MICROBIOLOGY AND IMMUNITY

THE PROBLEM OF DETECTION OF TISSUE ANTIGENS

COMMUNICATION II. ON THE CONDITIONS OF DETECTION OF COMPONENTS OF ANTIGENIC MIXTURES BY ANAPHYLAXIS

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When using the anaphylactic reaction to distinguish tumor antigens from the antigens of normal tissues, it is necessary after sensitizing the animal to a tumor preparation to desensitize it to normal antigens, and only after this, proceed with the test injection of the tumor preparation. Therefore two questions arise: 1) how to carry out the desensitization to be confident of its completeness; 2) how does such desensitization with respect to the predominant antigen affect the subsequent reaction of the animal to a "minor" antigen, i.e., one making up only an insignificant portion of the antigenic complex?

To answer the first question it is necessary to ascertain the relation between the shocking and desensitizing dose.

METHODS

For this purpose guinea pigs were sensitized to horse serum, which we injected in the amount of 0.5 ml per 100 g body weight (or 120 mg protein for a 300 g animal). After 3 weeks we titered out the minimum dose of antigen which when injected (intravenously) would produce fatal anaphylactic shock. This proved to be 0.01 ml of serum per 100 g body weight. Proceeding from this, experiments were carried out to investigate the conditions of desensitization, the results of which are presented in the table.

RESULTS OF EXPERIMENTS

From the table it may be seen that following the (intravenous) injection of antigen in the amount of 3-4 times the fatal dose, three out of 19 guinea pigs succumbed; the majority of the remainder responded with a pronounced anaphylactic reaction after which they showed themselves desensitized to 3 and even to 5 fatal doses. However, animals into which we injected 10 shocking doses to test the completeness of desensitization, gave a definite reaction, and pigs receiving 30 and more doses succumbed.

Only after double desensitization (not less than 5 shocking doses) did the insensitivity reach 30-35 doses, which contained 60-70% of the amount of antigen used for the sensitization.

It must be mentioned that about a day after double desensitization the sensitivity of the pigs was noticeably restored, and they reacted to 5 shocking doses.

In another analogous experiment the sensitization was carried with a so-called "weak" antigen, i.e., a preparation of nucleoprotein from horse liver in the amount of 1 ml, i.e., 20 mg protein for a pig weighing 300 g. In this case the shocking fatal dose was relatively large – from a half to all the sensitizing dose.

On desensitizing with half of the lethal dose a definite anaphylactic reaction was observed; such a reaction was observed in 5 of 8 guinea pigs into which we injected 1/5 of the shocking dose. All of these animals after

Relation Between Shocking and Desensitizing Doses

No. of pig	Reaction to desensitization with 3-4 MLD	Investigation of completeness of desensitization by various dosages			Repeated attempt at desensitization 30 MLD	Reaction after 24 hours to 5 MLD
		3-5 MLD	10 MLD	30 MLD and more		
1	Died					
2	"					
3	"					
4	+++			Died		
5	+++					
6	+++					
7	+++		++		−	++
8	+++	±			−	++
9	++		++		−	++
10	++	−			−	++
11	++	−			−	++
12	++	+			−	
13	++	−			++	
14	++	−			+	
15	++	+			+ (++)	
16	+ (++)	±			+	
17	+ (++)	±			+ (++)	
18	+ (++)	+			+	
19	+	+			+ (++)	

Explanation: +++ pronounced anaphylactic shock with severe respiratory difficulties, loss of ability to stand, convulsions; ++ definite anaphylactic reaction with pronounced symptoms of bronchial spasm (convulsive coughing); + anaphylactic symptoms in the form of scratching, sneezing, running motions, without definite symptoms of bronchial spasm; ± negligible reaction; − no symptoms.

this showed themselves to be desensitized to a repeated injection of half of the shocking dose, but on the third injection of antigen in the amount of 2-4 lethal doses 3 pigs out of 9 reacted. In addition it was also noticed in this experiment that, even after triple desensitization, among a part of the guinea pigs after 24 hours sensitivity to the antigen was restored, and they reacted to a single shocking dose.

For investigation of the second question – the effect of complete desensitization to the predominant major components of the antigen complex on the sensitivity of the animals to minor components – guinea pigs were sensitized with a mixture of human serum and horse serum (two "strong" antigens), mixed in the proportions of 50 : 1 (0.5 ml human serum to 0.01 ml horse serum per 100 g body weight). The shocking dose to the "minor" antigen, i.e., horse serum, amounted, for animals not subjected to desensitization, to 0.01 ml per 100 g of body weight. After profound desensitization to the "major" antigen (in this case, to horse serum) the animals did not react to this dose of antigen or even to three times this amount, and only with doses increased 6-10 times (0.06-0.1 ml per 100 g of animal) was it possible to record a definite reaction.

Another series of experiments gave analogous results; in these the animals were sensitized with a mixture of horse and rat serum, also in the proportions of 50 : 1, but in this case the "minor" antigen was rat serum, the "major," horse serum. The shocking dose of the "minor" antigen in this case amounted to 0.025 ml per 100 g of body weight, and for the "major" antigen amounted to 0.01 ml. After triple desensitization of the animals to the "major" antigen with increasing doses, beginning with 3/4 of the shocking dose of the antigen in question, loss of sensitivity to the horse serum was achieved. During this the sensitivity to the "minor" antigen also decreased, so that a definite anaphylactic reaction could be obtained only by the injection of 0.1 ml and more of rat serum.

In a third series of experiments guinea pigs were sensitized to two "weak" antigens, i.e., to nucleoprotein from organs of horse and rat; these were mixed in the proportions of 50 : 1 (horse nucleoprotein in the amount of 20 mg of protein and rat in the amount of 0.04 mg of protein, for an animal of 300-350 g weight). The shocking (lethal) dose to the "major" antigen in this case reached 1 ml of the preparation (20 mg of protein).

On the first desensitization with 0.25 ml the guinea pigs gave a definite anaphylactic reaction (++); even on the injection of 0.1 ml such reactions were observed in 5 out of 8 animals. Repeated injection of 0.25 ml did not produce a reaction; no reaction was observed either in pigs into which in a third of the experiments we injected 1 ml of the preparation, but at a dose of 2 ml, 2 pigs out of five reacted (+ and ++). In respect to sensitivity to the "minor" antigen, those pigs which were not subjected to desensitization to the "major" antigen died on the injection of 1-2 ml of rat nucleoprotein. A dose of 1.5-2 ml elicited severe shock and death of the animals within a few minutes, but on the injection of 1 ml of the preparation the animals responded by slow shock; death occurred in the course of 8-10 hours. Smaller doses (0.5 and 0.25 ml) elicited a definite anaphylactic reaction (++) which led to the death of the animal.

For guinea pigs subjected to triple desensitization to horse nucleoprotein, the lethal dose of rat antigen did not increase, and they succumbed following the injection of 1-2 ml of the corresponding preparation. But not all animals reacted to smaller doses (0.5 and 0.25 ml).

DISCUSSION

From the foregoing material it follows that sensitizing animals to a complex antigen and employing desensitization from removal of the sensitivity to one component, it is necessary, in the first place, to carry out desensitization starting with a shocking dose of the component in question, and, in the second place, establish the completeness of desensitization to not less than 10 shocking doses (preferably to values larger than this). In such work one must consider that repeated desensitization to the antigen which appears to predominate quantitatively leads to a decrease in the sensitivity of the animals to the "minor" antigen, which forces an increase in the dose of the preparation used in the test injection. Consequently the situation with tumor preparations, referred to above, is more complicated, since this necessarily involves an increase in the introduction of single doses of "major" antigen. It would seem that "weak" antigens (and in connection with tumors they present great interest) have some advantage, since in this case it is evidently possible to carry out desensitization with smaller doses of "major" antigen (in terms of the shocking dose), and this leads to a less marked decrease of sensitivity to the "minor" antigen. However, in this case the disadvantageous aspect consists in the fact that on sensitization with small quantities of "weak" antigen the shocking dose exceeds the sensitizing 10 times. Therefore on sensitizing animals with a mixture of two weak antigens, mixed in the proportions of 50 : 1, it is possible to obtain a shock reaction for the "minor" antigen, if the mixture (or complex preparation) is introduced in such amounts as contain not less than 20 shocking doses of the "major" antigen. Aside from the fact that this limit is difficult to attain technically, we are forced to the conclusion that with "weak" antigens it is necessary to resort to profound desensitization.

Decrease in the sensitivity to a "minor" antigen as the result of repeated desensitization to a "major" antigen is unrelated, evidently, to the phenomenon of sensitization decay. Preliminary introduction of some other serum in place of the "major" antigen, in amounts not producing any reaction, had no effect on sensitivity to the "minor" antigen. Obviously, following repeated desensitization there is a possibility of "exhaustion" (or "weakening") of the reacting system. The results of experiments with a mixture of two weak antigens correspond to this. As was shown above, in this case desensitization to the "major" antigen was accompanied by a significantly less pronounced reaction than in experiments with a strong antigen; accordingly the sensitivity to the "minor" antigen was lowered notably less by desensitization.

The results of the experiments summarized here deserve, it seems to us, serious attention when employing the anaphylactic reaction to detect antigenic differences between normal and tumor tissues.

It should be noted that in the experiments presented here only one method of desensitization was employed, one which is used in studies on tumor antigens which appear in the literature. Other methods and rates of administering the antigen should be studied.

Therefore, the use of the anaphylactic reaction for the demonstration of components of an antigenic mixture (or components of a complex antigen), resorting to desensitization of the animals to the predominant

components, makes it necessary to carry out desensitization starting with amounts corresponding to the shocking dose, and to check completeness of desensitization (with 10 and more shocking doses).

Repeated desensitization to the predominant antigen may lead to a decrease in sensitivity of the animal to a "minor" antigen.

These data should be taken into account when applying the anaphylactic reaction to the demonstration of antigenic differences between tumor and normal tissues.

SUMMARY

Utilization of the anaphylactic reaction for distinguishing tumor antigens from antigens of normal tissues requires the preliminary investigation of 2 problems: 1) how should desensitization against "major" antigens be performed in order to be sure of its completeness, and 2) how does such desensitization affect the sensitivity of the animal to "minor" antigens? Experiments were carried out on guinea pigs which were sensitized by mixtures of two antigens (foreign serums, tissue nucleoproteins) mixed in different proportions. It was demonstrated that the desensitizing dose of a "major" antigen is several times as great as the shocking dose, and therefore that repeated injection is necessary to bring about complete desensitization. This causes a pronounced decrease of the sensitivity to the "minor" antigen. Therefore it is necessary to increase the dose of the preparation containing the mixture of antigens (for example, a tumor extract) in order to obtain a definite anaphylactic reaction. These findings should be taken into consideration in employing anaphylaxis for the demonstration of antigenic differences between tumors and normal tissues.

LITERATURE CITED

[1] Ioffe, V. I., Biull. Eksptl. Biol.i Med. 1957, 44, No. 9, 12-15.*

* Original Russian pagination. See C. B. Translation.