

Specific passive desensitization

VI. By specific homologous and heterologous antisera

VII. In actively sensitized guinea pigs

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Homologous and heterologous anti-CEA sera provided complete specific passive desensitization (protection) against intravenous doses of antigen in 19 of 24 passively sensitized guinea pigs. This is an unusually low percentage of effective specific passive desensitization.¹⁻³ All of the twelve control guinea pigs, which were given saline, had fatal or near-fatal reactions to the intravenous antigen (Experiment VI). Specific passive desensitization with anti-CEA rabbit serum protected ten actively sensitized pigs against the intravenous doses of CEA which produced severe anaphylactic reactions in the ten control pigs which had been given normal rabbit serum (Experiment VII). In neither experiment, however, did specific passive desensitization prevent reactions to aerosols of antigen. All of the sensitized and "desensitized" control guinea pigs reacted to the aerosols. The fact that specific passive desensitization is systemic but not local³ is reconfirmed by these experiments. Also, this study increases the necessity to confirm the reports of successful clinical passive desensitization with human blocking antibody.⁴

Specific passive desensitization¹⁻³ with anti-CEA rabbit serum will prevent reactions to intravenous doses of antigen which routinely elicit fatal or near-fatal anaphylactic reactions in passively sensitized guinea pigs. The doses of antiserum employed have ranged from 10 to 33 multiples of the amount which regularly sensitizes guinea pigs passively. The antiserum desensitizes effectively when given into the vein, heart, or abdomen, or when given subcutaneously.² The intraperitoneal route is preferred because it is convenient and desensitization occurs overnight and lasts for 5 or 6 days.

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An unexpected feature of specific passive desensitization with heterologous antiserum was its failure to protect against inhaled antigen in spite of its predictable protection against intravenous antigen.³ This posed the important question of the difference between homologous and heterologous antisera. Bernton, Chambers, and Querry¹ reported effective relief of pollenosis by injections of human antiragweed serum concentrates from normal subjects who had been immunized with ragweed extract. Previous passive desensitization experiments had been done with passively sensitized guinea pigs and heterologous rabbit antisera.¹⁻³

The following experiments were designed to determine whether or not passive desensitization, with homologous or heterologous antisera, would prevent reactions to both inhaled and intravenously administered antigen in passively and actively sensitized guinea pigs.

The results show that neither homologous nor heterologous antisera prevented reactions to aerosols but that both antisera prevented reactions to (i.e., passively desensitized the animals against) normally fatal intravenous injections of antigen. The significance of these findings will be discussed.

MATERIALS AND METHODS

The aerosols were administered in a rectangular box, 5 by 8 by 10 inches, with glass top and sides, loosely fitted to a grooved wooden base to permit escape of air and aerosol fog. Two DeVilbiss No. 40 nebulizers were inserted into the aerosol chamber through small holes in the wooden base. Compressed air, filtered through water, was used to nebulize the various dilutions of egg white, which were used as the aerosol antigen. A dense aerosol fog was maintained by a continuous flow from the nebulizers.

Egg white was used instead of crystalline egg albumin (CEA) because of the large quantities employed, the ease of preparation, and because it was known to be an effective challenging agent. It was assumed, for purposes of these experiments, that the minor antigens in egg white would not play important roles.

In previous experiments here and elsewhere,⁵ hyperventilation from excitement and, what seemed to be, a conditioned reflex had made it difficult to interpret minor reactions to aerosols of specific antigens. This was effectively avoided by using an aerosol of normal saline for 10 minutes before each exposure to the specific egg white fog. Two to four pigs were challenged at the same time. They were put into the fog-filled chamber quickly. The onset of initial symptoms was timed from the insertion of the pigs. They were removed from the aerosol immediately upon the appearance of cough, dyspnea, or scratching of the nose, to lessen the chance of anaphylactic death. In spite of this precaution, there were three near-fatal reactions.

Experience had shown that most passively sensitized guinea pigs reacted within 10 minutes or less to an aerosol of a 1:1,000 or 1:100 concentration of egg white. The 1:1,000 concentration was used initially. If the pig did not react within 10 minutes, it was exposed immediately to a 1:100 concentration. A pig which did not react to the 1:100 concentration of egg white aerosol during this

initial test for sensitization was not considered satisfactory for the experiment.

After the desensitizing procedures, this sequence of aerosol challenges was repeated. If the pigs did not react to the weaker 1:1,000 or 1:100 aerosols, they were exposed to the stronger 1:20 concentration of egg white aerosol.

The intensity of the reactions to the various aerosols is described in the footnotes of the tables. The specific or nonspecific passive desensitizing intraperitoneal injections of the antiserum, normal serum, or normal saline were given shortly after recovery from the first reactions to the aerosols. The intravenous challenges with 0.2 mg. CEA were given shortly after recovery from the second aerosol egg white challenges.

Three criteria were used for combining groups of pigs in the tables: (1) the first concentration of aerosol which elicited a reaction; (2) the concentration of the egg white antigen which elicited the second aerosol reaction; and (3) the presence or absence and severity of the reactions to the intravenous CEA challenge. This is well illustrated in the second, third, and seventh groups of pigs in Table I and the first group in Table II.

Pools of anti-CEA guinea pig and anti-CEA rabbit sera were obtained from

Table I. Specific passive desensitization of passively sensitized guinea pigs* by homologous and heterologous antisera

Sensitized guinea pigs (No.)	Aerosol challenge			Passive desensitization	Interval (hours)	Aerosol challenge			Reaction to 0.2 mg. CEA, intravenously†
	Egg white concentration†	Duration (minutes)	Reaction‡			Egg white concentration†	Duration (minutes)	Reaction	
Heterologous serum§									
1	1:1,000	6	2+	5.0 ml.	24	1:1,000	10	2+	None
3	1:1,000	6-9	2+	anti-CEA	24	1:100	1-2	2+	None
5	1:100	1-4	2+	rabbit	24	1:100	1-3.5	2+	None
2	1:1,000	4.5-7	2+	serum,	24	1:1,000	10	2+	AD
1	1:1,000	9	2+	intraperitoneally	24	1:100	1	2+	AD
Homologous serum									
1	1:1,000	3	2+	5.0 ml.	24	1:100	0.5	2+	None
7	1:100	1-3	2+3+	anti-CEA	24	1:100	1-7.5	2+3+	None
1	1:100	1	3+	guinea	24	1:1,000	10	2+	None
1	1:100	8	2+	pig	24	1:20	2	2+	None
1	1:1,000	9	2+	serum,	24	1:100	2	2+	AD
1	1:100	1	2+	intraperitoneally	24	1:1,000	9	2+	AD
Controls									
8	1:1,000-1:100	0.5-9	2+3+	Normal saline,	24	1:1,000-1:100	2-10	2+3+	AD
4	1:1,000-1:100	0.5-9	2+3+	intraperitoneally	24	1:100	2-3.5	2+3+	3+

*Passively sensitized with 0.1 or 0.15 ml. anti-CEA rabbit serum.

†Challenged with antigen (egg white) aerosol one month after sensitizing doses (see text).

‡Reactions: 1+, Mild definite dyspnea

2+, Moderately severe

3+, Severe dyspnea, convulsions, lying on side, recovered

AD, Anaphylactic death.

§Desensitizing antiserum dose one hour after recovery from reaction to aerosol.

Table II. *Specific passive desensitization of actively sensitized guinea pigs**

Sensitized guinea pigs (No.)	Aerosol challenge†			Passive desensitization	Interval (hours)	Acrosol challenge			Reaction to 0.2 mg. CEA, intravenously‡
	Egg white concentration	Duration (minutes)	Reaction			Egg white concentration	Duration (minutes)	Reaction	
Antiserum									
6	1:100	1.5-3.0	2+	5.0 ml.	24	1:100	1.0-2.0	2+	None
1	1:100	1.5	2+	Anti-CEA	24	1:1,000	7.0	2+	None
1	1:100	2.0	3+	rabbit	24	1:100	2.0	2+	None
1	1:1,000	3.5	2+	serum,	24	1:100	0.5	2+	None
1	1:1,000	5.5	2+	intraperitoneally§	24	1:100	0.5	2+	1+
Controls									
1	1:1,000	4.5	2+	5.0 ml.	24	1:1,000	7.5	2+	AD
2	1:1,000	8.0-9.0	2+	Normal	24	1:100	2.0	2+	AD
2	1:100	2.0	2+	rabbit	24	1:100	2.0-3.0	2+	AD
2	1:1,000	5.0-6.0	2+	serum,	24	1:100	1.0-2.0	2+	3+
1	1:100	2.0	3+	intraperitoneally	24	1:100	3.0	3+	3+
1	1:100	2.0	2+		24	1:100	3.0	2+	2+
1	1:1,000	2.0	2+		24	1:100	6.0	2+	2+

*Actively sensitized with 0.2, 0.6, and 1.0 mg. CEA on consecutive days.

†Challenged with antigen (egg white) aerosol one month after sensitizing doses (see text).

‡Reactions: 1+, Mild definite dyspnea

2+, Moderately severe

3+, Severe dyspnea, convulsions, lying on side, recovered

AD, Anaphylactic death.

§Desensitizing antiserum dose one hour after recovery from reaction to aerosol.

||Control normal serum dose one hour after recovery from reaction to aerosol.

animals which were immunized with repeated intraperitoneal injections of CEA until they developed ring-test precipitin titers of 1:1,000 or stronger.

The animals were sensitized passively with intraperitoneal doses of 0.1 or 0.15 ml. anti-CEA rabbit serum. In the second experiment the pigs were sensitized actively by 0.2, 0.6, and 1.0 mg. of CEA, intraperitoneally, on three consecutive days.

Specific passive desensitization by specific homologous and heterologous antisera: Experiment VI

Thirty-six guinea pigs were sensitized passively with intraperitoneal doses of 0.1 or 0.15 ml. of anti-CEA rabbit serum. The next day they were challenged with an aerosol of egg white (the antigen). All the pigs reacted to the first or second challenge within 10 minutes. Approximately one hour after recovery from those reactions, 12 of the animals were given intraperitoneal desensitizing doses of 5.0 ml. anti-CEA rabbit serum. Twelve others were given equal intraperitoneal doses of anti-CEA guinea pig serum. The other 12, the controls, were given 5.0 ml. of normal saline, intraperitoneally. Twenty-four hours later all of the animals were challenged with and reacted to the 1:1,000 or 1:100 concentration of egg white aerosol. Note that all of the control and experimental animals reacted similarly but not identically to the aerosols.

The 36 guinea pigs were challenged intravenously with 0.2 mg. CEA about an hour after recovery from the second aerosol reactions. Ten of the pigs

desensitized passively with the anti-CEA guinea pig serum were completely desensitized (protected) against the 0.2 mg. intravenous dose of CEA, which caused violent reactions in the control pigs; two died in anaphylactic shock. Nine of the pigs desensitized with the anti-CEA rabbit serum were completely protected (desensitized) against the same intravenous dose of CEA. Three died in anaphylactic shock. All of the control pigs died or nearly died (Table I) when given 0.2 mg. of CEA, intravenously.

Comment. It is obvious that, in the doses used, heterologous (rabbit) and homologous (guinea pig) anti-CEA sera provided complete passive desensitization against shocking intravenous doses of antigen (CEA) in 19 of 24 passively sensitized animals. But neither of the sera prevented reactions to aerosols of the antigen, which confirms previous observations.³

The failure to passively desensitize 5 of the 24 animals is unusual.¹⁻³ One possible explanation of this failure is that the intraperitoneal desensitizing doses of antisera could have been injected into the intestinal tract. This and other possibilities should be studied.

Four of the 5 animals, which were not protected, had been sensitized passively with 0.1 ml. of the antiserum. The other animal which reacted fatally to the intravenous CEA had been sensitized with 0.15 ml. antiserum. No significance has been attached to this observation.

Specific passive desensitization of actively sensitized guinea pigs: Experiment VII

Twenty guinea pigs, actively sensitized, were challenged one month later with aerosols of a 1:1,000 concentration of egg white. Those which did not react within 10 minutes were challenged with the 1:100 egg white dilution. The intensities of the anaphylactic responses and the time required to elicit each of them were recorded. Ten of the animals were given 5.0 ml. of pooled precipitating anti-CEA rabbit serum, intraperitoneally, shortly after recovery from the aerosol reactions. The other 10, the controls, were given 5.0 ml. of pooled normal rabbit serum. The only difference between the control and the experimental animals was this use of normal, instead of anti-CEA, rabbit serum in the control pigs.

The next day, the guinea pigs were challenged again with the 1:1,000 or 1:100 egg white aerosols. Reactions were considered negative unless they appeared within 10 minutes. The pigs were removed from the aerosol at the first definite sign of a reaction. They were challenged intravenously with 0.2 mg. CEA, the predetermined fatal or near-fatal dose, approximately one hour after recovery from the second reactions to the aerosols.

The results are summarized in Table II. Note that with 6 of the 10 experimental pigs, the only difference between the two aerosol exposures was a slight variation in the duration of the exposure before the reactions began. The other 4 animals had other minor differences. For example, one, which initially reacted only to the 1:100 egg white concentration, reacted to the 1:1,000 antigen aerosol after the desensitizing dose of antiserum. One had a near-fatal (3+) reaction to the 1:100 dilution of aerosol in the predesensitization period but had only a 2+ reaction after receiving the desensitizing dose of antiserum. Two of

the pigs reacted to the 1:1,000 dilution during the first egg white aerosol challenge but required a 1:100 concentration to elicit a comparable reaction after the desensitizing dose of antiserum.

One of the 10 experimental pigs had a definite but mild reaction to the intravenous challenge with CEA. The other 9 had no reaction whatever. In short, intraperitoneal anti-CEA rabbit serum provided virtually complete protection (specific passive desensitization) against normally shock-producing intravenous doses of CEA. On the other hand, intraperitoneally administered normal rabbit serum provided no protection except, perhaps, the slightly less-than-anticipated intensity (2+) of the reactions in 2 of the 10 control pigs. Heterologous normal rabbit serum usually provides nonspecific passive desensitization in an appreciable, but unpredictable, percentage of sensitized guinea pigs.¹⁻³

There was no significant difference between the pre- and postpassive desensitization reactions to the aerosols, when experimental and control animals were compared. However, in contrast to the protection of the experimental group, 5 of the control animals died of anaphylactic shock, 3 nearly died (3+ reactions), and the other 2 had moderately severe (2+) reactions following intravenous injection of 0.2 mg. CEA.

DISCUSSION

The failure of the homologous antiserum in Experiment VI to prevent reactions to aerosols of antigen was disappointing for two reasons. First, it failed to provide experimental confirmation of the principle of clinical specific passive desensitization against inhaled antigens.⁴ Second this experiment failed to explain the different effects of specific passive desensitization in guinea pigs and man. The failure of heterologous anti-CEA rabbit serum to prevent reactions to aerosol, in actively sensitized guinea pigs in Experiment VII, was anticipated from previous results³ in passively sensitized animals. Two, but only two, of the differences between specific passive desensitization in the guinea pig and in man are thus resolved by these experiments.

Two obvious differences between these and the clinical experiments remain. One is the intangible species differences between man and the guinea pig. The other is that concentration of antigen in the egg white aerosol is much greater and is much more readily available than the small amounts of bound antigen in inhaled pollen grains.

These guinea pig experiments seem to cast sufficient doubt upon the validity of the clinical experiments to justify repeating them with the use of rigid clinical controls. Perhaps the dispute over the protecting role of the "blocking antibody" could be clarified by controlled clinical experiments of this sort. The outcome of such experiments will determine whether specific passive desensitization should be added to or deleted from our armamentarium of specific therapy for inhalant hay fever and asthma.

The solution of this problem has become increasingly important since Patterson and co-workers⁶ reported that canine "blocking" antibody prevented reactions to aerosols of ragweed extract in three passively sensitized dogs.

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