ON THE PROBLEM OF FINDING TISSUE ANTIGENS

COMMUNICATION I. THE RELATIONSHIP BETWEEN THE SENSITIZING AND SHOCK DOSES OF ANTIGENS IN ANAPHYLAXIS

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The representatives of many branches of biology and medicine are interested in the characteristics of the antigenic composition of animal and human normal and pathologic tissues. Such immunological studes are useful in studying the processes of tissue metamorphasis and development in ontogenesis; they are helpful in determining the character of various pathological changes in tissues, and they have a place in comparative and evolutional zoology. The investigation of this question is especially important to the study of the problems involved in the tumorous process. However, it is admittedly very difficult, technically, to examine the antigenic composition of tissue, since this involves finding and differentiating the different individual components contained in a complicated antigenic complex. Therefore, highly sensitive methods of research must be used. A method permitting even the smallest inexactitude increases the possibility of "experimental error", thus decreasing the conclusiveness of the experimental results. Therefore, a knowledge and comparative evaluation of all the immunological methods available to research is of paramount importance to this question as a whole.

Experimental analysis of this question is the purpose of these articles. We were primarily interested in the immunological methods used in the study of tumors. It seemed more expedient, however, to temporarily shelve specific oncological problems (as well as the specific problems of other branches of science) and to consider the subject on a general immunological basis first. The first step, then, was to determine the conditions best suited to the disclosure of the individual components of an antigenic complex, depending on the quantitative relationships existing between these components, and to determine the research methods best suited to this purpose. The purpose of this work was the investigation of this question.

EXPERIMENTAL METHODS

A double set of model experiments were conducted, with "strong" or "weak" antigens, depending on the degree to which the physical immunological reaction effected by these antigens was usually expressed. We used some heterogeneous serum as the "strong" antigen and nucleoproteins from normal tissues as the "weak" antigen. Depending on the experimental conditions, we injected the antigens either separately or mixed in various ratios. We used the anaphylactic reaction and serological methods to disclose the antigens.

The nucleoprotein preparations were prepared by the method used in research to disclose specific tumorous antigens [1]. The tissue was ground up; to the resulting mass, a triple quantity (in relation to the tissue weight) of a physiological solution and then a 1% solution of NaOH to pH=9 were added; the material was then kept at a temperature of 4° until the following day. The fluid obtained after 2 -minute centrifugation at 3,500 revolutions perminute was acidified with a 10% solution of acetic acid to pH=6, the precipitate was separated by centrifugation, and the remaining fluid was acidified to pH=4.5. The precipitate was dissolved in distilled water and alkalized with a 1% solution of caustic alkali to pH=7.2. Merthiolate in a concentration of 1:10,000 was added

to the resulting solutions of nucleoprotein. The preparation was kept at a temperature of 4°. The protein content was determined by Kjeldahl's method (microdetermination). The relationship between the sensitizing and shock dose amounts had to be determined in order to find under what conditions the anaphylactic reaction could be used to disclose an individual component constituting only a small part of the antigenic complex.

Besides data on the well-known fact that a smaller dose of antigen is necessary for sensitization than is needed for the reacting injection, the literature also contains some information concerning the quantitative relationships between the sensitizing and shock doses. In the experiments of Doerr and Burger [2, 3], for example, the minimal sensitizing dose of serous englobulin (horse serum) was 0.0004 mg. However, 0.1 mg of the same antigen had to be injected in order to produce shock, i.e., 250 times more. Similar relationships (0.0039 and 0.1 mg) are also mentioned in connection with the albumin group, salted out at 56-60% saturation with ammonium sulfate. The minimal sensitizing dose for an albumin group precipitating at 66-99% saturation with ammonium sulfate was 0.08 mg, the shock dose, 1.9 mg.

In our experiments, separate groups of guinea pigs were sensitized to horse serum, which was injected intravenously in doses of from 0.025 to 0.0001 ml per 100 g of weight, (from 0.024 to 2.4 mg of protein) into guinea pigs weighing 300 g each.

The results of the experiments are given in the table.

EXPERIMENTAL RESULTS

The shock dose (see Table) was equal to the sensitizing dose only when sensitization was done with a comparatively large amount of serum: 0.01 ml per 100 g of weight, or 2.4 mg of protein per 300 g guinea pig.

Relationship Between Sensitizing and Shock Doses of the Antigen (Horse Serum)

Reacting dose in ml per 100 g of weight	Sensitizing dose in ml per 100 g of weight				
	0.025	0.01	0.0025	0.001	0.0001
0.1					Died
0.06 - 0.05		Died + + +		Died	+ + (+ + +)
0.03		+ + + (+ +)		+ + +	
0.01		+ + + + + +	Died + + (+ + +)	+ +	—
0.005	+ +		+ (+ +)	+ +	
0.0025 — 0.002	+		+ —	+	
0.001				+ +	—
0.0001					—

Symbols: +++ pronounced anaphylactic shock with acute asphyxiation, loss of ability to stand, convulsions; ++ clear anaphylactic reaction with signs of bronchial spasm expressed (convulsive cough); + anaphylactic signs in the form of scratching, sneezing, exhaustion without distinct signs of bronchial spasm; - no signs of anaphylactic shock.

If the sensitizing dose was decreased to 0.6 mg of protein per 300 g guinea pig, then a reacting dose of the same or even a double amount of the antigen did not cause an expressed reaction; anaphylactic shock was only observed (a lethal shock, in one case) when 0.01 ml of the antigen per 100 g of weight was injected, i.e., an amount 4 times greater than the sensitizing dose.

When the sensitizing dose was still smaller (0.001 per 100 g of animal weight), 30-50 times the amount of the sensitizing dose of antigens had to be injected in order to obtain a shock reaction, although a clear anaphylactic reaction (++) was observed with a reacting dose consisting of 5-10 times the sensitizing dose, i.e., 0.005-0.01 ml of serum per 100 g of weight. With a still smaller sensitizing dose — 0.0001 ml per 100 g of weight, the injection of a dose 100 times larger caused no reaction at all; only when the antigen was injected in a dose 500 times that of the sensitizing injection was a clear (approaching shock) reaction observed, while the reacting dose had to be increased to 1,000 times the sensitizing dose in order to cause lethal shock.

In another experiment, the guinea pigs were sensitized to nucleoprotein from a horse's liver (weak antigen), with a protein content of 20 mg per 1 ml.

To sensitize 300 g guinea pigs, 0.02 ml (0.04 mg of protein) was injected intraperitoneally. The reacting injection was done intravenously after 3 weeks, in doses of from 0.25 to 1 ml. Both of the guinea pigs injected with 1 ml of the preparation died. Two of the 3 guinea pigs injected with 0.5 ml died; shock was deferred in one of them, and it did not die until 12 hours after the reacting injection. A pronounced anaphylactic reaction was observed in the third guinea pig. When the amount of antigen injected in the reacting injection was still further decreased, the animals did not die; both of the guinea pigs injected with the 0.25 ml reacting dose manifested a clear anaphylactic reaction (++). Therefore, it was also necessary to increase the reacting dose to several times the amount of the sensitizing dose in order to obtain a definite result when the sensitization had been done with a small dose of weak antigen.

The known method used to find the differences in the antigenic properties of tumorous and normal tissue (by means of the anaphylactic reaction) is to sensitize the animal and then give a reacting dose consisting of so-called tumorous preparations, of which the actual tumor component constitutes only a small part. Even with a rather large sensitizing dose of the preparation (5-10 mg), the sensitizing dose of the tumor component itself is usually small. One should therefore take into account the data presented regarding the relationships between sensitizing and shock doses, since they show when one can conclude an observed anaphylactic reaction to be caused by the tumorous antigen and when one must refrain from such a conclusion.

SUMMARY

The relationship between the sensitizing and booster (shock) dose of antigens of different intensity (such as serum proteins and horse liver) was studied in experiments on guinea pigs. General interrelationships were established, which was manifested in the fact that the booster dose had to be increased, according to the gradual decrease of the sensitizing dose of the antigen.

LITERATURE CITED

[1] Medved N. N. and Shabad, L. M., Vestnik AMN, SSSR, 1950, No. 6.

[2] Doerr T. u. Berger W., Z. f. Hyg., 1922, Bd. 96, H. 1, p. 199.

[3] Doerr T. and Berger, W., Z. f. Hyg., 1922, Bd. 96, H. 1, p. 255.