Short Communications

Organization in vitro of Ovarian Cells Into Testicular Structures

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Summary. While it has been shown previously (Zenzes et al., 1978; Ohno et al., 1978) that when dissociated testicular cells are exposed to anti-H-Y antiserum in vitro they are prevented from reorganizing into testicular structures, forming ovarian follicular structures instead, the most conclusive evidence for the action of H-Y antigen would be the conversion of ovarian cells into testicular organization. Testing for H-Y antigen of the medium collected from cultivated testicular cells revealed a positive reaction. Dissociated ovarian cells of newborn rats cultivated in this medium reorganize into testicular structures. It is concluded that H-Y antigen is responsible for this histomorphologic change.

Introduction

Embryogenesis proceeds through a complex series of cellular interactions in which embryonic cells sort out and associate into specific multicellular groupings giving rise to tissues and organs. It is now generally accepted that molecular events occurring at the cell surface contribute to the control of these morphogenetic processes. In the case of mammalian gonadal differentiation it has been assumed that the Y-linked histocompatibility (H-Y) antigen determines development of the indeterminate gonad of an embryo into a testis. Individuals that possessed either testis or ovotestis invariably typed as H-Y antigen-positive, whereas individuals lacking testis but endowed with ovaries typed as H-Y antigen-negative, irrespective of their sex chromosome constitution (Wachtel et al., 1977; Ohno, 1977; Silvers and Wachtel, 1977). The first direct evidence of the testis-organizing function of H-Y antigen was recently presented in cell reaggregation experiments with newborn mouse (Ohno et al., 1978) and rat (Zenzes et al., 1978). Using the experimental design of Moscona (1957, 1961), we have shown that while under exposure to anti-H-Y antiserum dissociated single cells of newborn rat testis

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reassociate into ovarian follicular structures, untreated cells and those exposed to anti-H-1 antisera (the H-I system is the major histocompatibility system of the rat) reorganize into testicular structures (Zenzes et al., 1978). The most conclusive evidence for the testis-inducing capacity of H-Y antigen would be the organization of dissociated ovarian cells into testicular structures in the presence of this antigen in the culture medium. We here report that following rotation culture, dissociated ovarian cells of newborn rat incubated in a medium of cultivated newborn rat testicular tissue reorganize into testicular structures.

**Materials and Methods**

Newborn male and female rats of the strain SIV-50, kindly provided by the Gödecke AG, Freiburg, were used throughout the experiments. Ovaries were collected in Ca²⁺-, Mg²⁺-free Hank's solution, cut into small pieces, and incubated in a 0.1% trypsin solution for 15–20 min at 37°C. After the fragments had been washed three times in Hank's solution, MEM supplemented with 10% fetal calf serum (FCS, Gibco Laboratories, USA) was added. A free cell suspension was obtained by drawing the fragments back and forth in a Pasteur pipette, and the quality of the suspension was controlled by phase microscopy (Fig. 2a). After centrifugation at 150 g ovarian cells were resuspended in medium and placed in flat-bottomed siliconized test tubes with tight-fitting stoppers. Monolayer cultures of newborn rat testicular cells (10 tests per culture flask) were established in Falcon tissue flasks in MEM supplemented with 10% FCS. After 24 h of incubation at 37°C in a 5% CO₂-air mixture, the cultures were thoroughly washed with Hank's solution and maintained for another 24 h in 3 ml FCS-free medium. Subsequently, medium free of testicular cells was collected by centrifugation. This medium was tested for the presence of H-Y antigen by an indirect cytotoxicity test according to Scheid et al. (1972), using rat tail epidermis cells as targets. Anti-H-Y antiserum was raised in female rats of the Lewis strain by inoculating a total of about 14 million spleen cells from congenic males in 6 two-week intervals. As shown in Figure 1, testing of the medium for H-Y antigen gave a positive result; thus, under these culture conditions H-Y antigen is released from the testicular cells into the medium. For each experiment, two parallel cultures were set up, each containing dissociated cells of 150 ovaries suspended in 3 ml medium. One culture contained FCS-supplemented MEM medium

![Fig. 1. Demonstration of H-Y antigen released into the medium from cultivated testicular cells (adult rat). Indirect cytotoxicity test on rat tail epidermis cells.](image)