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Monoclonal antibodies against *Wuchereria bancrofti* microfilarial excretory-secretory antigens

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Abstract. A battery of monoclonal antibodies were produced against *Wuchereria bancrofti* microfilarial excretory-secretory antigens and their specificity was studied using different filarial antigens. Among the 1116 wells plated out, 42 % of the wells developed hybrids and 5 % of the hybrids showed anti *Wuchereria bancrofti* microfilarial excretory-secretory antigens. Specificity studies on the antibodies produced from 63 cloned and expanded hybrids showed 10 clones which were specifically positive only to *Wuchereria bancrofti* microfilarial excretory-secretory antigens.

Keywords. *Wuchereria bancrofti;* excretory-secretory antigens; monoclonal antibodies; enzyme linked immunosorbent assay.

Introduction

The application of hybridoma technology has a great potential in immunoparasitology to produce monoclonal antibodies of interest in diagnosis and immuno prophylaxis. Yoshida *et al.* (1981) and Potocnjak *et al.* (1980) have demonstrated the utility of monoclonal antibodies in the protection of mice against malarial infection. In schistosomiasis Grzych *et al.* (1980) have produced monoclonal antibodies demonstrateing *in vitro* cytotoxicity against schistosomules. Mitchell *et al.* (1983) and Cruise *et al.* (1981) have used selected monoclonal antibodies in the development of immunodiagnostic assays for schistosomiasis infection, des Moutis *et al.* (1983) have used monoclonal antibodies in detection of circulating antigen in onchocerciasis patients. Studies from our laboratory (Kharat *et al.,* 1982; Malhotra *et al.,* 1982 and Harinath *et al.,* 1984) have shown that *Wuchereria bancrofti* microfilarial excretory-secretory antigens (*Wb* mf ES Ag) were superior to somatic antigens in terms of specificity and sensitivity when used in immunodiagnosis of bancroftian filariasis. This communication presents successful production of monoclonal antibodies to *Wb* mf ES Ag and studies on their specificity against different filarial antigens.

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Abbreviations used: *Wb* mf ES Ag, *Wuchereria bancrofti* microfilarial excretory-secretory antigens; DMEM, Dulbecco's Modified Eagles Medium; i.p., intraperitonially; ELISA, enzyme linked immunosorbent assay; BSA, bovine serum albumin: DEA, diethonolamine; *Di* Ag, *Dirofilaria immitis* Ag; *Bm* mf Ag, *Brugia malayi* mf Ag; *Al* Ag, *Ascaris lumbricoides* Ag; *Sd* 2-4 Ag, *Setaria digitata* 2–4 Ag.

Materials and methods

Cells and media

The plasmacytoma cell line used in the fusion was Balb/c mouse derived, non secreting and azaguanine resistant (NS-1). Dulbecco's Modified Eagles Medium (DMEM, of GIBCO Laboratories, USA) supplemented with 10% fetal calf serum (K. C. Biologicals), azaguanine (0·1 mM), L-glutamine (4 mm) and gentamycin (0·1 %) was used to maintain the parental cell line (NS-1). The hybrids were grown in OPI-HAT medium *i.e.* DMEM supplemented with 10 % NCTC 109 medium (M. A. Bioproducts), 15 % fetal calf serum, bovine insulin, pyruvate, oxalo-acetate, hypoxanthine, thymidine and aminopterin. The same medium without aminopterin (OPI-HT medium) was used to maintain the cloned hybrids.

W. bancrofti microfilarial excretory-secretory antigens

Wb mf ES Ag was prepared as described by Kharat *et al.* (1982). The microfilariae were isolated by nucleopore membrane filtration (5 μm pore size) from night blood samples of microfilaraemiae cases and were maintained *in vitro* in medium 199 supplemented with organic acids and sugars of Grace's medium but without serum. The culture fluid was collected after every 24 h, centrifuged at 13000 *g* at 4°C for 30 min and concentrated 200 fold by ultrafiltration arid freeze-drying. The protein was estimated using Biorad protein assay kit.

Immunization schedule: Eight Balb/c mice were injected intraperitonially (i.p.) with 10 μg of *Wb* mf ES Ag in complete Freunds adjuvant (Grand Island Biological Company, USA). Second and third booster doses were given (i.p.) with 10 μg of antigen in incomplete Freunds adjuvant in the 4th, and 5th weeks. Filarial antibody in the serum of the immunized animals was monitored by enzyme linked immunosorbent assay (ELISA) and all the animals were showing the presence of filarial antibody (1:40 and above reciprocal of antibody titre). Last booster dose was given (i.p.) with 10 μg of antigenic preparation without any adjuvant in the eighth week and four days later spleens were removed for fusion.

Cell fusion and selection of hybrids: The procedure followed was as described by Kennett *et al.* (1978). The immune mice were sacrificed by cervical dislocation and the spleens were aseptically removed. Cells were removed from spleens by teasing them with two sterile forceps. Erythrocytes were removed by lysis with 0·85 % ammonium chloride solution for 2 min. The cells from each spleen were mixed separately with an equal number of NS-1 myeloma cells and they were pelleted together in a 50 ml round bottomed tube (Falcon). After removing the supernatant, the pellet was resuspended and 0·5 ml of fusion medium (50 μl DMSO, 350 μl polyethylene glycol 1000 and 650 μl DMEM) was added and the cells were centrifuged at 400 *g* at room temperature for 6 min. Hybrids and free cells were resuspended in 3 ml DMEM without serum and the final volume of the cell suspension was made with OPI-HT medium (26 ml to a spleen of 10^8 cells). The cells were plated out into 96 well flat bottomed tissue culture plates (Costar, Cambridge, Mass.). The plates were incubated at 37°C in a humidified atmosphere of 8 % CO2 in air. The next day, 1 drop of HT medium with 2X

aminopterin was added to each well to initiate the selection of hybrids. Four days later the HT medium was changed twice with an interval of 2 days between each change

Cloning and expansion of hybrids: The hybrids which were consistingly positive in ELISA for the production of anti *Wb* mf ES Ag antibodies were cloned by limiting dilution method in 96 well flat bottom micro culture plates (Costar), containing γ-irradiated (5000 rads) normal mouse spleen cells as feeder layers. When the clones became macroscopically visible, ELISA was run with the supernatants from 12 wells of the plate with 100 cells/well concentration. Depending upon the yields from these wells, 10 to 50 wells from 1 cell/well plate were screened in ELISA, and 2–4 positive clones from 1 cell/well plate were expanded for further analysis and storage.

Enzyme linked immunosorbent assay for screening culture supernatants: Indirect ELISA was done as described by Voller *et al.* (1979) for screening culture supernatants. Fifty μl of *Wb* mf ES Ag or other antigens diluted in 0·06 M carbonate buffer pH 9·6 were added to the wells of flat bottom PVC microtitre plates (Falcon) and incubated overnight at 4°C. After draining out the antigen solution, the wells were blocked by washing the plate three times with 0·5 % bovine serum albumin (BSA, cohn Fraction V, Sigma) in carbonate buffer. The plate was further washed 4 times with 0·01 M phosphate buffered saline pH 7·2 containing 0·05 % tween 20 (PBS/T). Fifty μl of neat culture supernatants from hybrids or NS-1 cells were added into the wells and incubated for 2 h at room temperature. After washing the plate 4 times with PBS/T, 50 μl of alkaline phosphatase labelled to affinity purified anti mouse IgG + M (Boehringer Mannheim Biochemicals) was added to the wells and incubated for 2 h at room temperature. Followed by final washing with PBS/T, 50 μl of the substrate consisting of *p*-nitrophenyl phosphate (Sigma chemical Co. USA) in diethonolamine (DEA) buffer pH 9·8 (5 mg tablet/5 ml DEA buffer) was added into wells. The optical density was measured by Titertek multiscanner (FLOWLABS) at 405 nm.

Characterization of the specificity of the immunoglobulins produced

The specificity of monoclonal antibodies was assessed by measuring their reactivity with *Wb* mf ES Ag, 5 other filarial antigens and one non-filarial helminthic antigen. The *Wb* mf soluble Ag was prepared as described by Kaliraj *et al.* (1978) and the *Wb* circulating filarial antigen-2 was prepared as described by Reddy and others (Reddy, M. V. R., Prasad, G. B. K. S. and Harinath, B. C., unpublished data). *Dirofilaria immitis* antigen (*Di* Ag), *Brugia malayi* mf Ag (*Bm* mf Ag), *Setaria digitata* 2–4 antigen (*Sd* 2–4 Ag) and the *Ascaris lumbricoides* adult worms Ag (*Al.* Ag) were obtained from Prof Willy F. Piessens and Izaskun petrelanda of Harvard School of Public Health, Boston, USA. All the antigens were coated on to microtitre plates (Falcon) at a concentration of 5 μg/ml except *Sd* 2-4 Ag which was coated at a concentration of 2 μg/ml. Negative controls included the supernatants screened in the wells coated with BS A alone and NS-1 cell culture supernatant incubated in the antigen coated wells.

Results and discussion

Among 1116 wells plated out in 2 experiments of cell-fusion, 42 % of the wells showed the development of hybrids. When the supernatants from these were tested for anti- *Wb*

mf ES Ag activity in indirect ELISA, 5 % of the hybrids showed antibody production. Hybrids from all the wells secreting anti *Wb* mf ES Ag antibodies were cloned, and 2–4 positive clones from each of the positive hybrids were selected for expansion and further studies.

Table 1 shows the results of the specificity studies on the antibodies produced by the expanded 63 clones using *Wb* mf ES Ag, 5 other filarial antigens and non filarial helminth antigen. The supernatants of the clones showing optical density two times or more than the reactivity in the wells coated with BSA alone were taken as positive. Based on these criteria 33 out of 63 clones remained positive (to secrete monoclonales) to *Wb* mf ES Ag. *Wb* mf soluble Ag, and *Al.* Ag had 19 clones secreting positive monoclonal antibodies to each of them. The other 4 filarial antigens, *Wb* circulating filarial Ag-2, *BM* mf Ag, *Di* Ag., and *Sd* 2-4 Ag had 7,1,12 and 2 positive clones respectively. Ten clones (*Wb* E9, *Wb* E 17 , *Wb* E 24, *Wb* E 25, *Wb* E 26, *Wb* E 33, *Wb* E 34, *Wb* E 35, *Wb* E 36 and *Wb* E 37) were specifically positive only to *Wb* mf ES Ag (table 2).

Table 1. Specificity of the monoclonal antibodies secreted by 63 expanded clones in indirect ELISA using different antigens.

Antigen	No. of clones* positive
Wb mf ES Ag	33
Wb mf soluable Ag	19
Wb circulating filarial Ag-2	7
Di Ag	12
Bm mf Ag	1
Sd 2-4 Ag	2
Al adult Ag	19

* Clones with optical density values more than twice to their reactivity in the wells coated with BSA only.

Studies on the diagnostic utility of *Wb* mf ES Ag (Kharat *et al.,* 1982, Malhotra *et al.,* 1982; Harinath *et al.,* 1984) have shown that the *Wb* mf ES Ag is highly useful in immunodiagnosis of filariasis. Two antigen fractions reacting differently with different stages of infection have been isolated from *Wb* mf ES Ag by ultramembrane filtration (Reddy *et al.,* 1984). It will be of interest to characterize the *Wb* mf ES Ag to understand their importance in diagnosis and in anti-parasite immunity of the host. But our previous attempts to raise precipitive antibodies to *Wb* mf ES Ag in rabbits were unsuccessful (Reddy, M. V. R. and Harinath, B. C. unpublished data). However from this study using *Wb* mf ES Ag, the first one as far as we are aware, it can be envisaged that monoclonal antibodies can be raised *in vitro* against *Wb* mf ES Ag (table 2). The monoclonal antibodies thus produced have great potential in immunodiagnostic studies and in characterizing the ES antigens. The studies are in progress in that direction.

Table 2. Relative reactivities of 10 *Wuchereria bancrofti* microfilarial excretory-secretory antigen monoclonal antibodies with other filarial and non-filarial antigens.

Clone code No.	Optical density values (405 nm) in ELISA with							
	Wb mf ES Ag	*Wb* mf S Ag	*Wb* CFA-2 Ag	*Di* Ag	*Bm* mf Ag	*Sd*2-4 Ag	*Al* ad Ag	BSA
Wb E9	1·13	0·86	0·6	0·46	0·70	0·65	0·75	0·5
Wb E17	0·86	0·58	0·52	0·42	0·57	0·50	0·67	0·43
Wb E24	0·12	0·04	0·02	0·04	0·00	0·00	0·06	0·04
Wb E25	0·14	0·04	0·01	0·05	0·00	0·00	0·01	0·04
Wb E26	0·12	0·03	0·01	0·05	0·00	0·00	0·05	0·03
Wb E33	0·746	0·46	0·23	0·24	0·50	0·43	0·35	0·36
Wb E34	0·63	0·45	0·26	0·21	0·40	0·38	0·30	0·31
Wb E35	0·11	0·04	0·04	0·07	0·00	0·02	0·08	0·05
Wb E36	0·10	0·06	0·04	0·07	0·02	0·02	0·08	0·05
Wb E37	0·10	0·07	0·04	0·06	0·00	0·04	0·06	0·04

Wb mf S Ag, *Wuchereria bancrofti* microfilarial soluble antigen; *Wb* CFA-2, Ag, *Wuchereria bancrofti* circulating filarial antigen-2; *Sd*2-4-*Setaria digitata* antigen-24; *Al* ad Ag, *Ascaris lumbricoides* adult antigen.

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