

523 Anti-idiotypic antibody (Ab2) against human monoclonal sperm immobilization antibody (Ab1) for contraceptive vaccine. M. Shigeta, M. Kawakita, S. Isojima, Dept. Obst. and Gynec., Hyogo Medical College, Hyogo.

Since patients with sperm immobilizing antibody (SI-Ab) are healthy except infertility, a specific antigen which induces SI-Ab could be a good candidate for contraceptive vaccine. For purifying a specific antigen, we established the human monoclonal SI-Ab (H6-3C4) and analyzed the corresponding antigen epitope which had a structure of neolactosamin (Sialyl-N-acetyllactosamine). As the massproduction of carbohydrate antigen is limited by DNA recombinant technology, we attempted an immunization using anti-idiotypic antibodies, following Jerne's network theory. In this study, Balb/c mice were immunized with purified human IgM (Ab1) obtained from serum free culture supernatant of hybridoma H6-3C4 and anti-idiotypic antibodies (Ab2) were selected by absorption with unrelated human IgM coupled sepharose beads. Sera from rats and rabbit immunized with purified Ab2 contained human sperm immobilization antibodies (Ab3). The result showed that Ab2 contained internal image of sperm immobilizing antigen for rat and rabbit and possibly for human. These results indicated that anti-idiotypic antibody specific for human SI-Ab could be useful for contraceptive vaccine instead of purified human sperm antigen in animals and human.

524 Establishment of a transformat producing human sperm immobilizing antibodies on a large scale and constitutively using the cDNA expression vector. H. Sawai, N. Yamasaki, S. Komori, H. Kasumi, S. Isojima, Dept. of Obst. and Gynec., Hyogo Medical College, Hyogo.

For the purpose of searching and analyzing corresponding antigen of the sperm immobilizing antibody (SI-Mab), we used the recombinant DNA technique and planned to establish a transformat, which can produce human SI-Mab on a large scale and constitutively. Step 1: Making λ gt11 cDNA library from En46 clone (class-switched IgG1 sperm immobilizing antibody producing cells). Step 2: Screening this library using human immunoglobulin JH and V λ . Step 4: Insertion of H and L chain cDNA into the expression vector plasmid BCMGS-Hyg and BCMGS-Neo. Step 5: Transfection of these plasmids into X63.Ag8.653 by electroporation. The transfected cells (SI-BCMGS) produced sperm immobilizing antibodies. We confirmed the production of these antibodies by the method of Northern blot and Western blot. Although En46 decreased its ability to produce antibodies in a culture medium without selective reagents, in that condition SI-BCMGS kept it constitutively for a month. The amount of produced antibodies was three times as much compared with En46.

525 Negatively charged phospholipids as an corresponding epitope of sperm agglutinating monoclonal antibody. Y. Tsuji, H. Fukuda, A. Iuchi, S. Isojima, Dept. Obst. and Gynec., Hyogo Medical College, Hyogo.

To clarify cross reactive antigens between human trophoblast and sperm, villis of hydatidiform mole were immunized to BALB/c mice. The spleen cells of immunized mice were fused with NS-1 myeloma to make monoclonal antibodies which react to sperm and trophoblast cells. One monoclonal antibody, termed 9C12 demonstrated sperm agglutinating activity and reacted to normal trophoblast cells. The corresponding epitope of MAb 9C12 was examined by thin layer chromatography immunostaining against total lipids fraction extracted from normal placenta. MAb 9C12 reacted to negatively charged phospholipids, such as cardiolipin, phosphatic acid, phosphatidyl inositol and phosphatidyl serine, but did not react to neutral phospholipid, phosphatidyl choline. It is interesting that all of the previous identified epitope of anti sperm antibodies are negatively charged materials, such as sialic neolactosamine, sulfogalactose and negatively charged phospholipids in present work.