Supplementary Materials

Methods

Characteristics of subjects with ABPM

Relevant clinical information is summarized in Supplemental Table 1. Evidence of mold hypersensitivity based on skin testing was as follows: Patient 1 (P1): Aspergillus, Cephalsporium, Mold mix #1 (GREER), Mold mix #2 (GREER); patient 2 (P2): Alternaria, Aspergillus, Helminthosporium, Cladosporium, Eppicoccum, Penicillium; patient 3 (P3): Aspergillus, Epicoccum, Hormodendrum, Penicillium, Alternaria.

The first patient (P1) is an adolescent male with history of cystic fibrosis. He has significantly elevated total serum IgE as well as evidence of *Aspergillus* precipitins, in the setting of recurrent exacerbations that improved with steroids. Of note, while he has evidence of a IgE sensitization to multiple molds, his skin testing to other common aeroallergens was negative. His surveillance sputum cultures grew *Aspergillus fumigatus* at least 5 times, but, to date, no other fungal species were isolated.

The second patient (P2) is an adult male with cystic fibrosis. The diagnosis of ABPM was made based on markedly elevated serum IgE and evidence of mold sensitization by skin test, including *Apergillus* spp. Interestingly, serum testing for *Aspergillus* precipitans was negative. This patient repeatedly grew a number of *Aspergillus* spp, including *Aspergillus fumigatus*, *Aspergillus terreus*, and *Aspergillus nidulans* from surveillance sputum cultures.

The third patient (P3) is an elderly man with longstanding history of asthma, without evident bronchiectasis. He has had markedly elevated total IgE and evidence of *Aspergillus* sensitization based on significantly elevated *Aspergillus* specific IgE in ImmunoCAP. He had a broad pattern of allergic sensitization including animal dander, grasses, weeds, and trees in addition to mold sensitization. He was not evaluated for the presence of *Aspergillus* precipitins. He grew *Aspergillus fumigatus* a single time from sputum culture.

Human hybridoma generation: expansion of human B cells

IgE secreting human hybridomas were generated with using an overall scheme similar to that we have published previously for making IgG mAbs, but with many modifications (16-17). See Supplemental Figure 1 for a graphic overview of our human hybridoma generation scheme. Previously cryopreserved samples were thawed rapidly in a 37°C water bath and washed in 10 ml

of prefusion medium (ClonaCell-HY 03801; Stemcell Technologies). The cells were counted and viability was assessed with trypan blue staining (Gibco 15250-061) before plating. For every 2 million viable cells, the following was then added: 30 ml of warmed prefusion medium, 20 μl of CpG stock (2.5 mg/ml; ODN 2006), 1 μl each of mouse anti-human kappa (Southern Biotech; 9230-01) and mouse anti-human lambda (Southern Biotech; 9180-01), and 1 million gamma irradiated NIH3T3 fibroblast line genetically engineered to constitutively express cell-surface human CD154 (CD40 ligand), secreted human B cell activating factor (BAFF) and human IL-21 (kindly provided by Dr. Deepta Bhattacharya; Washington University in St. Louis, St. Louis, MO). The mixture then was plated into 96-well flat bottom plate (Falcon, 353072) at 300 μl/well, and plates were incubated at 37°C with 5% CO2 for 6 days, prior to screening for IgE secretion using an ELISA.

Human hybridoma generation: IgE screening ELISA

Omalizumab was used as a capture antibody, coating 384-well black ELISA plates (Greiner, 781076) at a concentration of 10 μg/ml in carbonate binding buffer overnight at 4°C. The plates then were blocked with 100 μl of blocking solution/well and incubated at room temperature for 1 h. Blocking solution was prepared with 10 g of powdered milk, 20 ml of goat serum, and 100 ml of 10X phosphate-buffered saline (PBS), mixed to a 1 liter final volume with distilled H_2O. Plates were washed five times with PBS, and 100 μl of supernatant was transferred from one well of a 96-well plate containing B cell lines, using an VIAFLO-384 electronic pipetting device (Integra Biosciences). The plates were incubated at room temperature for 1 h prior to five washes with PBS. Secondary antibody (mouse anti-human IgE Fc; Southern biotech, 9160-05) was applied at a 1:1,000 dilution in blocking solution using 25 μl/well, and the plates were again incubated at room temperature for 1 h. After 10 washes with PBS, fluorogenic peroxidase substrate solution (QuantaBlu; Thermo Scientific 15162) was added at 25 μl/well, as per manufacturer instructions, and the plates were incubated at room temperature for 30 min before addition of stop solution. Relative fluorescence intensity was determination on a Molecular Devices plate reader set for excitation and emission maxima of 325 nm and 420 nm, respectively.

Human hybridoma generation: electrofusion of B cells with myeloma fusion partner

HMMA2.5 nonsecreting myeloma cells (kindly provided by Marshall Posner, Mount Sinai,

New York, NY) were counted and suspended as 10 million cells/ml in warmed cytofusion medium composed of 300 mM sorbitol (Fisher, BP439-500), 0.1 mM calcium acetate (Fisher, AC21105-2500), 0.5 mM magnesium acetate (Fisher, AC42387-0050), and 1.0 mg/ml of bovine serum albumin (Sigma, A2153). After 8 days in 96-well plates, cells were pipetted gently into microcentrifuge tubes containing 1 ml of warmed cytofusion medium. B cells and HMMA2.5 cells were centrifuged at 900 *g* for 3 min, the supernatants were decanted, and the pellets were suspended in 1 ml of cytofusion medium; this process was repeated three times to ensure equilibration to the cytofusion medium. The cytofusion medium then was decanted gently from each sample tube such that 100 μl remained and the pellet was retained. HMMA2.5 cells were then suspended in cytofusion medium to achieve a concentration of 10 million cells/ml. Then, 125 μl of HMMA2.5 cell suspension was added to each sample tube and the mixture was pipetted into cytofusion cuvettes (BTX, 450125), and the cuvette placed in a BTX cuvette holder (BTX Safety stand, model 630B). Cytofusion was performed using a BTX ECM 2001 generator (BTX; 45-0080) with the electrical discharge programed to run with following settings: a prefusion AC current of 70 V for 40 s, followed by a DC current pulse of 360 V for 0.04 ms and then a postfusion AC current of 40 V for 9 s. After fusion, the cuvettes were incubated at 37°C for 30 min. The content of each cuvette was then added to 20 ml of hypoxanthine-aminopterin-thymidine (HAT) medium containing ouabain, composed of the following: 500 ml of post- fusion medium (Stemcell Technologies, 03805), one vial 50x HAT (Sigma, H0262), and 150 μl of a 1 mg/ml stock of ouabain (Sigma, 013K0750). Fusion products then were plated at 50 μl/well into 384-well plates (Nunc, 164688), followed by incubation at 37°C for 14 days before screening hybridomas for IgE antibody production by ELISA.

Human hybridoma generation: mAb production and purification

Wells containing hybridomas producing IgE antibodies were cloned biologically by indexed single cell flow cytometric sorting into 384-well culture plates. Once clonality was achieved, each hybridoma was expanded in postfusion medium (Stemcell Technologies, 03805) until 50% confluent in 75-cm^2 flasks (Corning, 430641). MAb was expressed by large-scale growth of the hybridoma in serum free medium (Gibco Hybridoma-SFM; Invitrogen, 12045084). After harvesting cells in 75-cm^2 flasks with cell scraper, the hybridomas were washed in serum-free medium and split equally among four 225-cm^2 flasks (Corning, 431082) containing 250 ml of

serum-free medium. IgE antibody was then purified by immunoaffinity chromatography (Omalizumab covalently coupled to GE Healthcare NHS activated HiTRAP; 17-0717-01) and visualized by SDS-PAGE for purity

ImmunoCAP and Western blot analysis

An antibody was considered positive if it bound to *A. fumigatus* in ImmunoCAP (>1.0 kUA/L) and/or one or more *A. fumigatus* extracts in ELISA (RFU >5 x background). It should be noted that we chose a much higher cutoff for defining ImmunoCAP positivity than the standard cutoff for serum analysis. Since we were using IgE mAbs at a known concentration, we were screening for allergen presence in the assay, not measuring IgE antibody. For each positive mAb, Western blot analysis was performed against all four extracts. Immunoprecipitation followed by mass spectrometry analysis was attempted for each mAb which bound to one or more extracts in ELISA and/or in Western blot.

Mold extracts obtained from GREER, Hollister-Stier, ALKB, and Allergy Laboratories were mixed with loading buffer, separated by SDS-PAGE under nonreducing/nondenaturing conditions and transferred to PVDL membrane. After blocking overnight at 4^{o}C, monoclonal IgE containing supernatant from hybridoma cultures was added for 1 hour at room temperature. After washing in PBS, HRP conjugated mouse anti-human IgE secondary antibody was added at 1:1,000 dilution in blocking solution and the blot incubated for 1 hour at room temperature. Blots were visualized using chemiluminescent substrate (Supersignal Pico; Thermo Scientific 34577) on a GE Healthcare ImageQuant LAS 4000 Imager.

Supplemental Table 1: IgE hybridoma reactivity

Subject code	IgE Hybridoma	*A. fumigatus* ImmunoCAP (kUA/L)	*A. fumigatus* extract reactivity ELISA				*A. fumigatus* extract reactivity WB				Immuno-precipitation
			GREER	HS	ALKB	AlrgyL	GREER	HS	ALKB	AlrgyL	
P1	**18F12**	*0.46*	-	-	-	-	-	-	-	-	N/A
	17A5	*1.84*	-	-	+	-	-	-	+	-	+
	10C4	*10.2*	-	+	-	+	-	+	-	+	+
	4F8	*0.16*	-	-	+	-	-	-	+	-	+
	8G2	*0.28*	-	-	-	-	-	-	-	-	N/A
	20F7	*<0.1*	-	-	-	-	-	-	-	-	N/A
	5E2	*0.31*	-	-	-	-	-	-	-	-	N/A
	10E8	*0.36*	-	-	-	-	-	-	-	-	N/A
	5A3	*0.30*	-	-	-	-	-	-	-	-	N/A
	6G9	*0.37*	-	-	-	-	-	-	-	-	N/A
	2B3	*<0.1*	-	-	-	-	-	-	-	-	N/A
	15C12	*2.55*	-	-	-	-	-	-	-	-	ND
	1C9	*9.39*	+	+	+	+	+	+	+	+	+
	5B7	*0.16*	-	-	-	-	-	-	-	-	N/A
	7C7	*0.18*	-	+	+	-	-	-	-	-	N/A
P2	**4D3**	*0.19*	-	-	-	-	-	-	-	-	N/A
	1D3	*6.51*	+	+	-	+	+	+	-	+	+
	21H12	*0.21*	-	-	-	-	-	-	-	-	N/A
	12C8	*5.63*	-	-	-	-	-	-	-	-	ND
	5H12	*0.50*	-	-	-	-	-	-	-	-	Sorrel
	8D6	*8.68*	-	-	-	-	-	-	-	-	ND
	16C5	*0.16*	-	+	+	-	-	-	-	-	ND
	4E12	*36.2*	+	+	+	+	+	+	-	-	+
	7E9	*0.12*	-	-	-	-	-	-	-	-	N/A
	1C1	*<0.1*	-	-	-	-	-	-	-	-	N/A
	2C8	*16.2*	-	-	+	-	-	-	-	-	+
	5C6	*>100*	-	-	+	-	-	+	-	+	+
	12G3	*0.36*	-	-	-	-	-	-	-	-	N/A
	12G11	*0.18*	-	-	-	-	-	-	-	-	N/A
	14H4	*2.99*	-	-	+	-	-	-	+	-	+
	9G9	*0.33*	-	-	-	-	-	-	-	-	N/A
	13A1	*0.37*	-	-	-	-	-	-	-	-	N/A
	2G11	*0.79*	-	-	+	-	-	-	-	-	ND
	18B7	*<0.1*	-	-	-	-	-	-	-	-	N/A
	3H5	*44.7*	-	-	-	-	-	-	-	-	ND
	24F3	*0.53*	-	-	-	-	-	-	-	-	N/A
	22H5	*7.51*	-	-	-	-	-	-	-	-	ND
	21E2	*6.34*	-	-	+	-	-	+	-	-	+
	15D8	*0.16*	-	-	-	-	-	-	-	-	N/A
	1C6	*<0.1*	-	-	-	-	-	-	-	-	N/A
	4H4	*0.30*	-	-	-	-	-	-	-	-	N/A
	19A4	*<0.1*	-	-	-	-	-	-	-	-	N/A
	3D4	*5.88*	-	-	+	-	-	+	-	-	+
	3F3	*61.6*	-	-	+	-	-	-	+	-	+
	20H10	*0.32*	-	-	-	-	-	-	-	-	N/A
	12D6	*0.30*	-	-	-	-	-	-	-	-	N/A
	5B5	*>100*	+	+	+	+	-	-	-	-	+
	13H5	*0.38*	-	-	-	-	-	-	-	-	N/A
	8F5	*0.13*	-	-	-	-	-	-	-	-	N/A
	7B4	*0.18*	-	-	-	-	-	-	-	-	N/A
	9C3	*>100*	-	+	+	+	-	-	-	-	+
	3F8	*1.92*	-	-	-	-	-	-	-	-	ND

	13B5	*0.16*	-	-	-	-	-	-	-	-	N/A
P3	**BH1**	*0.42*	-	-	-	-	-	-	-	-	N/A
	10H2	*0.19*	-	-	-	-	-	-	-	-	N/A
	23H7	*0.29*	-	-	-	-	-	-	-	-	N/A
	9E1	*1.63*	-	-	-	-	-	-	-	-	ND
	3B1	*0.12*	-	-	-	-	-	-	-	-	N/A
	7D4	*0.44*	-	-	-	-	-	-	-	-	N/A
	21D12	*0.14*	-	-	-	-	-	-	-	-	N/A
	2H4	*0.21*	-	-	-	-	-	-	-	-	N/A
	11B1	*0.45*	-	-	-	-	-	-	-	-	N/A
	5E3	*0.23*	-	-	-	-	-	-	-	-	N/A
	4C7	*0.24*	-	-	-	-	-	-	-	-	N/A
	5A9	*0.22*	-	-	-	-	-	-	-	-	N/A
	19E9	*0.16*	-	-	-	-	-	-	-	-	N/A
	14G1	*1.51*	-	-	-	-	-	-	-	-	ND
	11E3	*1.26*	+	+	+	+	-	-	-	-	ND
	14B10	*0.45*	-	-	-	-	-	-	-	-	N/A
	12F1	*<0.1*	-	-	-	-	-	-	-	-	N/A

All human IgE mAbs with detectable binding in ImmunoCAP or to commercial extract in ELISA or Western blot are shown for each subject. *Aspergillus fumigatus* ImmunoCAP was considered positive if >1.0 kUA/L. IgE mAb binding to *Aspergillus fumigatus* allergenic extracts in ELISA are shown: (-) no binding detected, (+) binding >5 times background. MAb binding to *Aspergillus fumigatus* allergenic extracts in Western blot are shown: (-) no binding detected, (+) binding detected. If binding was detected by any method, immunoprecipitation was attempted, listed are the target proteins identified by mass spectrometry. ND, not determined; N/A, not attempted.

Supplemental Figure 1

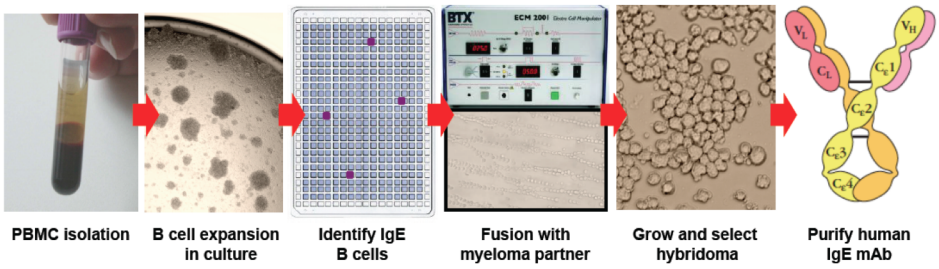

Figure 1. Human IgE hybridoma generation schema. After PBMCs are isolated from subject's blood, cells are grown in 96 well tissue culture plates for 6 days before primary screening for IgE by ELISA. Cells within wells containing IgE are then fused to a myeloma partner by electrical cytofusion. Human hybridomas are selected for in HAT medium and biologically cloned using flow cytometric single cell sorting. Finally, IgE secreting hybridomas are grown in serum free medium before monoclonal IgE antibody purified by Omalizumab chromatography.

Supplemental Figure 2

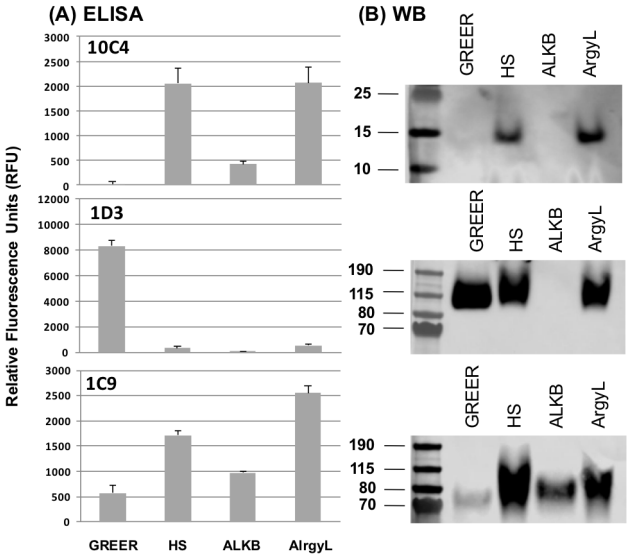

Figure 2. Identification of *Aspergillus* target proteins in commercial extracts using human IgE mAbs. Indirect fluorescence ELISA (A) and Western blot (B) analysis were performed on commercially manufactured allergenic extracts from GREER, HS, ALKB, and AlrgyL using human IgE mAbs to *Aspergillus fumigatus* proteins. Detection of *Aspergillus fumigatus* galactomannoprotein, the target protein of IgE mAb 10C4, is observed in extracts from HS, AlergyL and to a much lesser extent ALKB. Acetylhexosaminidase, the target protein of mAb 1D3, is detectable in all extracts except ALKB. The presence of glycosyltransferase, the target protein of IgE mAb 1C9, is observed in all four extracts. All ELISA assays were performed in triplicate, binding was deemed as positive if greater than 5 times background.

Supplemental Figure 3

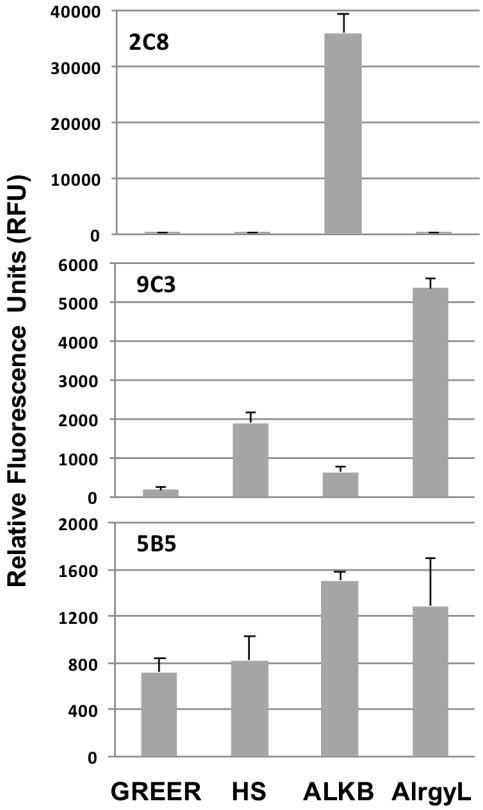

Figure 3. Identification of *Aspergillus* target proteins in commercial extracts using human IgE mAbs. Indirect fluorescence ELISA analysis was performed on commercially manufactured allergenic extracts from GREER, HS, ALKB, and AlrgyL using human IgE mAbs to *Aspergillus fumigatus* proteins. Endochitosanase, as detected by mAb 2C8, is only detectable in ALKB extract. *Aspergillus* peptidase protein, the target protein of IgE mAb 9C3, is detectable in extracts from HS, ALKB, and AlrgyL, but is not present in GREER. The target protein of mAb 5B5, endoglucanase, is present in all four manufactured extracts. All ELISA assays were performed in triplicate, binding was deemed as positive if greater than 5 times background.

Supplemental Figure 4

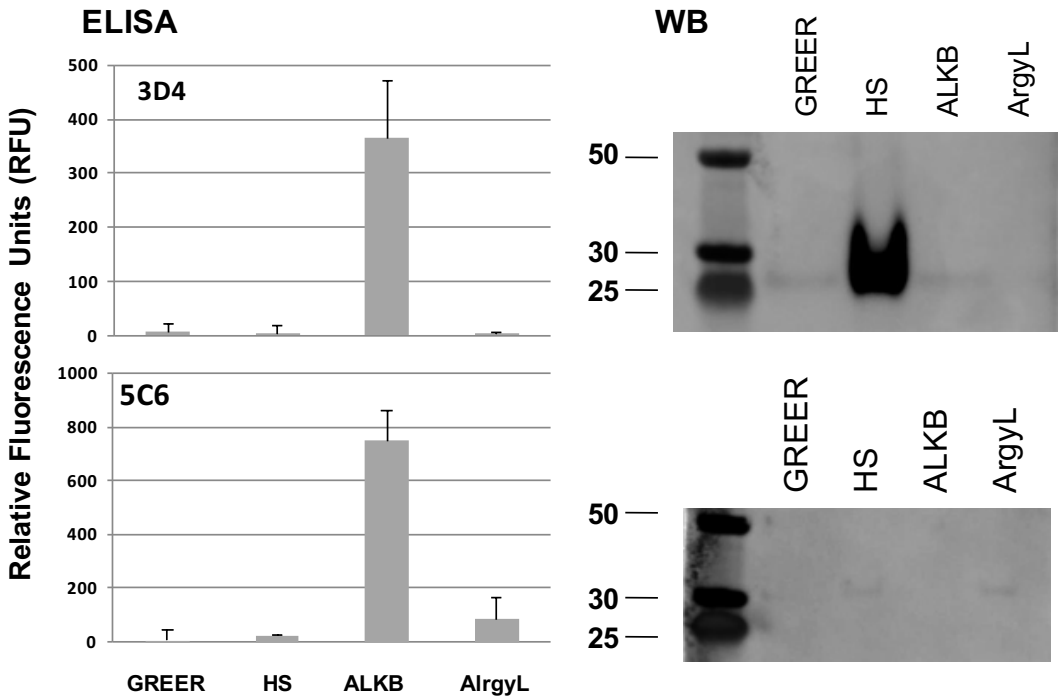

Figure 4. Identification of *Aspergillus* target proteins in commercial extracts using human IgE mAbs. Indirect fluorescence ELISA and Western blot analysis were performed on commercially manufactured allergenic extracts from GREER, HS, ALKB, and AlrgyL using human IgE mAbs to *Aspergillus fumigatus* proteins. Detection of *Aspergillus fumigatus* pectate lyase, the target protein of IgE mAb 3D4, is observed in extracts from HS and ALKB. Glucanase, the target protein of mAb 5C6, is detectable in all extracts except GREER. All ELISA assays were performed in triplicate, binding was deemed as positive if greater than 5 times background.

Supplemental Figure 5

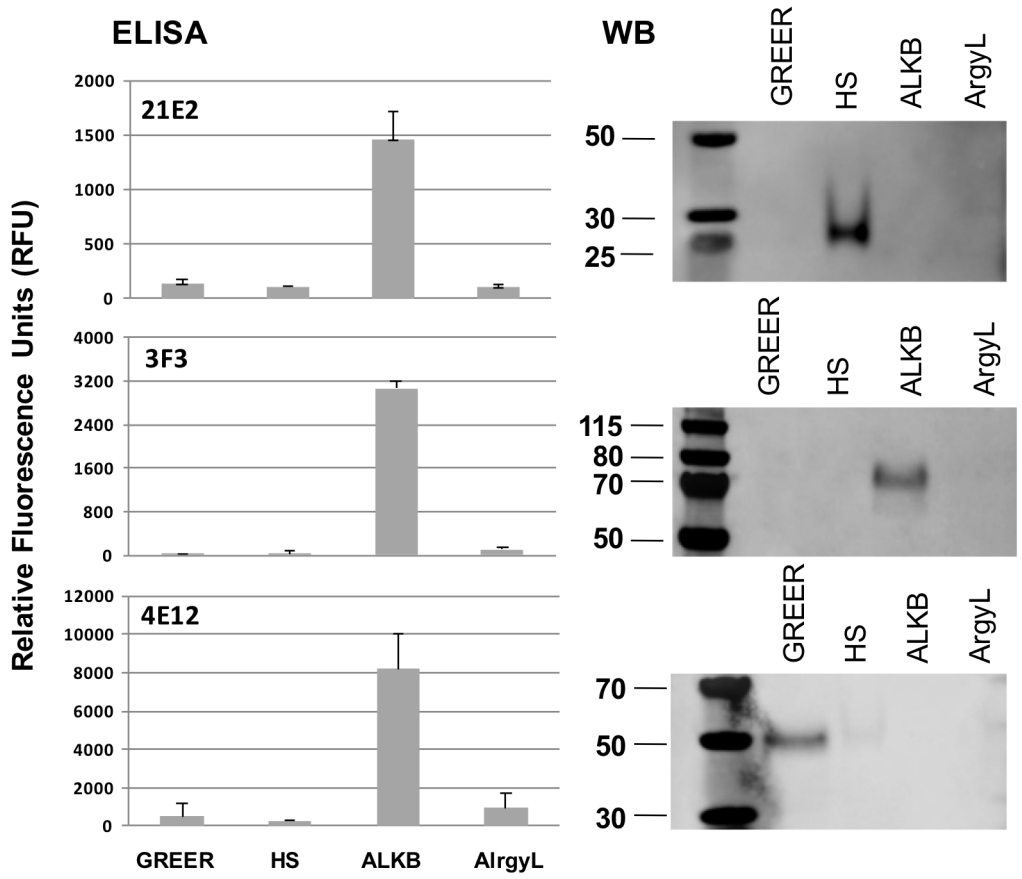

Figure 5. Identification of *Aspergillus* target proteins in commercial extracts using human IgE mAbs. Indirect fluorescence ELISA and Western blot analysis were performed on commercially manufactured allergenic extracts from GREER, HS, ALKB, and AlrgyL using human IgE mAbs to *Aspergillus fumigatus* proteins. Detection of *Aspergillus fumigatus* pectate lyase, the target protein of IgE mAb 21E2, is observed in extracts from HS and ALKB. Glycanosyltransferase, the target protein of mAb 3F3, is detectable only in ALKB extract. The presence of lactonase, the target protein of IgE mAb 4E12, is observed in all four extracts. All ELISA assays were performed in triplicate, binding was deemed as positive if greater than 5 times background.

Supplemental Figure 6

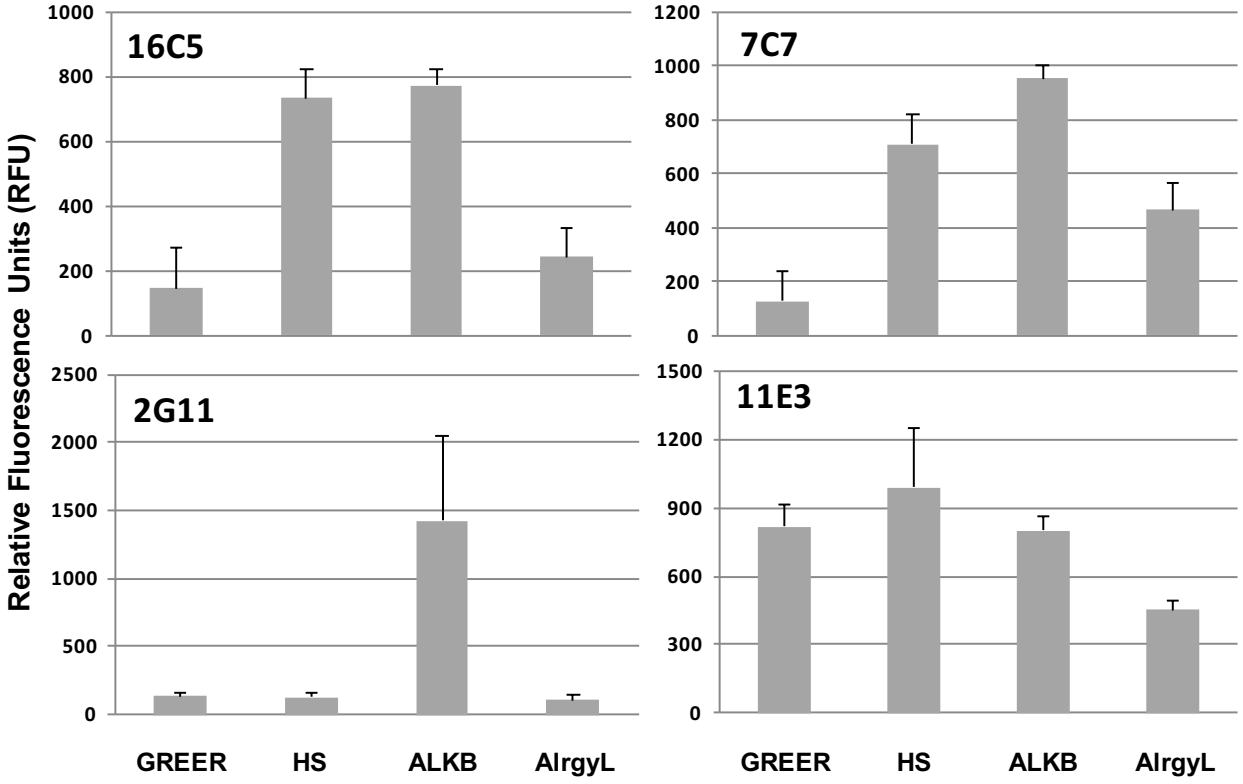

Figure 6. Identification of *Aspergillus* target proteins in commercial extracts using human IgE mAbs. Indirect fluorescence ELISA was performed on commercially manufactured allergenic extracts from GREER, HS, ALKB, and AlrgyL using human IgE mAbs to *Aspergillus fumigatus* proteins. Binding was detected for IgE mAbs 16C5 and 7C7 to HS, ALKB. Binding was only observed with ALKB extract and mAb 2G11, and to all four extracts with mAb 11E3. All ELISA assays were performed in triplicate, binding was deemed as positive if greater than 5 times background.