A STUDY OF THE CORRELATION BETWEEN MINIMAL SENSITIZING AND BOOSTER DOSES OF THE TISSUE AND SERUM ANTIGENS UNDER DIFFERENT CONDITIONS OF SENSITIZATION

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As the anaphylactic reaction with desensitization is currently being employed in different experiments, it is of interest to determine its sensitivity (particularly in relation to tissue antigens), the regularity with which the threshold booster doses change as the sensitizing doses are changed; and finally to study all the changes which these indices undergo under the influence of desensitization.

These problems were investigated by V. I. Ioffe et al. [1,2,3]. In their study of the sensitivity of the reaction, they took as a criterion of a positive result, not the anaphylactic phenomena of different degrees of intensity, but the death of the animal.

That is why, in their work, the minimal booster dose was reduced to the minimal lethal dose, i.e., it was grossly overestimated and did not, therefore, reflect the sensitivity of the reaction.

A. M. Gardash'yan [1] showed that after being desensitized to a "strong" antigen, the animals reacted to a "weak" antigen in a sensitizing dose 20,000 times smaller than that of the "strong" antigen. Moreover, 0.0005 ml (0.035 mg of protein) was the minimal amount of serum antigen at which the booster dose was equal to the sensitizing dose. These findings were similar to our own (see below).

A. M. Gardash'yan and Z. A. Avenirova [2] showed that under sensitization with a 3-component mixture, desensitization to 1 mg of rabbit γ-globulin revealed the other components: γ-globulin of a donkey (sensitizing dose 0.09 mg) and the Brown-Pierce tumor extract (sensitizing dose 0.009 mg of protein). However, as desensitization to all the serum antigens of a rabbit (which might conceivably have been in the Brown-Pierce tumor extract) was not carried out, the reaction to this extract could have been provoked not by tissue but by serum antigens.

Epshtein and Kligman [6] showed that when human skin was treated with a mixture of 2 allergens, one of which was 100 times stronger and used in a concentration 100 times greater than the other, the percentage of sensitization achieved with the weaker antigen was 3 times less than when it alone was used. There was no diminution in sensitivity under sensitization with a mixture of 2 allergens of equal strengths. Similar results were obtained by A. Fenyves [7]. When guinea pigs were sensitized with a mixture consisting of equal amounts of protein and horse γ-globulin, the sensitizing effect of the first diminished and that of the second remained the same both in mixtures with protein and with bull γ-globulin. The author concludes that, in the combined action of antigens, the qualitative and not the quantitative correlation is of decisive importance.

The present study carried out at the suggestion of L. A. Zil'ber had the following aims:

1) To determine the laws governing the changes in the minimal booster dose under successive increases in the sensitizing doze.

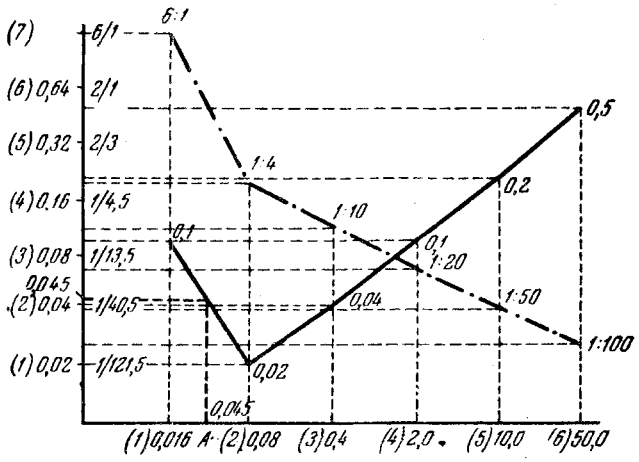
2) To determine the threshold of the sensitizing dose and the threshold of the booster dose of tissue antigens.

3) To determine whether desensitization to serum antigen injected together with tissue antigen but in amounts 100-200 times greater reduces the sensitivity of the reaction in relation to tissue antigen (i.e., whether under these conditions the thresholds of sensitizing and booster doses of tissue antigen undergo a change).

We studied two pairs of antigens: related (human liver and γ-globulin) and unrelated in the antigenic sense (mouse liver and human γ-globulin).

METHOD

Dry human and horse γ-globulin were obtained from the I. I. Mechnikov Institute of Vaccines and Sera. Antigens from mouse liver and human liver were obtained in the usual way, that is, by thoroughly washing the organs (mouse liver was washed in vivo, human liver in vitro), homogenizing in a blender, extracting the protein at pH 9, following a double precipitation of the protein at pH 4.5, by dissolving the precipitate at pH 7.2 and conserving in Merthiolate. The amount of protein was determined by the Kjeldahl-Lourie methods, the antigens were titrated on protein. All the preparations were checked before sensitization for sterility and toxicity.



Correlation between sensitizing and minimal booster doses of horse γ-globulin. A denotes the point at which the sensitizing dose is equal to the minimal booster dose (0.045 mg of protein). Along the X axis, sensitizing doses; along the Y axis, on the left, minimal booster doses (all the doses are given in milligrams of protein); on the right, the ratio between minimal booster and sensitizing doses. All the magnitudes are given on a logarithmic scale (the figures in brackets are absolute magnitudes). —— Minimal booster doses; - - - - - - ratio of minimal booster doses to sensitizing doses.

Guinea pigs (weighing 240-330 g) were sensitized subcutaneously in the inguinal region, desensitizing and booster injections being given 28-35 days after sensitization. The booster dose of tissue antigens was 1 mg, thus when used together with serum antigens for sensitization, not less than 1-2 mg of the latter were required for desensitization. In those tests in which the sensitizing dose of serum antigen was greater than 1 mg every 1-2 sensitizing doses were followed by desensitization. After a number of preliminary tests the following scheme of desensitization to human γ-globulin was adopted: first day - subcutaneously, in the morning one sensitizing dose (not less than 1 mg), in the evening two sensitizing doses (not less than 2 mg); second day - intraperitoneally, in the morning two sensitizing doses, in the daytime four sensitizing doses, in the evening eight sensitizing doses; Third day - intravenously 1-2 sensitizing doses (not less than 1-2 mg).

With such a procedure, 1-2 intravenous injections were sufficient for full desensitization to serum antigen (in conditions where the sensitizing dose did not exceed 6-10 mg). Booster injections were given intravenously 40-80 minutes after checking the completeness of desensitization. The reaction was determined by the usual four point system, i.e., only an absolutely distinct reaction was regarded as positive (+), i.e., one marked by intense, recurrent scratching accompanied by a disturbed general condition, ruffled appearance, unrest and cyanosis of the feet. Stronger reactions (+++ and more intense ones) occurred infrequently as the animals were sensitized only with very small doses (hundred thousands of a milligram of protein).

RESULTS

To establish the correlation between the minimal booster and sensitizing doses, each group of guinea pigs (4-6 animals) was sensitized with one dose of horse γ-globulin, moreover each subsequent dose was five times greater than the preceding one (from 0.016 to 50 mg of protein, see figure). The minimal booster dose in each group was determined. It was found that with a sensitizing dose of 0.016 mg the threshold of the booster dose was 0.1 mg, i.e., 6 times greater; when the sensitizing dose was 0.08 mg, the threshold of the booster dose decreased to 0.02 mg, and then as the sensitizing dose was increased, it also increased.

As in these conditions the sensitizing dose underwent a fivefold increase and the threshold of the sensitizing dose increased only by 2-2½ times, an increase in the sensitizing dose therefore reduced the correlation between the booster and the sensitizing doses.

The figure shows that the point at which the sensitizing dose is equal to the minimal booster dose is 0.045 mg (for horse γ-globulin). Thus for tissue proteins which are weaker anaphylactogens than horse γ-globulin, this point would be shifted on the graph to the right, and it can be assumed that its magnitude will be as many times greater 0.45 - 0.05 mg as the tissue protein is weaker than horse γ-globulin (see below).

TABLE 1. Minimal Sensitizing and Minimal Booster Doses of Antigen of Mouse Liver Injected Together with Human γ-Globulin after Desensitization to the Latter

	No. of guinea pigs	Sensitizing dose	Booster dose	Result			Threshold of sensitizing dose	Threshold of booster dose
				-	+	++		
Sensitization with mouse liver antigen; booster injection with mouse liver antigen	6	0.001	1	6	-	-		
	6	0.003	1	5	1	-	0.01	
	3	0.01	1	-	2	1		
	3	0.03	1	-	3	-		
	4	0.03	0.6	4	-	-		1
Sensitization with mouse liver antigen and human γ-globulin (in the ratio of 1:200). Desensitization with human γ-globulin (1.5-6mg); booster injection with mouse liver antigen	3	0.003 + 0.6	1	3	-	-	0.03	
	3	0.01 + 2.0	1	3	-	-		
	3	0.03 + 6.0	1	-	3	-		
	2	0.03 + 6.0	0.6	-	2	-		0.6
	2	0.03 + 6.0	0.3	2	—	-		

Note. All the doses are given in mg of protein.

TABLE 2. Minimal Sensitizing and Minimal Booster Doses of Human Liver Antigen Injected with Human γ-Globulin after Desensitization to It.

	No. of guinea pigs	Sensitizing dose	Booster dose	Result			Threshold of sensitizing dose	Threshold of booster dose
				-	+	++		
Sensitization with human liver antigen. Desensitization with human serum (2 mg). Booster injection of human liver antigen	3	0.002	0.6	3	-	-		
	3	0.006	0.6	2	-	1	0.02	
	3	0.02	0.6	1	-	2		
	2	0.06	0.6	-	2	-		
	2	0.06	0.3	2	-	-		0.6
Sensitization with human liver antigen and human γ-globulin (in the ratio of 1:100) Desensitization with human serum (2 mg) and human γ-globulin (1-6 mg). Booster injection of human liver antigen.	3	0.006 + 0.6	0.6	3	-	-	0.06	
	4	0.02 + 2.0	0.6	2	1	1		
	3	0.06 + 6.0	0.6	-	1	2		0.6
	5	0.06 + 6.0	0.3	4	1			

Note. All the doses are given in mg of protein.

To determine the threshold sensitizing and booster doses, guinea pigs were sensitized by a series of doses which increased in a threefold geometric progression so calculated that the initial doses would be too small for sensitization (in preliminary tests it was established that liver antigen in a dose of 0.001 mg of protein when the booster dose was 1-2 mg did not result in sensitization).

The animals were sensitized with mouse liver antigen in doses of 0.001, 0.003, 0.01 and 0.03 mg of protein. Each dose was administered to 3-6 animals. Desensitization was unecessary as the mouse liver had been thoroughly washed in sterilized water to remove sera antigens and introduced through the v.porta and v.inferior. The minimal sensitizing dose of mouse liver (when the booster dose was 1 mg) was 0.01 mg, whereas the minimal booster dose (when the sensitizing dose was 0.01-0.03 mg) was 1 mg (Table 1).

In combined sensitization with mouse liver antigen ("weak" antigen) and human γ-globulin ("strong" antigen) in a ratio of 1:200 (see Table 1) after desensitization to the latter the threshold of the sensitizing dose of mouse liver antigen was 0.03 mg (i.e., there was a threefold increase) whereas the threshold of the booster dose even decreased somewhat to 0.6 mg.

To study the relation of a pair of related antigens (human liver and γ-globulin) we first determined the threshold doses of human liver antigen by administering it alone. Before injecting the booster dose desensitization was carried out for every 1.5-2 mg of human serum so as to exclude the effect of serum antigens (Table 2).

The threshold of the sensitizing dose was 0.02 mg (when the booster dose was 0.6 mg) and the threshold of the booster dose was 0.6 mg when the sensitizing dose was in the range of 0.02-0.06 mg.

When human liver antigen and γ-globulin in a ratio of 1:100 were used for sensitization, desensitization was carried out with a mixture of the same dose of human serum, as in the preceding test, and human γ-globulin (Table 2). The result was similar to that obtained with an unrelated pair of antigens: the threshold of the sensitizing dose showed a threefold increase (0.06 mg), the threshold booster dose did not change (0.6 mg).

It should be emphasised that if, for horse γ-globulin in a sensitizing dose of about 0.05 mg of protein the minimal booster dose becomes equal to the sensitizing dose, so, for tissue antigen (e.g., from human liver) in approximately the same sensitizing dose (0.06 mg) the corresponding minimal booster dose is 0.6 mg, i.e., 10 times greater than the sensitizing dose. Consequently, human liver antigen is 10 times weaker than horse γ-globulin. Thus, if for horse γ-globulin, 0.05 mg of protein represents the level at which the minimal booster dose becomes equal to the sensitizing dose, then for tissue antigen this dose will be approximately 10 times greater, i.e., 0.5-0.6 mg of protein. For the investigator this is of practical significance: if in a test the booster dose of tissue antigen is equal to the sensitizing dose then one may assume that to produce a reaction in a guinea pig under these conditions the dose must be not less than 0.5-0.6 mg of protein.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
