NORMAL ANTIBODIES DURING REGENERATION IN INVERTEBRATE ANIMALS

COMMUNICATION 1. NORMAL ANTIBODIES AGAINST REGENERATING TISSUE IN THE RAY OF Asterias rubens

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Experimental researches in vertebrates [3-5] have shown that the processes of regeneration developing after disturbance of the integrity of the tissues (fractures, blood loss, burns, freezing, partial ablation of an organ) are accompanied by the production of specific autoantibodies. It might be expected that a similar process would also be observed in invertebrate animals. If such a pattern were established it would enable progress to be made toward the explanation of the nature of the primary immunological response and of the role of normal antibodies in morphogenetic processes [1].

In order to investigate the problem of the possible detection of specific autoantibodies to regenerating tissue in invertebrate animals we carried out an experiment on the starfish Asterias rubens, whose marked regenerative powers are well known.

EXPERIMENTAL METHOD

For the experiment we used 30 uninjured starfish and 30 starfish with a regenerating ray. In control experiments we used the polychaete worm Arenicola marina (20 specimens).

In order to verify that normal antibodies were present in the celomic fluid of the above-mentioned invertebrate animals, the ring precipitation test was carried out. As "serum" we used a lyzate of the cells of intact starfish and of others from which one ray had been removed, prepared in distilled water. The lyzate was prepared from the residue of the celomic fluid of the starfish after centrifugation at 2000 rpm for 5 minutes. The supernatant fluid was poured off into another test tube, and distilled water in the same volume was added to the residue. After careful mixing for 30 minutes, chemically pure sodium chloride was added to the solution to make the salt concentration of the solution equal to that of sea water (0.25 g NaCl to 100 ml sea water). The lyzate prepared in this way and also the supernatant fluid were used in the experiment in undiluted form.

Tissue extracts (antigens) for the ring precipitation test were prepared in filtered sea water in a concentration of 1:10. For the experiment we prepared antigens from the tissues of the uninjured starfish, of the regenerating ray of the starfish, and from the tissues of the annelid worm. All antigens were used in a dilution of 1:100. Into the control tubes filtered sea water was added instead of one of the reagents listed above.

The results of the ring precipitation test were read after the tubes had been kept for 5-10 minutes at room temperature. Each experiment was repeated 2-3 times. The degree of the reaction was expressed according to the following scale: – no ring; ± indistinct ring; + fine ring; ++ clear, dense ring.

During the performance of the ring precipitation test certain peculiarities in its course were observed. In all the experiments with untreated tissue fluids the reactions were positive only if these fluids were prepared not later than 18 hours before carrying out the test. In the experiments with tissue fluids prepared 12, 8, 6 or fewer hours before the test, there were no positive reactions. On the other hand, the lyzate of the cells from the celomic fluid of the starfish, used as serum, reacted even when it was prepared ex tempore. When carrying out the experiments we therefore used lyzate prepared ex tempore and tissue extracts prepared not later than 18 hours before the test.

EXPERIMENTAL RESULTS

The results of the experiments are shown in the table.

Ring Precipitation Test Between "Sera" of an Uninjured Starfish and a Starfish with a Regenerating Ray and Tissue Extracts from an Uninjured Starfish, a Starfish with a Regenerating Ray and the Annelid Worm Arenicola marina

Undiluted "serum" / Antigen (1:100)	"Serum" of uninjured starfish		"Serum" of starfish with regenerating ray		Sea water
	celomic fluid	lyzate of cells	celomic fluid	lyzate of cells	
Uninjured Asterias rubens	±	++	–	+	–
Asterias rubens with regenerating ray	–	–	±	++	–
Arenicola marina	–	++	–	++	–
Sea water	–	–	–	–	

It can be seen from the table that the celomic fluid of the uninjured starfish reacted with the tissue extract of the same starfish to a degree ± , but did not react with the tissue extract of the starfish with the regenerating ray.

The lyzate of the cells from the celomic fluid of the uninjured starfish reacted with the tissue extract of the same starfish, giving a clear, dense ring evaluated as ++, but did not react with the tissue extract of the starfish with the regenerating ray. The celomic fluid of the starfish with the regenerating ray reacted with the tissue extract of the same starfish (±) but did not react with the tissue extract of the uninjured starfish. The lyzate of the cells from the celomic fluid of the starfish with the regenerating ray reacted with the tissue extract of the uninjured starfish, forming a fine ring (+), and with the tissue extract of the starfish with a regenerating ray, forming a clear, dense ring (++). The celomic fluids of the starfish, both uninjured and with a regenerating ray, did not react with the tissue extract of the annelid worm. The lyzate of the uninjured starfish and of the starfish with the regenerating ray reacted distinctly with the tissue extract of the annelid worm, giving a dense ring (++).

Thus the celomic fluids of both the uninjured starfish and the starfish with the regenerating ray reacted weakly with the corresponding tissue extracts and did not react at all with the other extracts. This may evidently be explained by the fact that the greater part of the normal antibodies is contained in the cells and that only an insignificant part of them is present in the celomic fluid. Increased destruction of the cells leads to the release of these antibodies into the solution. This is confirmed by the fact that the lyzate of the uninjured starfish reacted only with the tissue extracts from its own tissues (or the tissues of another uninjured starfish) and with the tissue extracts of the annelid worm.

The lyzate of the starfish with the regenerating ray reacted with the tissue extracts of its own regenerating tissue (or the regenerating tissue of another starfish), and also with the tissue extracts of the uninjured starfish and of the annelid worm. In other words, the cells present in the celomic fluid of the starfish with the regenerating ray contained normal antibodies against its own regenerating tissue or the regenerating tissue of another starfish, but these antibodies could not be detected in the uninjured starfish.

The results obtained thus suggest that the process of regeneration in invertebrate animals is accompanied by the appearance of autoantibodies against the regenerating tissue in the cells of the celomic fluid. However, cross tests with these autoantibodies and tissue extracts of other species of invertebrates do not yet permit final conclusions to be drawn regarding their specificity. Further experiments with adsorption of nonspecific antibodies are required.

SUMMARY

The possibility of detection of specific autoantibodies to the regenerating tissue was studied. Experiments were carried out on Asterias rubens with employment of the ring precipitation tests. Cavital fluids with their cellular elements of intact and regenerating Asterias rubens served as "sera" and tissue extracts of their tissues as "antigens." The results of experiments have demonstrated that in the cavital fluid of the starfish with regenerating ray there are antibodies to their own regenerating tissues or those of other Asterias rubens. No such antibodies were revealed in the cavital fluids of intact starfish.

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