Interaction of Starfish Embryonic Cells with the Complex of Okadaic Acid and Monoclonal Antibody Specific to Okadaic Acid

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Okadaic acid is a very potent and specific inhibitor of protein phosphatase 1 and protein phosphatase 2A.1,2 Picard et al.3 showed that microinjection of okadaic acid into a starfish oocyte induced germinal vesicle breakdown via activation of maturation-promoting factor, which is a protein kinase the catalytic subunit (p34*CSK*) of which is activated just before M-phase and is central in driving cell cycle events in eukaryotic cells. They also showed that microinjection of okadaic acid into a single blastomere of a starfish embryo at the four-cell stage during early development arrested cell division of the blastomere. We recently found that an external application of okadaic acid at a concentration of 8 μg/ml or greater to fertilized eggs of the starfish, Asterina pectinifera, arrested development of the embryos at the 128-cell stage.4 Cytological examination found that premature chromosome condensation took place in each of the blastomeres of the treated embryo at the 64-cell stage, suggesting that this was the root cause of the arrest of further development.

We here describe the results of experiments done to examine whether a mouse monoclonal antibody, OA-3, raised against ovalbumin-conjugated okadaic acid5 is capable of neutralizing the action of okadaic acid on the starfish embryo. The antibody belongs to the IgG1 class with a kappa chain.

Various amounts of OA-3 were added to each 100 μl of artificial sea water (ASW; Jamarin Laboratories) containing 0.8 μg of okadaic acid and the mixture was incubated at 37°C for 1 hr before 30 μl of Protein A Cellulofine beads (Seikagaku Kogyo) were added. The mixture was kept stirred for 1 hr at room temperature and then centrifuged at 400 x g for 2 min to remove the beads. To the supernatant was added a small number of fertilized eggs and it was examined whether they were able to develop to the blastula stage. The result shown in Fig. 1 demonstrates that okadaic acid was removed from the solution by immobilized antibody, suggesting the antibody OA-3 was capable of binding okadaic acid in ASW. Next, 100-μl solutions of ASW containing 0.8 μg of okadaic acid and various amounts of OA-3 were incubated at 37°C for 1 hr and then at 20°C for 1 hr. To the solutions were added a small number of fertilized eggs, which were allowed to develop for 8 hr. As shown in Fig. 1, none of the embryos blastulated. It was possible that the fertilization envelope deprived the antibody of antigen-binding capacity. Therefore, soon after hatching the solutions containing the antigen-antibody complex were added to blastulae and we examined their fate in development. None of the blastulae reached the gastrula stage.

To establish whether or not the release of okadaic acid was due to the degradation of OA-3, 100-μl solutions of ASW containing 0.8 μg of okadaic acid and 600 μg of OA-3 were incubated at 37°C for 1 hr and then at 20°C for 4.5 hr. To each solution was added 100 fertilized eggs, which were allowed to develop for 4.5 hr. The embryos and the ASW were separated and their contents of OA-3 were measured by an enzyme-linked immunosorbent assay by the procedure described previously.6 It was found that the OA-3 present in the embryonic lysate and the ASW were nearly the same as those present in the original solutions which were devoid of embryos. The results described above suggest that the cell membrane of the embryonic cell or the hyaline layer surrounding the outer surface of the embryonic cell dissociates the antigen-antibody complex. Okadaic acid, being hydrophobic, can enter cells by diffusing across the lipid bilayer of the cell membrane, the interior of which is hydrophobic.

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References