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Experiments with Immunization of Mice with Blastocysts by an Intrasplenic Route

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ABSTRACT

A few attaching blastocysts from CBA/H mice were irradiated from a Cesium source and transferred into the spleen of male DBA_2 mice. A booster immunization was performed after four weeks. Blood samples for preparation of antiserum to test for the presence of immunoglobulins directed against blastocyst surface determinants were obtained by a retro-orbital puncture. Specific antibodies were detected with a protein A-gold method, modified for transmission electron microscopy of air-dried blastocysts. The results showed that CBA/H blastocyst incubated in DBA_2 immune serum were positive for protein A-gold labelling, while control blastocysts only possessed a few irregularily scattered gold particles. Thus, it seems as a deposition of antigens in the spleen tissue with persistence of the antigens at this site will result in detectable antibodies in the peripheral blood.

INTRODUCTION

Raising antiserum against blastocyst antigens is laborious, since a conventional immunization requires thousands of blastocysts (1,4,6). A more rational way could be the use of a spleen cell culture, that is, a few blastocysts (the antigens) are co-cultured with spleen cells which then are used to produce monoclonal antibodies (2). In the present report we describe experiments with a technique where the blastocysts were deposited directly into the spleen tissue, where they seemed capable to trigger an antibody response.

MATERIAL AND METHODS

Blastocysts were obtained from CBA/H mice in delayed implantation. The blastocysts were recovered 18 h after an injection of oestrogen, that is, when they were about to attach onto the uterine surface. The flushings were made with PBS containing 1 per cent serum from the future host or 500 IE/ml heparin. Since our prime goal was to obtain antibodies against surface constituents

of attaching trophoblast cells, the blastocysts were irradiated, before the transfer into the spleen, with 5000 R from a Cesium source to block their ability to grow.

Recipients were 4 male mice, aged two months, of the DBA_2 strain. The spleen was reached through a dorso-lateral incision, and the caudal end of the spleen was carefully taken out of the abdominal cavity. A micropipette containing 2-3 donor blastocysts in a small amount of PBS with recipient serum or heparin was inserted underneath the splenic capsule, and the blastocysts were extruded from the pipette. The fluid remaining in the pipette was examined in order to determine whether all of the blastocysts had been extruded. After a successful transfer the spleen was replaced in position, and the incision was closed. After four weeks, a booster immunization was performed by transfer of similarily treated blastocysts. Between one and three weeks later, blood samples were taken from each recipient animal by retro--orbital puncture, and serum was prepared for testing for the presence of immunoglobulins directed against trophoblast determinants.

The presence of specific antibodies was detected with a protein A-gold method (3,5) modified for transmission electron microscopy of blastocysts (Svalander, Ljung and Nilsson, in preparation). Blastocysts in the same functional state as those used for immunization were flushed from the uterine horns of CBA/H mice with a fixative of 0.5% glutaraldehyde in PBS into a watchglass. The blastocysts were left in the fixative for a maximum of 20 min, washed four times in PBS and incubated in the DBA_2 immune serum diluted 1:20 for 45 min at room temperature. Controls were incubated in PBS or in DBA_2 non-immune serum. After three subsequent washings in PBS, the blastocysts were incubated in rabbit anti-mouse-Ig antiserum diluted 1:50 for 30 min at room temperature, and washed three times in PBS. The blastocysts were then transferred to the protein A-colloidal gold solution, in which they were left for 30 min at room temperature. After this labelling, the blastocysts were washed three times in PBS and twice in double-distilled, ultrafiltered water. Finally, they were placed on a hexagonal grid (Hex 700 TB, Polaron Eq. Ltd., England) without membrane in a microdroplet of water, and were left to dry in the air. The air-dried blastocysts were examined in a Philips 400 STEM electron microscope with field emission gun operated at 100 kV to evaluate the number of gold particles.

RESULTS AND COMMENTS

Blastocysts flushed from CBA/H mice 18 h after an injection of estrogen and incubated in DBA_2 immune sera were positive for protein A-gold labelling. The particles observed in the micrographs imaged gold colloid from both the upper and lower surface of the flat blastocyst. The particles were

irregularily scattered in the field of view. The control blastocysts, which were incubated in PBS or in DBA_2 non-immune serum instead of the immune serum at the first incubation step, possessed only a few irregularily scattered gold particles. Thus, it seems as a deposition of antigens in the spleen tissue with persistence of the antigens at this site will result in a detectable amount of antibody in the peripheral blood. This preliminary finding is now further tested using, among other things, gel plugs with bovine serum albumin as antigen.

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