ANTIGENIC PROPERTIES OF ARTICULAR CARTILAGE

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The work of I. I. Mechnikov and his pupils [9, 10] not only had a decisive influence on the theory and practice of the immunity of infectious diseases, but laid the foundations for the theory of tissue and organ immunity. The work of Soviet scientists [1-8] played a large role in the subsequent development of immunology.

The situation with respect to the problem of lessening the antigenic differences of tissues and organs when they are transplanted from one person to another is definitely less satisfactory. The usual outcome of all homoplastic transplants is complete resorption of the transplants, or in the most favorable cases temporary clinical survival. Biological survival of homotransplants in the clinic has so far not been achieved because of antigenic differences of tissues and organs.

It is clear from the literature that with the aid of immunological reactions, it has been established that there are specific antigenic differences within the species, in the organs and tissues of animals and man (skin, nervous tissue, thyroid, and others). Regarding the presence of specific antigenic differences in hyaline cartilage, especially articular cartilage, one finds conflicting data. Meanwhile an answer to this question would have not only theoretical interest, but also great significance in practical medicine (homoplasty).

Considering the urgency of the problem of homoplasty of cartilage, we have been studying, since 1950, homoplasty of articular cartilage in experiments on dogs.

To eliminate the antigenic properties of articular cartilage transplants, we utilized various methods of preservation. Following storage of the transplants at 0° in serum of the recipient for 7 days (gradual cooling to 0°), biological survival was observed in two cases. But these were the usual type of experiment on transplanted tissues, where immunological reaction of the body was evaluated by the survival of the transplant. For a more precise evaluation of the immunological reaction, we decided in 1955 to investigate the antigenic properties of fresh and preserved articular cartilage, by the complement fixation (CF) reaction.

In parallel, similar experiments were set up for the determination of the specific antigenic properties of skin.

METHODS

We carried out experiments on 18 adult rabbits, which were divided on the basis of sex and weight into five groups. The immunization was carried out by the usual method.

Obtaining monospecific serum against articular cartilage and skin. Into the three rabbits of the first group we injected an extract of articular cartilage (antigen) which had been stored at 0° for 7 days; into the three rabbits of the second group we injected an extract (antigen) of fresh articular cartilage; into the three rabbits of the third group we injected an extract (antigen) of skin which had been stored at 0° for 7 days; into the six rabbits of the fourth group we injected an extract of fresh skin. Three rabbits of the fifth (control) group were not immunized.

Material for immunization was taken from young rabbits from a single litter, at the age of 40-50 days.

After removing the hair, we removed, under sterile conditions, skin and articular cartilage from the epiphsis of the femur. We cut up the material thus obtained finely with scissors, then ground it in a porcelain mortar with hypertonic salt solution (for 1 g of tissue 10 ml of a 1.7% solution of common salt). The suspension was allowed to stand 2 hours, after which we centrifuged it for 10 minutes at 2000-2500 rpm. We diluted the centrifuged liquid (antigen) with distilled water to the concentration of physiological saline. We put the extracts prepared in this way, with sterile precautions, into ampules of 3 ml and used them for immunization and for the CF reaction. Animals were selected which were nearly the same weight (2280-2317 g), and their food and quarters were of the same type.

All immunizations lasted four weeks. On the first 3 days we injected, with sterile precautions, the antigen (extract of cartilage and skin) intravenously in 1 ml amounts. After a 4-day interval we carried out a second course of immunization. On the first day we injected 1 ml intraperitoneally, and on the following 2 days 1 ml intravenously, followed by a 4-day interval. Three cycles like the second were carried out. On the 8th day after the last intravenous injection, blood was taken from all immunized and control rabbits. The serum obtained was used for the CF reaction with antigens from cartilage and skin, fresh and preserved.

It should be remarked that in order to determine the immunizing dose of the antigen we tried 2 ml of the extract (antigen) of cartilage and skin, stored at 0°, or fresh. On the first day we injected the above mentioned amount intravenously into each experimental animal. About 8-10 minutes after injection of the antigen 2 rabbits of the group died of anaphylactic shock. The others remained normal. As a result the immunizing dose was lowered to 1 ml, and the fourth group of experimental animals replenished with three new rabbits.

However, in spite of the reduction in dose of antigen, on the third week of immunization 3 rabbits of this group died of anaphylactic shock about 3-5 minutes after intravenous injection of the extract. Thus we were able to carry out a complete course of immunization to the antigen from fresh skin with only one rabbit (No. 10).

We carried out the complement fixation reaction by the usual method. By this reaction, using the antibody of the sera, we estimated the antigenic properties of the articular cartilage and skin with which we had immunized the experimental animals. For the CF reaction we diluted the antigens 10, 100, and 200 times (see table).

RESULTS

As can be seen from the table, a positive complement fixation reaction was obtained with the 1 :100 dilution in the group of animals (Experiment No. 2) which were immunized with an extract of fresh articular cartilage. Fresh articular cartilage, accordingly, possesses well-defined antigenic properties.

Articular cartilage stored at 0° for 7 days possesses distinctly weaker antigenic properties than does fresh cartilage, which is shown by the CF results in the first experiment. In this experiment, the CF reaction was positive in two rabbits at a dilution of 1 :10 in the first 15 minutes. In rabbit No. 3 the CF reaction was negative. Consequently antibody to the injected antigen was hardly formed in the blood of the animals injected with the extract of preserved articular cartilage. On the basis of this observation it may be stated that storage of articular cartilage at low temperatures lowers its antigenic properties significantly.

In the fourth experiment, where we used an extract of fresh skin for immunizing the rabbits, 5 rabbits out of 6 died of anaphylactic shock during the immunization. Only one rabbit (No. 10) survived the complete course of immunization. The CF reaction in this rabbit was negative, as may be seen from the table. On the basis of the death from anaphylactic shock of 5 rabbits in the fourth experiment, it may be suggested that fresh skin possesses pronounced antigenic properties. The negative CF reaction in rabbit No. 10 is evidently explained by a nonreactivity peculiar to this animal.

In the third experiment in which we immunized the rabbits with an extract of preserved skin, all the animals survived. However, the CF reaction was positive in all rabbits at dilutions of 1 :10 and 1 :100. This experiment showed that skin possesses pronounced antigenic properties. Storage at low temperatures perhaps lowers this property, but does not remove it completely.

The reliability of the CF results obtained in the experiment is supported by study of the sera of all the control groups. As is evident from the table, none of the sera of the control animals contained specific antibodies to any of the four antigens.

Results of Complement Fixation (CF) Reaction for the Determination of the Antigenic Properties of Articular Cartilage and Skin (fresh and stored at 0° for 7 days)

No. of expt.	Antigen used to obtain immune serum	No. of rabbit	Control without antigen		Dilution of Extract					
					1 : 10		1 : 100		1 : 200	
			Complement fixation reading after							
			15 min.	45 min.	15 min.	45 min.	15 min.	45 min.	15 min.	45 min.
1	Extract of preserved articular cartilege									
		1	h	h	+++	h	h	h	h	h
		2	h	h	++	h	h	h	h	h
		3	h	h	h	h	h	h	h	h
	Control	17.18,19	h	h	h	h	h	h	h	h
2	Extract of fresh articular cartilege		h	h	++++	++++	++++	++	h	h
		5	h	h	++	++	+++	+	h	h
		6	h	h	+++	++	++	h	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h
3	Extract of preserved skin	7	h	h	++++	++++	++++	+++	h	h
		8	h	h	++++	++++	+++	++	h	h
		9	h	h	++++	++++	++++	+	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h
4	Extract of fresh skin	10	h	h	h	h	h	h	h	h
	Control	17,18,19	h	h	h	h	h	h	h	h

Note. The complement fixation results are expressed in the usual way: ++++ indicates absence of hemolysis, h, complete hemolysis, +++, ++, and + intermediate degrees of hemolysis.

The decrease in the antigenic properties of cartilage as a result of storage at low temperatures suggests the possibility of further improvement in methods of preservation with the aim of weakening the antigenic properties for use in homoplastic transplants.

SUMMARY

Fresh articular cartilage possesses marked antigenic properties. Storage at low temperatures (0°C) lowers its antigenic properties considerably.

Data from complement fixation tests show that the antigenic properties of fresh and stored skin are considerably more pronounced that those of fresh articular cartilage.

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