Preparation and Application of Monoclonal Anti-P30 Antibody

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Since 1975, the technique of cell hybridisation or fusion has been applied to a large number of problems in biomedical science. In recent years the monoclonal antibody has been used in forensic science. Ryouichi Tsuda (1984) [1] identified seminal stain by sandwich ELISA using monoclonal gamma-sm antibody. Sensabaugh (1978) [2] successfully found a human semen specific protein (HSSP) from human semnial plasma. The HSSP was proved to be a 30000-dalton semen-specific glycoprotein of prostatic origin and thus named as P30. The present article describes the method of preparation of monoclonal P30 antibody and its application to the examination of semen or seminal stain.

MATERIALS AND METHODS

BALB/c mice about 8 weeks old were immunized by intraperitoneal and intradermal injections for three times, each with 100ug of purified human seminal protein P30 which were isolated and characterized in our lab. [3] For the two injections, the P30 was emulsified with an equal amount of complete Freund's adjuvant and the interval between the first and second injection was two weeks. The third injection with no adjuvant was given 4 weeks after the second injection and 3 days prior to the cell fusion.

The fusion procedure was a modification of routine method. 10^7 SP2/0 myeloma cells were mixed with 10^8 immunized spleen lymphocytes, centrifuged at 800 r.p.m. for 5 mins, suspended in serum-free RPMI 1640 medium and recentrifuged at 800 r.p.m. for 5 mins. Then, the supernatant was discarded and 0.7 ml of 50% polyethyleneglycol (PEG 1500) were added to the sedimented cells. The cell suspension was stirred carefully for 60 sec. 20 ml of serum-free medium were added to the fusion tube slowly and centrifuged at 800 r.p.m. for 5 mins. The supernatant was discarded. The cells were re-suspended in 40 ml HAT 1640 medium, distributed onto four 96-well microculture plates and incubated in 5% CO_2, 37 C, for 10-15 days. The supernatant of each well was assayed for antibody using ELISA.

The solid-phase binding assays for specific antibody were used: 1. solid phase binding antigen (P30 3ug/ml) - supernatant -enzyme labelled goat anti mouse IgG-colored product; 2. solid phase binding antibody (serum of guinea pig anti-P30 [4] 1: 3000) - antigen (P30 3ug/ml) - supernatant - enzyme labelled goat anti mouse IgG-colored product.

For cloning of hybridoma, the limiting dilution method was used.

For ascites growth, BALB/c mice were injected with 0.4ml liquid paraffin at an interval of 1 week and approximately 10^6 hybridoma cells were injected intraperitoneally. Typically, the mice were dying from the resulting tumors after 8 days. The mice were killed and the ascites fluids were obtained by washing the peritoneal cavity with saline.

Two methods for the examination of seminal stain using P30 McAb were established: 1. solid phase binding P30 McAb (ascites 1:10000) - seminal stain extract - serum of rabbit anti P30 [4] - enzyme labelled goat anti rabbit IgG-colored product; 2. solid phase binding antibody (serum of guinea pig anti P30 1:3000) - seminal stain extract - P30 McAb (supernatant)- enzyme labelled goat anti mouse IgG - colored product.

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RESULTS AND DISCUSSION

BALB/c mice were immunized with P30 and 12 immunized mice spleen cells were fused with SP2/0 myeloma cells. From each fusion, several hundred clones were obtained. The fusion rate was mean 80%. The supernatant from each well in which cell growth could be noted was tested for specific P30 McAb by ELISA and the positive rate was mean 2.4%.

Nine mouse McAb directed against the human seminal specific protein P30 have been isolated. These McAb only recognized and bound to P30 and P30 in the seminal plasma, and did not reacted with any other human body fluid (e.g. vaginal secretion, blood, serum, saliva, urine and colostrum) and semen from goat, cat and cock.

All of 9 P30 McAb are IgG class and IgG1 subclass determined by double diffusion.

The supernatant of hybridomas and the ascites were further tested for their antibody titers by ELISA. The titers of supernatants were 16, 32, 80 and 320 fold and the titers of ascites were 1×10^4 - 6.4×10^4 fold. The titers of hybridomas which had been cultured in vitro for 3-6 months did not reduce.

The methods for the identification of semen or seminal stain using the P30 McAb have been established successfully. Seminal stain extract diluted to 32×10^4 fold could be detected. The concentration of P30 in seminal stain extract as low as 3ng/ml could be detected.

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