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The production and characterization of monoclonal antibodies to the fungus *Aspergillus versicolor*

Abstract Fungal exposure measurements in indoor environments require accurate and precise monitoring methods. Such techniques may be based on monoclonal antibodies (Mabs) and enzyme-linked immunosorbent assays (ELISA) and here we report the cross-reactivity patterns of Mabs produced against *Aspergillus versicolor*. Balb/c mice were immunized with the particulate fraction of homogenized spores and 46 Mabs (35 IgM, nine IgG_3, two IgG_1) were produced and tested for cross-reactivity against 55 fungal species. None of the Mabs was found to be species-specific for *A. versicolor*. Several Mabs strongly cross-reacted with most *Aspergillus*, *Penicillium* and *Eurotium* species and some Mabs also cross-reacted with *Paecilomyces variotii* and several *Cladosporium* and *Stachybotrys* species. Our results show that antibody responses in mice against spores of *A. versicolor* are dominated by highly cross-reactive antibodies of the IgM isotype. The widespread cross-reactivity suggests that the specificity of antibodies to be used for the detection of fungi in environmental samples need to be thoroughly characterized in order to avoid ambiguities in the interpretation of monitoring results. Furthermore, accurate estimates of spore concentrations may require the application of species-specific Mabs in order to avoid bias in result interpretation because of the differential reactivity of cross-reactive Mabs with different fungi.

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Key words: Monoclonal antibodies; Fungi; Enzyme-linked immunosorbent assays; *Aspergillus*; Exposure measurements.

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Practical Implications

Producers of monoclonal or polyclonal antibodies for the detection of fungi in environmental or clinical samples need to verify antibody reactivity patterns and accurately report that information to potential users. Furthermore, immunoassays based on mouse or human serum or purified immunoglobulin fractions need to consider antibody cross-reactivity as a potential confounding factor during interpretation of results.

Introduction

Exposures to aerosols containing fungal spores have been associated with a variety of adverse health effects such as allergies, mycotoxicoses and infections (Kurup, 2003; Nordness et al., 2003; Yang and Johanning, 2002). Some of the most important fungi include *Aspergillus versicolor*, *Penicillium brevicompactum* and *Penicillium chrysogenum*. These fungi are not only among the most commonly found species in domestic and indoor work environments but are also known to produce a variety of harmful mycotoxins (Hocking, 2001; Pitt, 2001). Thus, accurate and precise information on the presence of fungi in the environment would help to improve patient management and also help to better understand causal relationships between fungal exposure and adverse health effects.

Current diagnostic and monitoring techniques for fungi are based on either fungal culture, spore counts using light microscopy or the detection of indicator molecules for total fungal biomass, such as (1–3)-β-D-glucan or ergosterol (Miller, 2001; Pasanen, 2001). More recent applications include the production of monoclonal antibodies (Mabs) and the design of DNA primers and probes. While fungal culture and spore counts are still the most straightforward and common methods for sample analyses, the results are highly dependent on the environmental growth conditions during sample analyses, the viability of the aerosol and the degree of the taxonomical skills of the investigator. The resultant variability and lack of comparability may compromise the validity and the reproducibility of the results and prevent the development of standardized sample analyses techniques for the definition of exposure guidelines.

Similarly, while the detection of indicator molecules may provide reliable estimates of absolute amounts of reference molecules in the sample,

variable expression patterns per unit biomass among different fungal species may not allow accurate estimates of total fungal biomass in the sample. Furthermore, biomass-dependent techniques do not account for species-specific pathogenicity factors such as the differential expression of mycotoxins or distinct abilities of certain species to invade or colonize the exposed host.

Our approach towards the development of objective monitoring techniques for fungi in indoor environments aims at the development of species-specific detection methods based on immunological techniques using Mabs and enzyme-linked immunosorbent assays (ELISA) for sample analysis. Our previous work on *Stachybotrys chartarum* has shown that species- and spore-specific Mabs can be successfully produced (Schmechel and Lewis, 2001).

In this paper we describe the production of Mabs against spores of *A. versicolor*. *A. versicolor* was selected as model fungus because it is one of the most prevalent fungi found in indoor environments, serves as an indicator organism in buildings with moisture problems and in addition to its allergenic properties may also produce harmful mycotoxins (Nielsen, 2003; Engelhart et al., 2002; Meklin et al., 2002; Sudakin, 1998). The unique advantage of Mab-based techniques is their potential to provide objective and quantitative estimates of target fungi. However, due to problems associated with antibody specificity and the lack of standardized sample collection and processing methods, their current application for monitoring purposes of fungi is limited (Schmechel et al., 2003; Chapman et al., 2000). Also, previous work with Mabs against *S. chartarum* suggests that since spores and mycelia may express different amounts of a given epitope per unit biomass, Mabs should not only be species-specific but also spore- or mycelium-specific in order to maintain valid estimates of the extent of fungal contamination (Schmechel and Lewis, 2001). The influence of the metabolic activity/quiescence on epitope expression and thus data interpretation also requires further research.

Materials and methods

Cultivation of fungi and antigen preparation

Fungi were grown on malt extract agar plates for 10 days at room temperature (RT). Spores were collected inside a biohazard fume hood from air-dried cultures by applying 1 g of glass beads (0.45–0.5 mm diameter, Braun, Melsungen, Germany) per Petri dish. The lid was put back in place and the plates were gently shaken five to 10 times back and forth which allowed the beads to roll across the spore-covered agar. We found that following this procedure ample spores were attached to the beads. Spore-covered beads were poured into a 50 ml tube and spores were then eluted off the beads by suspending the beads in 20 ml of either phosphate buffered saline (PBS) for antigen preparation or carbonate coating buffer (CCB) for ELISA-based cross-reactivity tests of Mabs. The elution process was enhanced by briefly shaking the tube a few times and spores were then separated from the beads by decanting the spore suspension after the beads had settled out.

Production of monoclonal antibodies

Spores of *A. versicolor* were collected in 5 ml PBS and 1 ml aliquots were homogenized using a Mini-Bead Beater (Biospec Products, Inc., Bartlesville, OK, USA) and 1 g of glass beads per aliquot (0.45–0.5 mm in diameter, B. Braun Biotech International GmbH, Melsungen, Germany). Spores were homogenized three times for 1 min intervals and kept on ice for 2 min between treatments. Microscopic analysis showed that the resulting homogenate was a mixture of spore debris and intact spores. Aliquots were combined and washed twice by centrifugation at $4100 \times g$ for 10 min and two female BALB/c mice were immunized, intraperitoneally, with 0.5 ml of PBS-diluted spore homogenate containing the appropriate number of spore equivalents as determined by hemacytometer counts before homogenizing the spores. The initial antigen concentration of 3×10^8 spores per mouse was gradually reduced to 1×10^6 between the first and the sixth immunization and mice were finally boosted with 5×10^6 spores per mouse 3 days before harvesting splenic lymphocytes. We used the particulate fraction of spore homogenates which included spore wall fragments in order to be able to raise Mabs which would bind to the outer spore wall. Such Mabs could then be used for sample processing not only based on ELISA but alternatively on fluorescent or flow cytometric assays. Furthermore, many types of fungal spores are covered by hydrophobins which are highly diverse among different fungi (Kershaw and Talbot, 1998) and are at the same time difficult to extract (Wösten and de Vocht, 2000) and may thus have been missed as potential specific antigens when using conventional spore extracts.

Antibody-producing cell lines (hybridomas) were produced according to standard techniques (Harlow and Lane, 1988) using SP2/0-AG14 myeloma to immortalize antigen-reactive murine plasma cells. Culture supernatants (CSN) were tested by antigen-mediated indirect ELISA as described below using spores of *A. versicolor* as antigen. Positive hybridomas were cloned twice by limiting dilution and aliquots were stored in liquid nitrogen.

Heavy and light chain isotypes were determined according to the manufacturer's instructions using specific rat anti-mouse antibodies (BioSource International, Camarillo, CA, USA).

Cross-reactivity ELISA

For hybridoma screening with spores of *A. versicolor* and cross-reactivity tests with all fungi, spores were harvested into carbonate coating buffer, pH 9.6 (CCB) and uniform spore suspensions were obtained by sonication for 10 s (Sonicator 3000; Misonix, Inc., Farmingdale, NY, USA). PolySorp ELISA plates (Nalge Nunc International, Naperville, IL, USA) were seeded with fungal spores in 100 μl per well. Harvested spore concentrations varied according to the degree of sporulation and specific concentrations used in the ELISA are listed in the legend of Figure 1. For some fungal species such as *Alternaria alternata*, *Epicoccum nigrum*, *Geotrichum candidum* and some *Fusarium* and *Stachybotrys* species comparatively lower concentrations of spores had to be used in ELISA because of poor *in vitro* sporulation. This may have resulted in lower antigen concentrations in ELISA for some species. The ELISA plates were incubated overnight at RT in a plastic box containing moist filter paper. Next morning, plates were washed twice for 5 min with 200 μl of washing buffer, PBST (PBS containing 0.05% Tween 20) and processed by the five following steps which were each separated by two washings: (i) plate blocking; incubation for 1 h at RT in 200 μl of PBST containing 1% non-fat dry Milk powder (PBSTM); (ii) hybridoma antibody incubation; incubation for 1 h at 37 °C in 100 μl of Mab culture supernatant diluted five times in PBSTM; (iii) secondary antibody incubation; incubation for 1 h at 37 °C in 100 μl of Biotin-SP-conjugated AffiPure goat anti-mouse IgG + IgM secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) at a dilution of 1/5000 in PBSTM; (iv) tracer molecule incubation; incubation for 1 h at 37 °C in 100 μl of alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Inc.) at a dilution of 1/5000 in PBSTM; and (v) substrate incubation time (SIT); incubation of 100 μl per well of *p*-nitrophenyl phosphate-containing buffer [5 mg substrate in 10 ml of buffer (1 M diethanolamine, 0.5 mM $MgCl_2$, pH 9.8)] for 30 min at RT. After incubation, the optical density (OD) was determined spectrophotometrically at 405 nm using an Ultra-Microplate Reader, Model EL_x800 (Bio-Tek Instruments, Inc., Winooski, VT, USA). The results represent the average OD of 4 ELISA well repeats which were corrected by subtracting the average OD of 4 ELISA background control wells. Assay background controls were processed in parallel but contained plain CSN instead of Mab CSN. Optical densities >0.1 were arbitrarily considered to be positive results.

Results

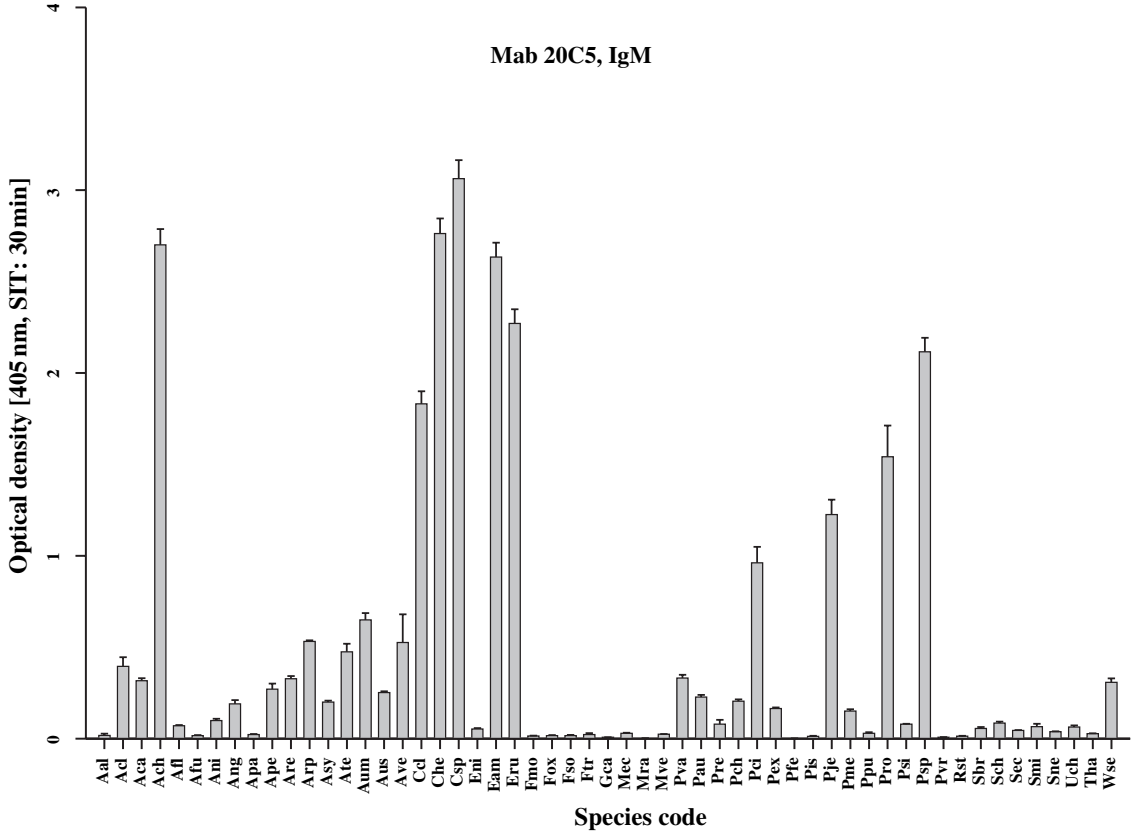
A total of 46 hybridomas were produced and isotyping showed that two hybridomas (17A7, 17G10) produced IgG_1 antibodies, nine hybridomas (6C8, 13D10, 16D6, 17A10, 17E9, 18A2, 18A3, 20D7 and 21E3) produced IgG_3 antibodies and the remaining 35 cell lines produced IgM antibodies. All hybridomas produced κ light chains.

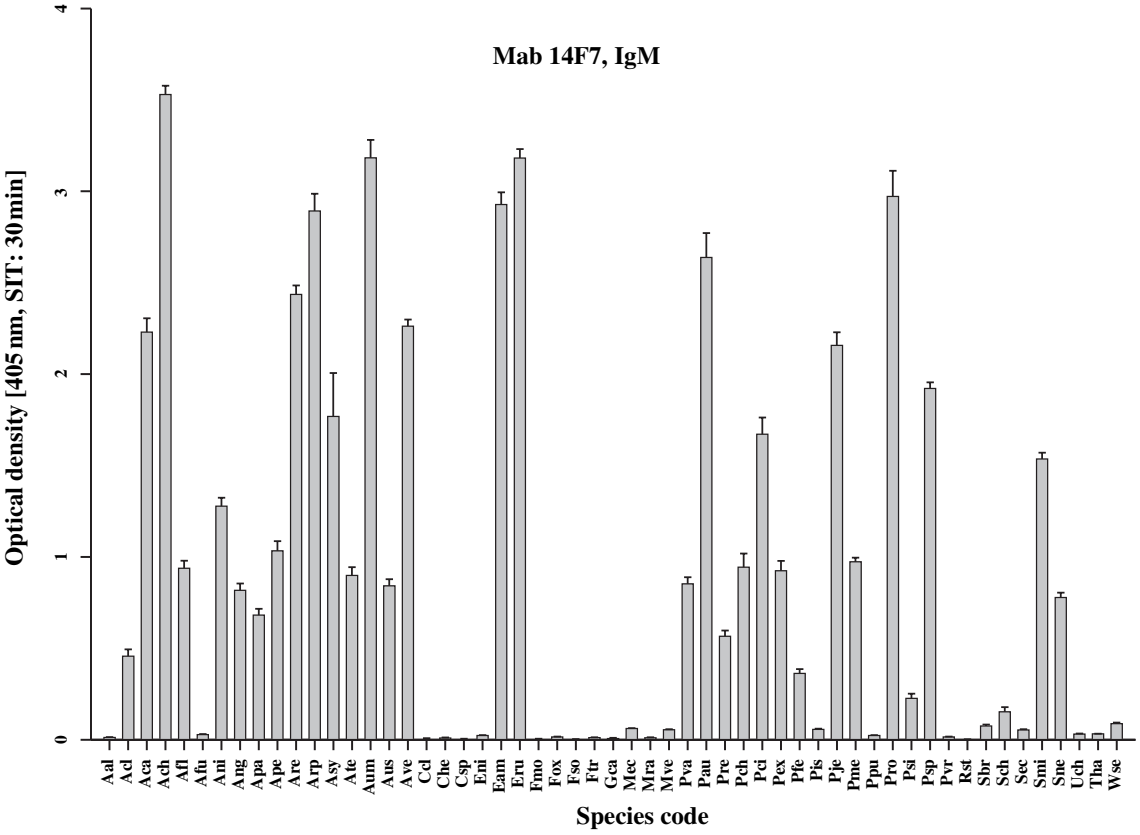
Table 1 provides a general overview of all Mab reactivities. It compares the reactivity of spores of each of 55 fungal species (table rows) with each of the 46 Mabs (table columns). Positive Mab reactions are indicated by an 'X'. Based on their overall qualitative and quantitative reactivity in the cross-reactivity ELISAs, Mabs were categorized into 14 distinct groups as indicated in the table head. The number in brackets specifies the number of Mabs found in each group that probably all react with the same epitope, i.e. the 14 groups are considered to recognize 14 distinct epitopes. For example, group 3 contained only a single Mab and group 12 contained six Mabs with identical cross-reactivity patterns. The last column in the table summarizes the number of groups (first number) and the total number of Mabs (second number) reacting with each fungal species. For example *A. clavatus* is recognized by eight groups of Mabs containing 37 Mabs and *A. fumigatus* or *A. alternata* are not recognized by any of the Mabs. The summaries in the last column can be considered to be indicators of antigenic relatedness. For example, *A. versicolor*, *A. sydowii*, *Penicillium jensenii* and *P. roqueforti* share reactivity with 12 of 13 groups of Mabs reacting with each fungus and they can be considered antigenically closer related to each other than they are for example, to *A. alternata* or several *Fusarium* species which are not recognized by any of the Mabs. Groups 5, 9 and 11 contained only IgG antibodies and groups 1, 2, 3, 4, 6, 7, 8, 10, 12 and 14 contained only IgM antibodies. Group 13 contained a mixture of IgG and IgM antibodies.

The bottom row of Table 1 shows the number of fungal species recognized by each group of Mabs and it indicates how common the epitope recognized by that group of Mabs is among the 55 tested fungal species. For example, the epitope recognized by Mab group 10 is expressed and thus shared by 33 fungi including several species of *Aspergillus* and *Penicillium*, as well as multiple species of *Paecilomyces*, *Stachybotrys* and *Wallemia*.

There was a general tendency of groups containing more Mabs to cross-react with more fungal species. This may be an indication that the immune response to spores of *A. versicolor* is dominated by cross-reactive epitopes.

None of the Mabs was found to be species-specific for *A. versicolor* and cross-reactivity especially among *Aspergillus*, *Eurotium* and *Penicillium* species was extensive. For example, Mabs of groups 10, 12, 13


Mab 20C5, IgM
Optical density [405 nm, SIT: 30 min]
0
1
2
3
4
Aal Acl Aca Ach Afl Afu Ani Ang Apa Ape Are Arp Asy Ate Aum Aus Ave Ccl Che Csp Eni Eam Eru Fmo Fox Fso Ftr Gca Mec Mra Mve Pva Pau Pre Pch Pci Pex Pfe Pis Pje Pme Ppu Pro Psi Psp Pvr Rst Sbr Sch Sec Smi Sne Uch Tha Wse
Species code


Mab 14F7, IgM
Optical density [405 nm, SIT: 30 min]
0
1
2
3
4
Aal Acl Aca Ach Afl Afu Ani Ang Apa Ape Are Arp Asy Ate Aum Aus Ave Ccl Che Csp Eni Eam Eru Fmo Fox Fso Ftr Gca Mec Mra Mve Pva Pau Pre Pch Pci Pex Pfe Pis Pje Pme Ppu Pro Psi Psp Pvr Rst Sbr Sch Sec Smi Sne Uch Tha Wse
Species code

and 14 reacted with all *Aspergillus* and *Penicillium* species other than *A. fumigatus*, *P. islandicum*, *P. purpurogenum* or *P. variabile* which were not recognized by any of the Mabs produced. However, selective reactivities of all other groups of Mabs with *A. candidus*, *A. clavatus*, *A. flavus*, *A. parasiticus*, *P. brevicompactum*, *P. fellutanum* or *P. simplicissimum* also indicated antigenic differences between species within both genera. Mabs of group 2 and especially group 8 broadly cross-reacted with several species of *Aspergillus*, *Cladosporium*, *Eurotium* and *Penicillium*. None of the Mabs was found to react with *A. alternata*, *E. nigrum*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *F. tricinctum*, *G. candidum*, *Memnoniella echinata*, *Mucor ramannianus*, *Myrothecium verrucaria*, *Rhizopus stolonifer*, *S. echinata*, *Ulocladium chartarum* or *Trichoderma harzianum*.

Figure 1 and 2 show quantitative ELISA results for individual Mabs to document the variability and magnitude of Mab cross-reactivity. Figure 1 demonstrates that the epitopes recognized by the two IgM isotype Mabs 20C5 and 14F7 are expressed at highly variable amounts on a per spore basis by different *Aspergillus* and *Penicillum* species as well as two *Eurotium* species which are closely related to the genus *Aspergillus*. Alternatively, the observed differential Mab reactivities for different fungi may be because of the expression of slightly different epitopes by different fungi which would result in differential Mab affinities. Mab 20C5 was also found to strongly cross-react with several *Cladosporium* spp. and Mab 14F7 was found to strongly cross-reacts with *Paecilomyces variotii*, *S. microspora* and *S. nephrospora*. The extent of individual Mab reactivities also show significant intra-genus antigenic diversity among species of *Aspergillus* and *Penicillium* such that some species of both genera shared more Mab-reactive epitopes with each other than they share with other species within their respective genus. For example, based on the reactivity of Mab 14F7, *A. versicolor* and *A. candidus* seem to be closer related to *P. jensenii* or *P. spinulosum* than to some other *Aspergillus* species such as *A. clavatus* or *A. terreus*. Most remarkably, *A. fumigatus* was not recognized by any of the Mabs. Figure 2 shows the cross-reactivities of two IgG isotype Mabs (17G10, IgG_1; 18A3, IgG_3). Although neither Mab showed noticeable cross-reactivity with any of the non-related fungi, both react differentially with several *Aspergillus*, *Penicillium* and *Eurotium* species. This suggests that even purified IgG fractions of animal or human sera may still be susceptible to experimental bias because of antibody cross-reactivity.

Discussion

The results show that species-specific Mabs against *Aspergillus versicolor* are difficult to raise because of widespread cross-reactivity among related fungi such as *Aspergillus* or *Penicillium* species. Even phylogenetically distant species such as *Cladosporium* and *Stachybotrys* or *Wallemia* were found to share multiple epitopes with *Aspergillus* and *Penicillium* species. We also observed that teleomorph stages of fungi such as *Eurotium* and anamorph stages such as *Aspergillus* express many common epitopes since all but one Mab reacted with two *Eurotium* species. Furthermore, Mabs reacting with many fungi (>25) by far outnumbered those reacting with lower numbers of fungi (<10). This seems to indicate that murine immune responses against spores of *A. versicolor* are dominated by cross-reactive epitopes. Also, only groups with highly cross-reactive Mabs contained Mabs derived from different animals which further strengthens the suggestion that cross-reactive epitopes may be conserved and dominate the immune responses against this fungus. The reasons for the dominance of shared epitopes are not entirely clear but may well have to do with the fact that cross-reaction might also provide cross-protection against a broader spectrum of fungi for the exposed host.

Also, the fact that *A. fumigatus* was not recognized by any of the 46 Mabs demonstrates its unique

Fig. 1 The reactivities of two IgM isotype monoclonal antibodies 20C5 and 14F7 with 55 fungal species commonly found in indoor environments. Each column represents the optical density (OD) after a substrate incubation time (SIT) of 30 min. ELISA plates were coated with spores overnight and antibody reactivity was determined the following day using an indirect alkaline phosphatase-based ELISA. The three-letter abbreviation denotes the species code and the number in brackets indicates the number of spores per ELISA well which was used during the cross-reactivity analysis. *Alternaria alternata* (Aal, 812), *Aspergillus clavatus* (Acl, 10^5), *Aspergillus candidus* (Aca, 1.32×10^4), *A. chevalieri* (Ach, 10^5), *A. flavus* (Afl, 10^5), *A. fumigatus* (Afu, 10^5), *A. nidulans* (Ani, 10^5), *A. niger* (Ang, 10^5), *A. parasiticus* (Apa, 10^5), *A. penicillioides* (Ape, 10^5), *A. restrictus* (Are, 10^5), *A. repens* (Arp, 10^5), *A. sydowii* (Asy, 10^5), *A. terreus* (Ate, 10^5), *A. umbrosis* (Aum, 10^5), *A. ustus* (Aus, 10^5), *A. versicolor* (Ave, 10^5), *Cladosporium cladosporioides* (Ccl, 10^5), *C. herbarum* (Che, 7.025×10^4), *C. sphaerospermum* (Csp, 6.175×10^4), *Epicoccum nigrum* (syn. E. purpurascens) (Eni, 2.25×10^3), *Eurotium amstelodami* (Eam, 10^5) and *E. rubrum* (Eru, 5×10^3). *Fusarium moniliforme* (Fmo, 10^5), *F. oxysporum* (Fox, 10^5), *F. solani* (Fso, 5×10^3), *F. tricinctum* (Ftr, 1.175×10^4), *Geotrichum candidum* (Gca, 2×10^3), *Memnoniella echinata* (Mec, 10^5), *Mucor ramannianus* (Mra, 3.75×10^3), *Myrothecium verrucaria* (Mve, 2.75×10^3), *Paecilomyces variotii* (Pva, 10^5), *Penicillium aurantiogriseum* (Pau, 10^5), *P. brevicompactum* (Pre, 10^5), *P. chrysogenum* (Pch, 10^5), *P. citrinum* (Pci, 10^5), *P. expansum* (Pex, 10^5), *P. fellutanum* (Pfe, 10^5), *P. islandicum* (Pis, 10^5), *P. jensenii* (Pje, 10^5), *P. melinii* (Pme, 10^5), *P. purpurogenum* (Ppu, 10^5), *P. roqueforti* (Pro, 10^5), *P. simplicissimum* (Psi, 10^5), *P. spinulosum* (Psp, 10^5), *P. variabile* (Pvr, 10^5), *Rhizopus stolonifer* (Rst, 10^5), *Scopulariopsis brumptii* (Sbr, 7×10^4), *Stachybotrys chartarum* (Sch, 3×10^4), *S. echinata* (Sec, 10^5), *S. microspora* (Smi, 6.33×10^3), *S. nephrospora* (Sne, 3.75×10^3), *Ulocladium chartarum* (Uch, 1.15×10^4) *Trichoderma harzianum* (Tha, 10^5), and *Wallemia sebi* (Wse, 10^5)

Table 1 Reactivity of 46 Mabs produced against spores of *Aspergillus versicolor*. Fifty-five fungal species were grown on malt extract agar and collected spores were tested in ELISA

	Group of monoclonal antibodies[a]														
Fungal species	1 (1)	2 (1)	3 (1)	4 (1)	5 (2)	6 (2)	7 (2)	8 (2)	9 (2)	10 (3)	11 (5)	12 (6)	13 (7)	14 (11)	SUM[b] (46)
Alternaria alternata															0
Aspergillus clavatus	X						X	X		X	X	X	X	X	8, 37
Aspergillus candidus			X	X				X		X	X	X	X	X	8, 36
Aspergillus chevalieri		X	X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Aspergillus flavus	X				X		X			X	X	X	X	X	8, 37
Aspergillus fumigatus															0
Aspergillus nidulans			X	X	X		X		X	X	X	X	X	X	10, 40
Aspergillus niger	X						X	X		X	X	X	X	X	8, 37
Aspergillus parasiticus	X						X			X	X	X	X	X	7, 35
Aspergillus penicillioides			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Aspergillus restrictus			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Aspergillus repens			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Aspergillus sydowii	X		X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Aspergillus terreus				X	X		X	X		X	X	X	X	X	9, 39
Aspergillus umbrosis			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Aspergillus ustus			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Aspergillus versicolor	X		X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Cladosporium cladosporioides		X						X							2, 3
Cladosporium herbarum		X						X							2, 3
Cladosporium sphaerospermum		X						X							2,3
Epicoccum nigrum															0
Eurotium amstelodami		X	X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Eurotium rubrum		X	X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Fusarium moniliforme															0
Fusarium oxysporum															0
Fusarium solani															0
Fusarium tricinctum															0
Geotrichum candidum															0
Memnoniella echinata															0
Mucor ramannianus															0
Myrothecium verrucaria															0
Paecilomyces variotii								X		X	X	X	X	X	6, 34
Penicillium aurantiogriseum			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Penicillium brevicompactum										X		X	X	X	4, 27
Penicillium chrysogenum			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Penicillium citrinum			X	X	X	X	X	X	X	X	X	X	X	X	12, 44
Penicillium expansum			X	X	X		X	X		X	X	X	X	X	10, 40
Penicillium fellutanum				X			X			X	X	X	X	X	7, 35
Penicillium islandicum															0
Penicillium jensenii		X	X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Penicillium melinii				X	X		X	X		X	X	X	X	X	9, 39
Penicillium purpurogenum															0
Penicillium roqueforti		X	X	X	X	X	X	X	X	X	X	X	X	X	13, 45
Penicillium simplicissimum										X		X	X		3, 16
Penicillium spinulosum		X	X	X	X		X	X	X	X	X	X	X	X	12, 43
Penicillium variabile															0
Rhizopus stolonifer															0
Scopulariopsis brumptii												X			1, 6
Stachybotrys chartarum										X					1, 3
Stachybotrys echinata															0
Stachybotrys microspora				X						X					2, 4
Stachybotrys nephrospora										X					1, 3
Ulocladium chartarum															0
Trichoderma harzianum															0
Wallemia sebi								X		X					2, 5
Number of fungi recognized	6	9	19	23	21	15	25	27	17	33	27	30	29	28	

X indicates reactivity; empty box indicates no reactivity.

[a]Mabs were grouped according to similar specificity profiles and the number in brackets denotes the number of Mabs in each group; group 1, Mab 16E1; group 2, Mab 17B7; group 3, Mab 3C6; group 4, Mab 4F4; group 5, Mabs 18A2, 18A3; group 6, Mabs 6C5, 4B11; group 7, Mabs 3E2, 19E11; group 8, Mabs 17E2, 20C5; group 9, Mabs 17A7, 17G10; group 10, Mabs 14F7, 18B2, 18E2; group 11, Mabs 13D10, 17A10, 17E9, 20D7, 21E3; group 12, Mabs 9G9, 12C1, 14D6, 18E1, 19C9, 21F4; group 13, Mabs 1B9, 6C8, 13C6, 16D6, 17A8, 17C8, 21C7; group 14, Mabs 2G2, 3E4, 8G10, 10E6, 12C6, 12E4, 14G2, 17E8, 18F7, 19D11, 20C6.

[b]The first number indicates the number of groups and the second the number of Mabs reacting with a particular fungus.

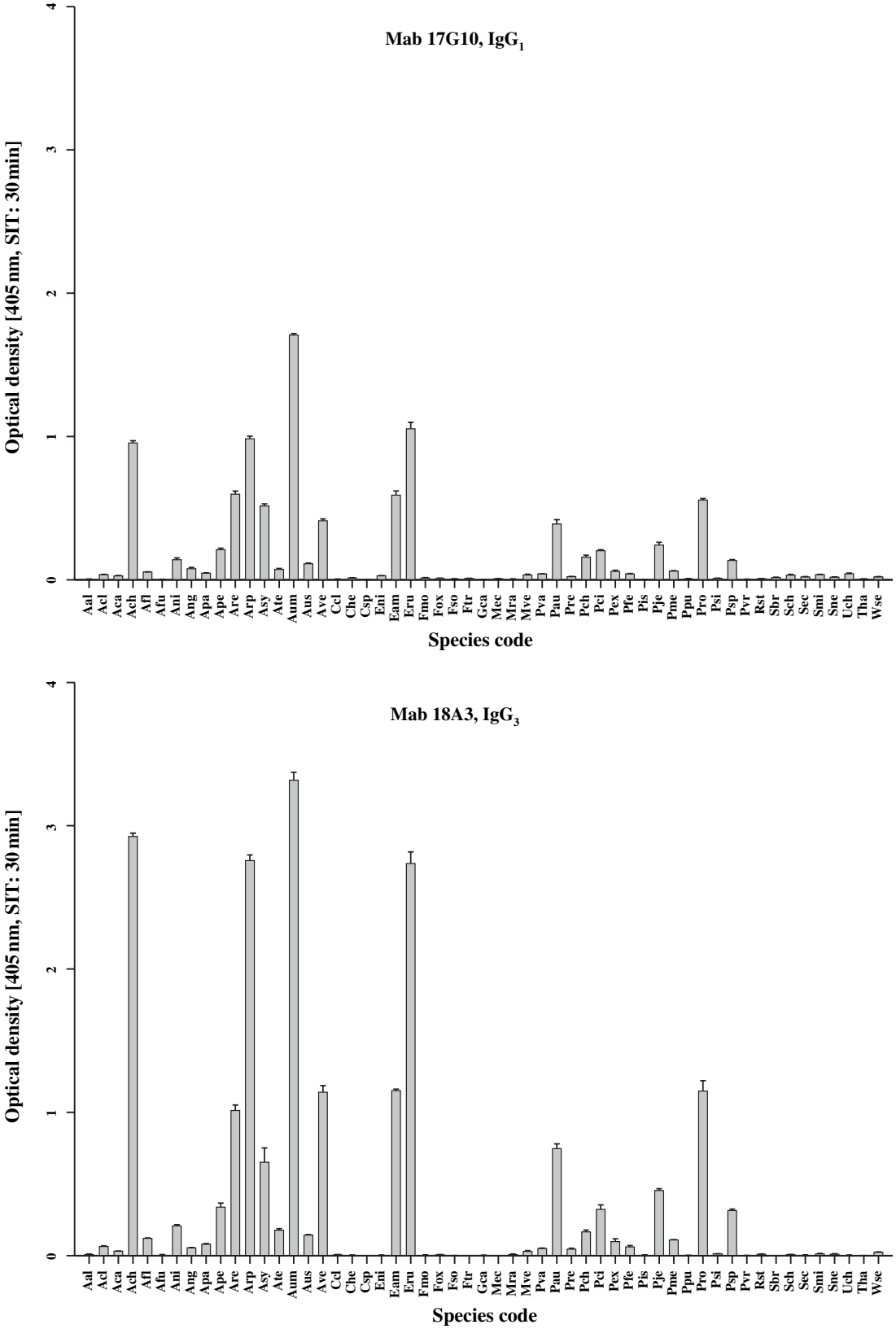


Fig. 2 The reactivities of two IgG isotype Mabs, 17G10 (IgG_1) and 18A3 (IgG_3), with 55 fungal species commonly found in indoor environments. Each column represents the optical density (OD) after a substrate incubation time (SIT) of 30 min. ELISA plates were coated with spores overnight and antibody reactivity was determined the following day using an indirect alkaline phosphatase-based ELISA. For the species code see the legend of Figure 1, p15

antigenic position within the genus of *Aspergillus*. Although no other consistent patterns of overall Mab reactivity for any other *Aspergillus* species was observed, antigenic profiling with Mabs may well become an additional tool in the future to investigate phylogenetic relationships within the genus *Aspergillus*.

The results also show that *Penicillium* species belonging to the subgenus *Biverticillium* such as *P. islandicum*, *P. purpurogenum* and *P. variabile* are clearly different in their antigenic composition from other *Penicillium* species since none of the 46 Mabs was found to cross-react with any of them. This not only confirms classical fungal taxonomy but also agrees with earlier reports based on the reactivity of polyclonal antibodies (Notermans et al., 1998).

Conclusions and implications

The immunodominance of cross-reactive antigens suggests that in order to produce species-specific Mabs immunization schemes need to be developed which direct the immune response toward unique epitopes. The immunization with immune complexes or the co-administration of antigen-reactive passive antibodies have been suggested to alter immune responses by recruiting a more diverse population of B cells into the antigen-responsive repertoire (Nie et al., 1997) and also to suppress immunodominant epitopes (Benkirane et al., 1987). We are currently investigating these possibilities for the production of species-specific Mabs against *A. versicolor*, *P. brevicompactum*, *P. chrysogenum* and *S. chartarum*. As an alternative approach, we have also recently started to investigate phage displayed peptide libraries as another source to obtain species-specific reagents for these fungi. Some of the more cross-reactive Mabs will be investigated as components of antibody 'cocktails' for the broad-spectrum detection of fungi in both initial screening assays to monitor hygienic standards for drinking water or food safety as well as the evaluation of remediation measures.

The observed differential cross-reactivities also imply that environmental monitoring results obtained with cross-reactive monoclonal or polyclonal antibodies need to be interpreted with great care. For example, in the absence of any other supporting analytical evidence, a positive ELISA result obtained for a given environmental sample may be because of the presence of any one or a mixture of all the fungi known to react with the antibody. Furthermore, the fact that cross-reactive Mabs may react differently with standardized amounts of spores of different fungi suggest that a given ELISA result may be equally because of the presence of many spores of a particular fungus expressing lower numbers of antibody-reactive epitopes per spore or because of the presence of fewer spores of another cross-reactive fungus expressing the epitope at higher densities. In order to avoid such analytical ambiguities we would recommend species-specific Mabs for the identification of fungi in environmental samples. Furthermore, users of commercial and non-commercial antibodies should carefully consult or experimentally obtain cross-reactivity patterns for antibodies to be used in environmental monitoring.

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