicts the results of an experiment concerned with the sequential changes in the CH_{60} and the release of SRS-A^{rat} in rats pretreated with this cobra venom fraction as compared with normal rats. Both parameters were markedly reduced during the first 3 days and gradually returned to normal within the next 3 days with SRS-A^{rat}-releasing capacity being fully restored to normal somewhat earlier than whole complement activity.

Since complement depletion suppressed the antigen-induced release of SRS-A^{rat}, the effect of the various modifying procedures on the CH_{50} of the rat

TABLE VIII

Inhibition of the Antigen-Induced Release of SRS-A^{rat} with Intraperitoneal Diethylcarbamazine

Diethylcarbamazine (mg/kg) intraperitoneally	Mean SRS-A^{rat} release	Inhibition
	units/rat	%
0	235	—
5	272	—
10	162	32
20	92	61
40	67	72

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against BSA for the release of SRS-A^{rat}, and they were challenged intraperitoneally with BSA. Diethylcarbamazine was injected intraperitoneally 30 sec before challenge with specific antigen.

was determined (Table VII). None of the modifying procedures significantly diminished the titer of whole complement in the rat except pretreatment with the cobra venom fraction.

Effect of Diethylcarbamazine on the Antigen-Induced Release of SRS-A^{rat}.—

The observation that diethylcarbamazine (Hetrazan) suppressed the antigen-induced release of SRS-A^{rat} in rats prepared with heterologous hyperimmune antiserum without interfering with the bioassay of SRS-A^{rat} (17) prompted an assessment of its action in rats prepared with homologous, hyperimmune antiserum. When diethylcarbamazine was administered intraperitoneally 30 sec before challenge with specific antigen by the same route, a dose of 20 mg/kg consistently gave greater than 60% suppression of SRS-A^{rat} release (Table VIII).

Since it had already been established that diethylcarbamazine did not interfere with antigen-antibody interaction in vitro (17), an experiment was devised

to show that diethylcarbamazine would not interfere with antigen-antibody interaction in vivo. If antigen-antibody interaction does indeed occur in vivo in the antigen-challenged, diethylcarbamazine-treated rat, then such an animal should be unresponsive to a second challenge with specific antigen at a time when diethylcarbamazine has been excreted to a noninhibitory level (Table IX). When animals prepared with homologous hyperimmune antiserum were challenged with antigen at 1 hr without being sacrificed and then rechallenged at 2 hr, the peritoneal fluid contained very little SRS-A^{rat} because the initial antigen challenge utilized most of the available antibody, and the SRS-A^{rat} released had been inactivated in vivo. When animals sensitized in the same manner were challenged with antigen at 1 hr in the pres-

TABLE IX

"Desensitization" of Rats Prepared for the Antigen-Induced Release of SRS-A^{rat} with Homologous Antibody by Pretreatment with Specific Antigen in the Presence and Absence of Diethylcarbamazine

Modifying procedures	No. of rats	SRS-A^{rat} release	Suppression
		units/rat	%
Antigen alone at 2 hr	3	380	—
Antigen + diethylcarbamazine at 2 hr	2	105	72.4
Antigen alone at 1 hr + repeat antigen dose at 2 hr	3	50	87.0
Antigen + diethylcarbamazine at 1 hr + repeat antigen dose alone at 2 hr	3	41	89.3

Each result represents a mean value for the rats in that group. Rats were prepared with hyperimmune antiserum against BSA for the release of SRS-A^{rat}, and they were challenged intravenously with BSA. Diethylcarbamazine (20 mg/kg intravenously) was injected 30 sec before challenge with specific antigen.

ence of diethylcarbamazine and then were rechallenged at 2 hr with antigen alone, there again was no appreciable release of SRS-A^{rat}. In the latter group, the failure of the repeat challenge to release SRS-A^{rat} cannot be attributed to the presence of diethylcarbamazine because this drug is excreted at a rate of 100 mg/kg/hr (18), and a 20 mg/kg dose is no longer inhibitory 15 min after administration (17). This dose of diethylcarbamazine effected a 72 % inhibition of the immunologic release of SRS-A^{rat} when administered at the time of challenge with specific antigen (Table IX). Thus, inhibition of the antigen-induced release of SRS-A^{rat} by diethylcarbamazine is associated with "desensitization" of the tissues since effective antigen-antibody interaction takes place but the reaction sequence initiated by this interaction is blocked by diethylcarbamazine at some subsequent step.

Inhibition of SRS-A^{rat} Release in the Actively Sensitized Rat.—

Inhibition of the antigen-induced release of SRS-A^{rat} was also achieved in actively sensitized rats by depletion of the circulating PMN leukocytes; greater than 95% suppression of the antigen-induced release of SRS-A^{rat} was observed when the absolute PMN leukocyte count was reduced by pretreatment with Ra anti-RPMN to less than 2% that of control animals (Table X). Diethylcarbamazine in a dose of 20 mg/kg intraperitoneally given at the time of challenge with specific antigen also markedly suppressed the immunologic release of SRS-A^{rat} in the actively sensitized rat (Table X).

TABLE X

Inhibition of the Antigen-Induced Release of SRS-A^{rat} in Actively Sensitized Rats

Modifying procedure	No. of rats	Peripheral blood				Peritoneal cells	Mean SRS-A^{rat} release	Suppression
		Total WBC per mm^3	Differential			Total cellular histamine		
			Poly	Lymph	Mono			
						μg/rat	*units/rat*	%
Control	3	18,400	27	70	3	9.2	1488	—
Ra anti-RPMN	3	7,300	1	94	5	0.40	69	95.4
Diethylcarbamazine	3	20,400	28	70	2	13.8	185	87.6

Each result represents a mean value for the rats in that group. Rats were hyperimmunized against BSA and challenged intraperitoneally with BSA for the release of SRS-A^{rat}. Ra anti-RPMN (3.0 ml intraperitoneally) was injected 16 hr before the experiment, and diethylcarbamazine (20 mg/kg intraperitoneally) was injected 30 sec before challenge with specific antigen.

DISCUSSION

Requirement for the PMN Leukocyte in the Reaction Sequence Leading to the Antigen-Induced Release of SRS-A^{rat}.—The implication of the mast cell in the elaboration of slow reacting substance of anaphylaxis is based upon the findings that, in guinea pig lung slices, the quantity of SRS-A^{gp} released appears proportional to the release of histamine (19) and that homologous γ_1-immunoglobulins prepare the tissue for the subsequent antigen-induced release of both mediators (1). A slow reacting substance has also been released in small quantities from isolated peritoneal mast cells (20). However, the ratio of histamine (mμg) to SRS (units) released from guinea pig lung was 5:1, whereas the ratio for isolated rat peritoneal mast cells was 240:1, suggesting that in the rat the mast cell may not be a major source of SRS-A (21). In the rat, the homocytotropic antibody–mediated release of histamine is not associated with the concomitant appearance of SRS-A^{rat} (1). The present experiments further corroborate the thesis that the mast cell is not a prerequisite for the antigen-induced

release of SRS-A^{rat} since depletion of free and tissue peritoneal mast cells by pretreatment with distilled water or a rabbit anti-rat mast cell antiserum had no effect on the subsequent immunologic release of SRS-A^{rat} in rats prepared with homologous hyperimmune antiserum (Table I). However, mast cell depletion did prevent the antigen-induced release of histamine following sensitization with antiserum containing homocytotropic antibody (Table II).

The role of the PMN leukocyte in the immunologic pathway leading to the formation and release of SRS-A^{rat} was initially investigated by studying the effects of leukocyte depletion. When leukopenia was induced in rats by pre-

TABLE XI

The Effect of Pretreatment with Nitrogen Mustard on the Antigen-Induced Release of Histamine and SRS-A^{rat} in the Rat Peritoneal Cavity

Modifying procedure	Sensitization with hyperimmune rat antisera	Sensitization with rat antiserum containing homocytotropic antibody
	SRS-A^{rat} release	Histamine release
	units/rat	*ug/rat*
Control	326	2.2
Nitrogen mustard	126	4.4

Each result represents a mean value for the three rats in that group. Rats were prepared with hyperimmune antiserum against BSA for the release of SRS-A^{rat}, and they were challenged intraperitoneally with BSA. Rats were prepared with rat antiserum containing homocytotropic antibody against Ea for the release of histamine, and they were challenged intraperitoneally with Ea. Nitrogen mustard (2.0 mg/kg intravenously) was injected 5 days before experiment. The mean peripheral WBC for control group releasing SRS-A^{rat} was 16,010/mm^3 and for control group releasing histamine, 15,970/mm^3, whereas the values for the nitrogen mustard–treated groups were 1020/mm^3 and 1070/mm^3 respectively.

treatment with nitrogen mustard (Table III), a marked suppression of the antigen-induced release of SRS-A^{rat} was observed. Although nitrogen mustard did not alter the peritoneal mast cells as assessed by morphologic examination and quantitative histamine analysis of the residual peritoneal cell population, it was important to establish the functional integrity of this cell population by their ability to participate in an immunologic reaction sequence. As described in Table XI, leukopenic rats retaining their peritoneal mast cells could be prepared for the antigen-induced release of histamine following sensitization with antiserum containing homocytotropic antibody, whereas the immunologic release of SRS-A^{rat} following preparation with hyperimmune antiserum was markedly suppressed. Thus, inhibition of SRS-A^{rat} release by nitrogen mustard can be attributed to the induced leukopenia and not to some nonspecific tissue unresponsiveness.

The depletion of leukocytes following pretreatment with nitrogen mustard involved lymphocytes as well as PMN leukocytes; thus, attempts were made to determine which cell type was the prerequisite cellular element for the immuno-

logic release of SRS-A^{rat}. Pretreatment of rats with a rabbit antiserum directed against rat thymic lymphocytes effected a greater than 80% reduction in the absolute lymphocyte count without significantly altering the population of PMN leukocytes, and no suppression of the antigen-induced release of SRS-A^{rat} was observed (Table IV). Conversely, pretreatment of rats with a rabbit antiserum directed against rat PMN leukocytes produced a profound neutropenia, moderate lymphopenia, and marked suppression of the antigen-induced release of SRS-A^{rat} (Table III). Rats depleted of circulating PMN leukocytes by pretreatment with a rabbit anti-rat PMN leukocyte antiserum were injected intravenously with 5.0 ml of pooled, washed, peritoneal exudate cells (> 70% PMN leukocytes) in an attempt to reconstitute their population of circulating leukocytes. Although only 25% restoration of the absolute peripheral PMN leukocyte count was achieved, this was associated with approximately a 20% restoration of the ability to prepare the rat with homologous, hyperimmune antiserum for the antigen-induced release of SRS-A^{rat}. Since further attempts to achieve complete repletion of the circulatng PMN leukocytes were unsuccessful, attention was directed towards demonstrating enhancement of SRS-A^{rat} release by a local increase in the number of PMN leukocytes. When the number of PMN leukocytes in the rat peritonal cavity was substantially increased by the induction of a peritoneal exudate in actively or passively sensitized animals a twofold to sevenfold increase in the subsequent antigen-induced release of SRS-A^{rat} was observed (Tables V and VI). Thus, the PMN leukocyte is implicated in the immunologic release of SRS-A^{rat} on the basis of inhibition of SRS-A^{rat} release by specific depletion of this cell type, partial restoration of SRS-A^{rat} release associated with partial repletion of the circulating PMN leukocytes, and enhanced SRS-A^{rat} release in the presence of an increased number of PMN leukocytes in the rat peritoneal cavity.

In preliminary experiments, the peritoneal exudates of actively sensitized rats were passively transferred to the peritoneal cavities of unsensitized rats, and, upon subsequent challenge with specific antigen, SRS-A^{rat} release was achieved. The activity of the donor exudate could not be easily attributed to passive sensitization by free antibody since the supernatant fluid following sedimentation of the cells did not prepare normal rats for SRS-A^{rat} release, and the interval of 5 min between transfer and antigen challenge was too brief a latent period to prepare the tissue for the immunologic release of SRS-A^{rat} (1). These observations are consistent with the essential role of the PMN leukocyte in the reaction sequence leading to the antigen-induced release of SRS-A^{rat}.

The precise role of complement in the immunologic release of SRS-A^{rat} has not been established. The complement system may be required for the generation of a leukotactic factor (22) or possibly enhancement of immune phagocytosis (23). A non–complement-dependent mechanism of inhibition has not been excluded, since crude cobra venom has been observed to release a slow

reacting material from perfused guinea pig lung (24) and thus could inhibit by depleting the substrate required for the formation and release of SRS-A^{rat}.

Separation of the Reaction Sequences Leading to the Antigen-Induced Release SRS-A^{rat} and Histamine.—The immunologic pathways to the antigen-induced release of histamine and SRS-A^{rat} in the rat peritoneal cavity can be separated in terms of the homologus immunoglobulins which prepare the tissues, the prerequisite cellular elements, and by pharmacologic inhibition studies. The immunoglobulin which prepares the rat for the antigen-induced release of SRS-A^{rat} is associated with the IgG class, while the homocytotropic antibody which sensitizes rat mast cells in vivo and in vitro for the antigen-induced release of histamine and serotonin represents an immunoglobulin class distinct from IgG, IgA, or IgM (1, 25). The elimination of the free and tissue peritoneal mast cells prevents the antigen-induced release of histamine following sensitization with antiserum containing the homocytotropic antibody of the rat (Table II) but in no way suppresses the antigen-induced release of SRS-A^{rat} (Table I). In contrast, the elimination of the circulating PMN leukocyte prevents the antigen-induced release of SRS-A^{rat} following preparation with hyperimmune rat antiserum (Table III) but has no effect on the antigen-induced release of histamine following sensitization with antiserum containing homocytotropic antibody (Table XI).

Finally, diethylcarbamazine administered intravenously or intraperitoneally suppresses the antigen-induced release of SRS-A^{rat} following preparation with hyperimmune antiserum but has no effect on the antigen-induced release of histamine after sensitization with antiserum containing the homocytotropic antibody. As expected, this agent has no effect on the lesion of passive cutaneous anaphylaxis mediated by homocytotropic antibody which is fully suppressed by a combination of histamine and serotonin antagonists (2). Preliminary studies indicate that only a combination of diethylcarbamazine, mepyramine, and methysergide will abolish the lesion of passive cutaneous anaphylaxis mediated by homologous hyperimmune antiserum (17); the requirement for such a combination indicates the complexity of this lesion and the precise contribution by the pathway to SRS-A^{rat} release remains to be determined. It is apparent from the studies concerned with the antigen-induced, intraperitoneal release of SRS-A^{rat} that diethylcarbamazine blocks at some point subsequent to antigen-antibody interaction and prior to the elaboration of SRS-A^{rat} (17). Thus, inhibition of the antigen-induced release of SRS-A^{rat} by diethylcarbamazine is associated with tissue "desensitization" because the antigen utilizes the available antibody in initiating a reaction that is then blocked at some subsequent step in the reaction sequence (Table IX).

SUMMARY

The polymorphonuclear leukocyte appears to be an essential cellular prerequisite for the antigen-induced release of SRS-A^{rat} in the peritoneal cavity of

rats prepared with homologous, hyperimmune antisera. Depletion of PMN leukocytes is associated with a marked suppression of SRS-A^{rat} release, whereas depletion of circulating lymphocytes or peritoneal mast cells does not influence the antigen-induced release of SRS-A^{rat}. A local increase in the number of PMN leukocytes produced by the induction of a peritoneal exudate was associated with an enhanced release of SRS-A^{rat}.

A distinct difference in the cellular requirements for the antigen-induced release of histamine and SRS-A^{rat} in the rat was observed. Homocytotropic antibody–mediated histamine release could be achieved in leukopenic rats but not in mast cell–depleted animals. Conversely, SRS-A^{rat} release was suppressed in leukopenic rats but was unaffected by mast cell depletion.

Diethylcarbamazine inhibited the antigen-induced release of SRS-A^{rat} following preparation with homologous, hyperimmune antisera but did not interfere with homocytotropic antibody–mediated histamine release. In preventing SRS-A^{rat} release, diethylcarbamazine did not interfere with antigen-antibody interaction since desensitization of tissues was possible in the presence of this inhibitor. This observation is consistent with the view that diethylcarbamazine inhibits the reaction sequence leading to the formation and release of SRS-A^{rat} at some step subsequent to antigen-antibody interaction.

These studies support the view that the immunologic pathways leading to the release of SRS-A^{rat} and histamine in the rat are distinctly different in terms of the immunoglobulins involved, the cellular prerequisites, and the effective pharmacologic inhibitors.

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