

Monoclonal Antibodies 3C3, 6D4, 7D11, 9G6, 24D11, 27C10, 27E2, and 29E5

Antigen Used for Immunization

Stachybotrys chlorohalonata (ATCC 201863) cytolytic antigens (cScp) were semi-purified from tryptic soy broth culture supernatants as previously described.⁽¹⁾

Method of Immunization

Mice were immunized intraperitoneally at biweekly intervals. Mice were primed with 50 µg of cScp emulsified in equal volumes of TiterMax (TiterMax USA, Norcross, GA). The antigen concentration was reduced by half for each of five subsequent booster immunizations. A final boost of 50 µg was given 3 weeks after the sixth immunization, and mice were sacrificed 3 days later for hybridoma production.

Parental Cell Line Used for Fusion

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

Selection and Cloning Procedure

Positive clones were identified using 1 µg/mL of cScp in an indirect ELISA.

Heavy and Light Chains of Immunoglobulin

Heavy chain—mu.

Light chain—not determined.

Specificity

Hybridoma tissue culture supernatants (CSN) were screened using an indirect ELISA as previously described.⁽²⁾ The specificity of MAbs was tested against hyphal extracts of seven *S. chartarum* isolates, eight isolates of other *Stachybotrys* species, as well as 39 related and non-related fungi commonly

found in indoor environments. All extracts were tested in a capture ELISA using rabbit polyclonal antibodies as solid-phase capture reagent. Specific binding activity was documented using Western blot analysis against mycelial extracts derived from *Stachybotrys chlorohalonata* and using fluorescent halogen immunostaining with various fungal species.

Specific Antigen Identified

Exoantigens produced by *Stachybotrys chlorohalonata*.

Availability

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

References

1. Vesper SJ, Magnuson ML, Dearborn DG, Yike I, and Haugland RA: Initial characterization of the hemolysin stachylysin from *Stachybotrys chartarum*. Infect Immun 2001;69:912.
2. Schmechel D, Simpson JP, Beezhold D, and Lewis DM: The development of species-specific immunodiagnostics for *Stachybotrys chartarum*: the role of cross-reactivity. J Immunol Methods 2006;309:150.

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