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PREPARATION AND PROPERTIES OF MONOCLONAL ANTIBODIES TO RECOMBINANT HBsAg PRODUCED BY SILKWORM LARVAE

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*Monoclonal antibodies to recombinant HBsAg produced by silkworm (*Bombyx mori*) larvae were prepared. The cross reactivity of the prepared antibodies was studied by solid-phase enzyme-linked immmunosorbent assay. It has been found that the prepared antibodies interact with recombinant and plasma HBsAg.*

Key words: recombinant HBsAg, hybridoma, monoclonal antibodies.

A specific marker of hepatitis B virus from acute and chronic infections is the virus surface antigen (HBsAg), a high-molecular-weight complex glycosylated lipoproteide. HBsAg occurs in the intact virion and also in 22-nm particles circulating in the blood of patients and carriers of the virus [1]. Hybridoma technology for preparing monoclonal antibodies (MAbs) has provided new capabilities for using HBsAg. According to the literature, MAbs to HBsAg can be used not only to study the structure of the hepatitis B virus antigen but also to provide a basis for preparing diagnostic formulations [2]. One of the important biotechnological aspects of using MAbs is the preparation of high-purity HBsAg formulations.

The goal of the present research was to prepare and study the properties of MAbs to recombinant HBsAg produced by silkworm (*Bombyx mori*) larvae. Recombinant HBsAg was prepared at the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan in the Molecular Genetics Laboratory [3].

Hybridomas were prepared by fusing murine myeloma X Ag 8.653 cells with splenocytes of BALB/c mice immunized with recombinant HBsAg in the presence of polyethyleneglycol (PEG). Hybridomas that produce MAbs to recombinant HBsAg were identified among the resulting clones by primary screening using enzyme-linked immunosorbent assay (ELISA). Then, these were cloned by limited culturing in the presence of macrophages isolated from the murine peritoneum. Then, productive clones were again identified by ELISA. This resulted in 16 clones that were selected and recloned. Productive clones were cultured mass in serial increments and grown in vivo in syngenic mice. Cells of various productive clones exhibited various capabilities to induce ascitic tumors in syngenic animals (Table 1). Cells of clones 1, 13, and 16 caused formation of solid tumors in the majority of innoculated animals. Cells of clones 10 and 15 survived poorly upon i.p. administration and caused low levels of ascitic fluid formation.

MAbs from ascitic fluids were obtained by precipitation twice with $(NH_4)_2SO_4$ with following desalting over a column of Sephadex G-25. High-purity MAb preparations were prepared and purified over a column of DEAE-cellulose as required.

The prepared ascitic fluids contained from 5 to 9 mg/mL of MAbs that were reactive to HBsAg. This is evident in the high titers for direct ELISA (Table 1).

The specificity of the prepared MAbs toward HBsAg antigenic determinants was found by direct ELISA by sorption onto plates of recombinant HBsAg from *B. mori* and a parallel quality control using commercial plasma antigen (ImBio, Russia) and recombinant vaccine "Engerix-B" (SmithKline Beecham, Belgium). The MAb titers determined by ELISA are given in Table 1. The results show that MAbs prepared toward recombinant HBsAg from *B. mori* cross react with plasma HBsAg and with recombinant vaccine. This indicates that all aforementioned antigens contain analogous antigenic determinants.

The sensivity of the HBsAg determination by ELISA was 0.25 ng/mL for most clones.

The results identified the four (3, 5, 9, 11) most promising clones that were then used to produce preparative amount of MAbs toward recombinant HbsAg.

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TABLE 1. Induction of Ascitic Tumors by Hybridomas and MAb Specificity

Clone No.	Amount		Average volume of ascitic liquid, mL	Antibody titer in ELISA upon reaction with		
	innoculated animals	prepared ascites		recombinant HBsAg from *Bombyx mori*	recombinant vaccine "Engerix-B"	plasma HBsAg
1	5	3 (2)	4,3	1×10^6	1.2×10^5	1×10^6
2	5	4 (1)	2.4	1.4×10^5	1.2×10^5	1×10^5
3	6	6	6.3	3.2×10^7	1.6×10^7	3.2×10^7
4	5	3 (1)	3.0	2.5×10^5	1.2×10^5	1×10^5
5	7	7	5.8	1.8×10^7	6.4×10^6	1.4×10^7
6	8	5 (1)	4.7	1.2×10^5	1.2×10^5	1×10^5
7	6	4	5.0	5.4×10^5	2.5×10^5	2.5×10^5
8	6	5	3.2	2.6×10^5	1.2×10^5	1.4×10^5
9	7	7	5.2	4×10^7	1.8×10^7	3.2×10^7
10	5	3 (1)	1.8	1×10^6	1.2×10^5	1×10^6
11	8	7	4.9	5×10^7	3.2×10^7	5×107
12	8	6	3.7	2.5×10^5	1.2×10^5	1.2×10^5
13	8	3 (2)	2.9	1.2×10^5	6.4×10^4	1×10^5
14	7	6 (1)	3.3	8×10^5	1.2×10^5	6.4×10^5
15	5	2	1.6	5×10^5	1.2×10^5	2.6×10^5
16	6	2 (1)	2.1	1.8×10^6	2.6×10^5	1.2×10^5

Note. The number of solid tumors is given in parentheses.

The results led to the conclusion that the prepared MAbs can be used to develop diagnostic tests for detection of HBsAg in blood of patients and carriers of the virus and also to prepare pure recombinant HBsAg.

EXPERIMENTAL

Mouse Immunization. BALB/c mice of weght 15-20 g were immunized with an injection of purified recombinant HBsAg (0.5 μg, isolated from *B. mori* larvae) in phosphate-buffered saline (0.5 mL, PBS, pH 7.0) containing total Freund adjuvant (50%). After one week the procedure was repeated with incomplete Freund adjuvant and then after one week with total Freund adjuvant. Three days before fusion an i.v. booster injection (0.1 μg) of recombinant HBsAg was carried out.

Fusion of cells in suspension was carried out using PEG solution (50%, MW 1500) in RPMI-1640 with DMSO (15%).

Hybrid cells were prepared as follows. Mice were sacrificed on the third day after the last immunization. Spleens were excised using sterile techniques, placed in nutrient medium RPMI-1640 containing penicillin (100 units/mL), homogenized, and centrifuged at 800 rpm for 5 min. The washed splenocytes were treated with myeloma cells in a 5:1 ratio. The moist precipitate of cells was treated carefully and dropwise with PEG solution (0.3 mL) over 1 min, incubated for 1 min, and treated with RPMI-1640 medium (5 mL) over 3 min and another portion (10 mL) over 5 min. The volume was adjusted to 50 mL. The mixture was centrifuged at 800 rpm for 5 min. After the fusion was finished, the precipitate of cells was cultured by selective HAT medium prepared from RPMI-1640 by adding fetal-calf serum (20%), glutamine (200 mM), and penicillin (100 units/mL). Then the cells were transferred to a 96-well plates in which murine macrophages had been deposited one day before and were cultivated in a CO_2-incubator with flowing CO_2 (5%). Clones of hybrid cells grew in one week.

Selection of Hybridomas. Hybridoma clones producing antibodies to recombinant HbsAg were revealed on the 14th day after fusion. The presence of the appropriate antibodies in the culture medium was verified by solid-phase ELISA on flexible PVC plates with immobilized recombinant HBsAg. Then producing clones were transferred into 96-well plates with previously deposited macrophages for limited cultivation of 3-5 cells per well. After a relatively high cell density (~50%) in the wells was achieved, producing clones were identified using the same solid-phase ELISA. Identified clones were transferred in new plates with previously deposited macrophages at 1 cell per well, repeating again the cloning process (recloning). Then, producing

clones were mass cultivated in serial increments and grown in vivo in BALB/c mice, where they induced formation of ascitic tumors in the peritoneum. MAbs specific to hepatitis B virus surface antigen were isolated from ascitic fluid.

Antibodies were isolated by precipitation twice with $(NH_4)_2SO_4$. For this, ascitic fluid was treated with an equal volume of PBS (pH 6.8). The resulting solution was treated with an equal volume of saturated $(NH_4)_2SO_4$ solution (buffered to pH 7.2), stirred for 1 h at room temperature, and centrifuged at 10,000 rpm for 10 min. The resulting precipitate was again dissolved in the minimal volume of PBS, treated with saturated $(NH_4)_2SO_4$ solution (2/3 the volume), stirred, and left overnight at +4°C. The precipitate was separated by centrifugation at 10,000 rpm for 10 min, dissolved in PBS (5 mM, pH 8.0), and placed on a column of Sephadex G-25 equilibrated with the same buffer. Protein fractions were collected from the column using a Uvicord (LKB, Sweden) instrument at wavelength 280 nm and placed on a column of DEAE-cellulose equilibrated with the same buffer. Antibodies were collected using NaCl gradient (0-0.25 M), desalted again, lyophilized, and used for further investigations.

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