MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 33, Number 5, 2014

DOI: 10.1089/mab.2014.0060

Monoclonal Antibody 1C7 Against *Chaetomium globosum* Enolase

Antigen Used for Immunization

A recombinant *Chaetomium globosum* enolase that was produced in a bacterial expression system was used for immunizations.

Method of Immunization

Three 5- to 7-week-old female BALB/cJ mice were immunized via intraperitoneal (IP) injection with a 50:50 (v/v) emulsion of 25 μg of the purified recombinant *C. globosum* enolase protein and TiterMax® Gold Adjuvant. Five subsequent booster IP immunizations containing 5 μg recombinant *C. globosum* enolase in sterile PBS were administered at biweekly intervals. The final boost was administered 3 days before the monoclonal antibody fusion.

Parental Cell Line Used for Fusion

SP2/0-AG14 myeloma cells (ATCC# CRL-1581).

Selection and Cloning Procedure

Individual spleens were aseptically removed from each mouse and single cell suspensions of the splenocytes produced. Fusion of splenocytes with SP2/0-AG14 myeloma cells was performed as previously described,[1,2] and hybridoma cells were cultivated in Dulbecco's Modified Eagle Medium. The supernatant fluid from confluent hybridomas was diluted 1:2 (v/v) in PBS 0.05% Tween-20 containing 3% non-fat dry skim milk powder and tested using a recombinant *C. globosum* enolase indirect ELISA. Hybridomas that were confirmed to produce recombinant *C. globosum* enolase-specific MAb were selected, grown in bulk, and then transferred to a 96-well tissue culture plate and cloned twice by limiting dilution. Single positive clones were rescreened using the indirect ELISA, selected, grown in bulk, and purified according to the method of Kent.[3]

Heavy and Light Chains of Immunoglobulin

An MAb isotype ELISA was used to identify the isotype of the screened MAb. MAb 1C7 was identified to be IgG_1 isotype.

Specificity

MAb 1C7 exhibited reactivity to immobilized recombinant *C. globosum* enolase in an indirect screening ELISA. The IgG_1 isotype MAb was then further assessed in SDS-PAGE and Western blot studies. MAb 1C7 bound to immobilized recombinant *C. globosum* enolase in Western blotting. Additional Western blot studies were designed to evaluate the reactivity of MAb 1C7 to *C. globosum* protein extract fractions, including spore wash, homogenized spore, and mycelial extracts. The highest MAb 1C7 reactivity was observed to a ~49 kDa band in the homogenized spore extract that corresponded to recombinant *C. globosum* enolase. In cross-reactivity studies, the greatest MAb 1C7 reactivity was observed to *C. globosum* UAMH strains 9683 and 841 as well as a putative enolase derived from *C. atrobrunneum*. Interestingly, MAb 1C7 did not react with enolase derived from *C. indicum* or *Aspergillus fumigatus*. Divergence with as few as two amino acids in the *A. fumigatus* enolase extract resulted in the loss of MAb reactivity. Based on these data, MAb 1C7 may additionally react with other closely related species within the fungal orders Sordariales and Hypocreales.

Specific Antigen Identified

Epitope mapping of MAb 1C7 demonstrated binding to the *C. globosum* enolase decapeptide, LTYEELANLY.

Availability

Tissue culture supernatant	Yes ✓	No
Ascitic fluid	Yes	No ✓
Hybridoma cells	Yes ✓	No

References

1. Kohler G, and Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256:495–497.
2. Nayak AP, Green BJ, Friend S, and Beezhold DH: Development of monoclonal antibodies to recombinant terrelysin and characterization of expression in *Aspergillus terreus*. J Med Microbiol 2012;61:489–499.
3. Kent UM: Purification of antibodies using ammonium sulfate fractionation or gel filtration. Methods Mol Biol 1999; 115:11–18.

Address correspondence to:

Dr. Brett James Green
Allergy and Clinical Immunology Branch
Health Effects Laboratory Division
National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
1095 Willowdale Road, M/S L-4020
Morgantown, WV 26505
Telephone: +1-304-285-6127
Fax: +1-304-285-6126
E-mail: dox6@cdc.gov