RESEMBLANCES AND DIFFERENCES BETWEEN HUMAN AND ANIMAL ORGAN-SPECIFIC ANTIGENS

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It has been shown by a number of authors [3, 5, 10, 11, 12, and others] that every organ possesses antigens specific to itself, in addition to general antigens characteristic of the given organism. Indisputable evidence of the existence of organ-specific antigens was afforded by the preparation of sera monospecific with regard to human organs [8].

The discovery of organ-specific antigens raised the question as to whether these antigens are identical for organs of analogous function of humans and of different animal species. It is known that the corresponding organs of different species of animals exhibit morphological and physiological resemblances.

The study of organ-specific antigens has yielded contradictory data. Some workers [1, 9, and others] reject the view that organ-specific antigens of different species resemble each other, and maintain that strict species-specificity prevails, while others [2, 14, 15] hold that organ specificity is not restricted by species differences, i. e., that the organ-specific antigens of different species are either identical or very closely related.

The majority of workers [3, 5, 10, 13, and others] admit the resemblance between organ-specific antigens, with the reservation that the antigens of some organs (brain, crystalline lens, posterior hypophysis) of different species are closely related, but of other organs much less so.

It thus seems that the question of the resemblance or the difference between the antigens of homologous organs of different species of animals and of humans has not yet received any definitive experimental answer. The present paper describes experiments directed towards this end.

Our objective was to determined whether there are any qualitative differences between human and animal organ-specific antigens.

For this purpose we prepared organ-specific immune sera against human organs, in rabbits. We examined these sera by the complement-fixation test with extracts of animal organs, and came to the same conclusion as many of our predecessors, that human and animal organs possess similar antigens. Our findings differed, however, insofar as we found that the reaction with extracts of animal organs was much weaker than of human organs. It remained to be shown whether this difference was a quantitative one only, or whether there exist qualitative differences between the antigens of different species.

EXPERIMENTAL METHODS

For the comparative study of organ-specific antigens we applied the reaction of binding of hemagglutinins, which we had previously used for the demonstration of human organ-specific antigens. This reaction is based on the secondary, nonspecific binding of α-agglutinins by specific antigen-antibody complexes.

This method permitted the examination of whole tissue brei, and not of extracts only. This was very convenient, as it gave us the opportunity of blocking heterogeneous antigens common to human and animal organs, which would interfere with the demonstration of specific antigens. Blocking of nonspecific antigens was effected by preliminary treatment of the tissues with various heterologous sera, in order to saturate the antigens.

We performed comparative experiments on human liver and spleen, and on the same organs of guinea pigs, dogs, pigs, and bulls. We used suspensions of the tissues in an equal volume of physiological saline for the reaction of binding of hemagglutinins. The organ-specific sera used for the hemagglutination reaction were diluted with 5 volumes of saline, and to 3 ml of diluted serum we added 0.3 ml of α-immune serum, diluted 1:10, and taken from a rabbit. The organ-specific serum so prepared was titrated against group A erythrocytes, undiluted, and in dilutions of 1:2, 1:3, 1:4, and 1:5. Serum diluted 1:5 usually gave a + or ++ reaction for agglutination of erythrocytes. The 50% tissue suspensions were measured by means of a pipet (0.5 ml portions) into agglutination tubes. These were then centrifuged for 10 minutes, and the supernatant solution was discarded. The centrifugate was taken for the experiments.

The experiments consisted of three stages.

In the first stage of the experiments we treated the tissue residues with various heterologous sera, for the preliminary blocking of heterogeneous antigens. Thus for the comparative study of human and animal spleen (Table 1) we used the following antisera: anti-spleen, anti-brain, anti-kidney, anti-stomach, and anti-myocardium.

The preliminary treatment of organ tissues consisted in addition to the centrifugates of 0.2 ml of the appropriate serum, which was mixed in with the brei, and the mixture was allowed to stand at room temperature for 30-45 minutes. It was then centrifuged, and the supernatant serum was titrated against Group A erythrocytes. A lowering of the titer of α-agglutinins in the serum was evidence that an immunological reaction had proceeded, whereas if there was no change in the α-agglutinin titer it was concluded that there had been no reaction.

The preliminary treatment was repeated with fresh portions of serum until all the heterogeneous antigens had been blocked, i. e., until the tissues gave no further lowering of the α-agglutinin titer of added serum.

We found that 4-5 additions of heterologous serum were required for full blocking of the common antigens of human organs, whereas 1 or 2 additions of serum sufficed for blocking antigens common to both human and animal organs.

The tissue breis were taken for further study after all these antigens had been fully saturated.

In the second stage, involving a specific reaction between organ-specific antigens and the corresponding sera, we added 0.1 ml portions of anti-liver serum to portions of human and animal liver tissue, mixed, and kept in the refrigerator for 15-16 hours.

The final, third stage consisted in assessing the extent of binding of α-agglutinins. The suspensions were centrifuged for 10 minutes at 2000-2500 r p m, and the supernatant serum was titrated against Group A erythrocytes.

EXPERIMENTAL RESULTS

Table 1 presents the results of comparative study of organ-specific antigens of human, guinea pig, dog, pig, and bull liver. It may be seen from this Table, which gives typical protocols of agglutination reactions, that preliminary treatment of human liver tissue with various heterologous sera does not remove its capacity of entering into specific reaction with anti-liver serum, as is shown by a regular fall in the α-agglutinin titer of the serum. Thus if the anti-liver serum initially agglutinated Group A erythrocytes at all dilutions, after it had been allowed to react with human liver tissue it scarcely agglutinated the erythrocytes at all. This is evidence that in the process of formation of the liver antigen-antibody complex practically all of the α-agglutinins were bound nonspecifically.

Quite different results were obtained with animal liver tissues previously treated with the same heterologous sera as were used for human liver tissue. The agglutinating power with respect to Group A erythrocytes was the same after anti-liver serum had reacted with animal liver as before. The unchanged α-agglutinin titer

TABLE 1

Results of Hemagglutination Reactions Performed in Connection with a Comparative Study of Human and Animal Liver Tissue

Expt. No.	Organ studied	Serum used for preliminary treatment	Serum used for specific absorption	Hemagglutination reaction with Group A erythrocytes of the serum after specific absorption, at dilutions				
				undiluted	1 : 2	1 : 3	1 : 4	1 : 5
	—	—	Anti-liver	+++	+++	++(+)	++	++
39	Human liver	Anti-spleen	»	+(+)	—	—	—	—
	Human liver	Anti-brain	»	+	±	—	—	—
	Guinea pig liver	Anti-spleen	»	+++	+++	++	++	+(+)
	Guinea pig liver	Anti-brain	»	+++	+++	+(+)	+	+
	Human liver	No treatment	»	—	—	—	—	—
	Guinea pig liver	No treatment	»	+(+)	+	±	—	—
	—	—	Anti-liver	+++	+++	+++	+++	++
44	Human liver	Anti-spleen	»	+	—	—	—	—
	Dog liver	Anti-spleen	»	+++	+++	+++	+++	++
	Dog kidney	Anti-spleen	»	+++	+++	++(+)	++(+)	++
	Dog spleen	Anti-spleen	»	+++	+++	+++	+++	++
	Human liver	No treatment	»	++(+)	+(+)	+	—	—
	Dog liver	No treatment	»	++(+)	+(+)	+	+	±
	—	—	Anti-liver	+++	+++	++	+(+)	+(+)
20	Human liver	Anti-spleen	»	++	+	±	—	—
	Human liver	Anti-spleen	»	++	+	±	—	—
	Human liver	Anti-brain	»	+(+)	±	—	—	—
	Human liver	Anti-stomach	»	++	±	—	—	—
	Pig liver	Anti-kidney	Anti-liver	+++	+++	++	+(+)	+(+)
	Pig liver	Anti-spleen	»	+++	++(+)	++(+)	++	+(+)
	Pig liver	Anti-brain	»	+++	+++	++(+)	++	+(+)
	Pig liver	Anti-stomach	»	+++	+++	++	++	+(+)
	Human liver	No treatment	»	+	—	—	—	—
	Pig liver	No treatment	»	+++	+	+	±	—
	—	—	Anti-liver	+++	++	+(+)	+	+
160	Human liver	Anti-kidney	»	+	—	—	—	—
	Human liver	Anti-myocardium	»	+	—	—	—	—
	Human liver	Anti-stomach	»	+	±	—	—	—
	Bull liver	Anti-kidney	»	+++	+(+)	+	+	+
	Bull liver	Anti-myocardium	»	+++	++	+(+)	+	+
	Bull liver	Anti-stomach	»	++(+)	++	+	+	+
	Human liver	No treatment	»	—	—	—	—	—
	Bull liver	No treatment	»	++(+)	+(+)	+	—	—

Note: +++, ++ (+), ++, +(+), +, ± represent decreasing degrees of intensity of the positive reaction; — represents absence of reaction.

Note: +++, ++(+), ++, +(+), +, and ± represent descending degrees of intensity of the positive reaction; — represents absence of reaction.

TABLE 2

Results of Hemagglutination Reactions Performed in Connection with a Comparative Study of Human and Animal Spleen Tissue

Expt. No.	Organ studied	Serum used for preliminary treatment	Serum used for specific treatment	Hemagglutination reaction with Group A erythrocytes of the serum after specific absorption, at the dilutions:				
				undiluted	1:2	1:3	1:4	1:5
	—	—	Anti-spleen	+++	++(+)	++(+)	++	+(+)
15	Human spleen	Anti-liver	»	++	—	—	—	—
	Human spleen	Anti-brain	»	++	—	—	—	—
	Guinea pig spleen	Anti-liver	»	+++	++(+)	++(+)	++	+(+)
	Guinea pig spleen	Anti-brain	»	+++	+++	++(+)	++	+(+)
	—	—	Anti-spleen	+++	+++	+++	+++	++
45	Human spleen	Anti-liver	»	++	+	+	—	—
	Human spleen	Anti-brain	»	++	+	±	—	—
	Human spleen	Anti-stomach	»	+++	++	±	—	—
	Dog spleen	Anti-stomach	»	+++	++(+)	++(+)	++	+(+)
	Dog kidney	Anti-stomach	»	+++	++(+)	++(+)	++	+(+)
	Dog liver	Anti-stomach	»	+++	+++	+(+)	+(+)	+(+)
	Human spleen	No treatment	»	++	+(+)	+(+)	+	±
	Dog spleen	No treatment	»	+++	++(+)	++(+)	++(+)	+(+)
	—	—	Anti-spleen	+++	+++	++(+)	++	+(+)
30	Human spleen	Anti-brain	»	+	—	—	—	—
	Human spleen	Anti-stomach	»	+	—	—	—	—
	Pig spleen	Anti-brain	»	+++	+++	++(+)	++	+
	Pig spleen	Anti-stomach	»	+++	+++	++(+)	++	+(+)
	Bull spleen	Anti-brain	»	+++	++(+)	++(+)	++	++
	Bull spleen	Anti-stomach	»	+++	+++	++(+)	++	+(+)
	Human spleen	No treatment	»	—	—	—	—	—
	Pig spleen	No treatment	»	++(+)	+	—	—	—
	Bull spleen	No treatment	»	+++	++	+	—	—

Note: +++, ++(+), ++, +(+), +, and ± represent descending degrees of intensity of the positive reaction; — represents absence of reaction.

is evidence of the absence of immunologically specific reactions between animal liver tissues and anti-liver serum.

Human and animal liver tissues not subjected to preliminary treatment (see Table 1) enter into immunological reaction with anti-liver serum (fall in α-agglutinin titer); this reaction evidently proceeds as a result of the presence of similar antigens in human and animal organs.

The abolition of the capacity of animal liver tissue to enter into immunological reaction with anti-liver serum after blocking of nonspecific antigens with heterologous sera is in contrast to human liver tissue, which retains this capacity after repeated treatment with heterologous sera; this reveals qualitative differences between human and animal liver antigens.

Table 2 gives the protocols of comparative studies of human, guinea pig, dog, pig, and bull spleen tissue.

The spleen tissue was subjected to preliminary treatment with anti-liver, anti-kidney, anti-brain, and anti-stomach sera. We used anti-spleen serum with α-agglutinins for the specific reaction.

The experiments showed that spleen tissue of animals did not, after the preliminary treatment, react with anti-spleen serum (α-agglutinin titer unchanged). It follows that human spleen tissue resembles human liver in differing qualitatively from animal spleen tissues.

We have thus been able, by means of the method of blocking of heterogeneous antigens common to human and animal tissues, and using the hemagglutination reaction, to show that human organ-specific liver and spleen antigens differ qualitatively from those of the animals studied by us. This qualitative difference is apparently due to species-specific properties of the organ-specific antigens.

It is known that species-specificity of organisms is determined chiefly by the serum proteins. Proteins identical with serum proteins have been found in human liver and tumor tissues [6].

Our experiments show that species-specificity of organs is determined not only by the serum proteins, but also by organ-specific antigens.

The possibility is not excluded that the species-specificity of organs is due to a number of different antigenic complexes. It is possible that each antigen of a cell bears the characters of species-specificity.

SUMMARY

A comparative immunological investigation of the tissues of liver and spleen of man, guinea pig, dog, pig and bull was conducted. It was demonstrated with the aid of a method of blocking of heterogeneous antigens (common for organs of man and animals) and by the reaction of hemagglutinin binding that organ-specific antigens of man differ qualitatively from the corresponding antigens of animals. Organ-specific antigens, evidently, determine together with the other antigens the species to which the organ belongs.

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