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HYBRIDOMA PRODUCING MONOCLONAL SPERM IMMOBILIZING ANTIBODY TO HUMAN SEMINAL PLASMA ANTIGENS

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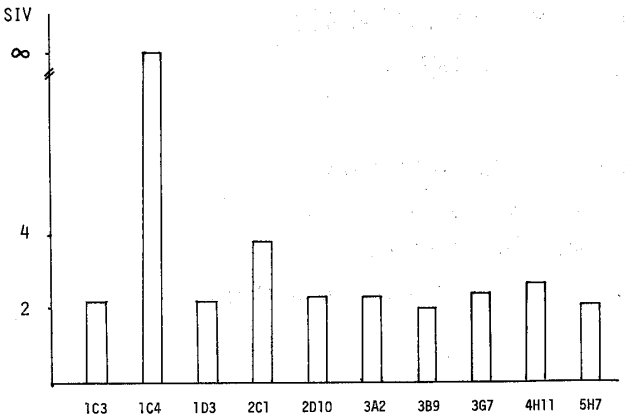
The sperm immobilizing antibodies found in the sera of some unexplained sterile women[1)2)] seemed to be a mixture of antibodies to several antigens in spermatozoa or seminal plasma. In order to examine the nature of antigen relevant to the sperm immobilizing antibodies, the purification of human spermatozoa and seminal plasma antigens was attempted. Isojima et al.[3)] discovered that at least four human seminal plasma specific antigens and two other seminal plasma antigens cross reacted with human milk were present in human azoospermic semen but they also proved difficult to isolate a single pure antigen from these materials. A large amount of monoclonal antibody to an individual antigen of human seminal plasma could make it possible by immuno-affinity chromatography with bound antibody, and thus the establishment of hybridomas producing monoclonal antibodies[4)] was studied.

Fisher rats were injected twice with 1 ml of human azoospermic semen (Prot: 10 mg) in complete Freund's adjuvant two weeks apart and then they were given three intraperitoneal booster injections with same material without adjuvant 2 weeks after the second injection with 2 days between injections. The immunized rats were proved to produce sperm immobilizing antibody on 7 days after the last injection, and spleen cells were taken from these animals 3 days after another intraperitoneal injection. These rat spleen cells (1×10^8) and mouse myeloma cells (P3/X63Ag8U1; P3U1[5)], 1×10^7) were mixed in Eagles modified minimal essential medium (MEM) and centrifuged in a 50 ml conical tube. The supernatant was discarded and the cells were resuspended in 1 ml of 50% (W/V) polyethylene glycol (PEG-1500)[6)] in serum-free MEM (pH 7.4). The suspension was stirred gently at 37°C for 60 sec. and then a further 1 ml of serum-free MEM was added within 30 sec. A further 10 ml of serum-free MEM was added during the next 5 min., and then the mixture was centrifuged. The precipitated cells were gently resuspended in 20 ml of RPMI 1640 medium containing 10% fetal calf serum, 10^{-5}M 2-mercaptoethanol and 2 mM glutamine. The cell suspension was distributed in wells of a Microtest II plate (Falcon No. 3042, Oxnard, Calif., U.S.A.) at inocula of 1×10^5 tumor cells per well. The cells were incubated in a CO_2 incubator at 37°C and the culture medium was replaced by hypoxanthine aminopterin thymidine (HAT) medium after 24 hrs. This HAT medium was replaced by fresh medium every two to three days. After 14 days, the HAT medium was replaced by hypoxanthine thymidine (HT) medium, which has the same composition as HAT, but without aminopterin. After 7 days, the medium was replaced by RPMI 1640 medium. All surviving cells after culture were transferred to Falcon

Fig. 1. 10 clones producing sperm immobilizing antibody from A3 cell culture



SIV: sperm immobilization value

multi well plates (Falcon No. 3008). After culture for 6 days, the medium in each well was tested for sperm immobilizing activity to human spermatozoa[2]. Nineteen of 89 fused cell cultures produced sperm immobilizing antibody and one of the cell cultures (A3) producing antibody which indicated the strong sperm immobilizing activity was distributed into 480 wells for further recloning and 10 of 114 clones established produced sperm immobilizing antibody (Fig. 1). The clone (1C4) producing the highest antibody titer was found to produce a large amount of IgG into culture supernatants and to contain two types of chromosome (M±SD: 85±5) which were derived from rat and mouse.

Intracellular IgG in the cells of clone 1C4 was strongly stained with FITC-conjugated rabbit anti-rat IgG antibody. Immunodiffusion tests were performed between the rabbit anti-rat IgG serum and the supernatants from hybridoma 1C4, 2C1 (low antibody producing cell line) and A3. The supernatant from 1C4 gave a single precipitin line with rabbit anti-rat IgG and it indicates that 1C4 clone has secreted a large amount of monoclonal antibody in the culture medium. The supernatant from hybridoma 1C4 gave positive sperm immobilization value (SIV: 2.0) to 160 fold dilution and γ-globulin prepared from the supernatant gave positive sperm immobilization value (SIV: 2.8) at the concentration of 1.1 μg/ml. Immunodiffision test also did show a single precipitin line between γ-globulin from the supernatant (Prot: 9.0 mg/ml) and human azoospermic semen (Prot: 20 mg/ml).

These results indicate that the established hybrid cell line (1C4) produced a large amount of monoclonal complement-dependent sperm immobilizing antibody to human seminal plasma antigen, and thus a single pure antigen in human seminal plasma which was relevant to sperm immobilization could possibly be isolated by immuno-affinity chromatography.

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