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Profile of Gene Expression in a Murine Model of Allergic Bronchopulmonary Aspergillosis

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Allergic bronchopulmonary aspergillosis (ABPA) results from the interactions of the *Aspergillus* allergens and immune system of the patients. We studied the gene expression profile in a mouse model of ABPA. Of the 12,000 genes studied, 1,300 genes showed enhanced expression and represent chemokine, cytokine, growth factor, signal transduction, and transmembrane receptor genes as well as genes related to arginine metabolism.

Allergic bronchopulmonary aspergillosis (ABPA) results from inhalation of *Aspergillus fumigatus* antigens and is characterized by a pronounced CD4⁺ Th2 lymphocyte response, which is evidenced by enhanced immunoglobulin E (IgE), eosinophils, and inflammatory responses (5). In recent years, there have been several attempts to delineate allergy-associated genes (9, 14, 15). As mice treated with *A. fumigatus* demonstrated features similar to those of human ABPA, we studied the profile of genes altered in the mouse model of ABPA (1, 3). The results indicate modulation of over 2,000 genes in mice, including enhanced expression of a number of Th2 cytokine genes.

Culture filtrate antigens from *Aspergillus fumigatus* were instilled into the nostrils of BALB/c mice with sterile micropipette tips as described before (6, 7). Serum IgE, *A. fumigatus*-specific IgG, and lung histology were studied as described before (6, 7).

RNA was isolated from the lungs of control and *Aspergillus*-exposed mice with TRIzol according to the recommended protocol of the manufacturer (Invitrogen Life Technologies, Carlsbad, CA). Fifteen micrograms total RNA was reverse transcribed (Invitrogen, Carlsbad, CA) and in vitro transcribed to obtain biotinylated cRNA using a transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). After hybridization to the MG_U74Av2 Gene-Chip (Affymetrix, Santa Clara, CA), the chips were automatically washed and stained with streptavidin-phycoerythrins using a fluidics system. The signal was amplified with biotinylated anti-streptavidin antibody, and the chips were scanned (Agilent Technologies, Palo Alto, CA). Gene transcript levels were determined from data image files using algorithms in the Micro Array Analysis Suite Software 5.0 and Data Mining Tools (Affymetrix; Santa Clara, CA). Fold differences in gene expression were calculated from the average normalized expression levels in the experimental and control groups.

Serum total IgE, *A. fumigatus*-specific IgG1, and eosinophils showed enhancement in the immunized mice compared to

preimmunization sera and sera from control mice (data not shown). All the *Aspergillus*-immunized mice showed significant histological changes similar to ABPA, while all controls were normal (6). As reported previously in a series of communications, the antibody and eosinophil responses as well as histological and immunohistochemical findings in the *A. fumigatus*-exposed mice were comparable to responses in human ABPA (3, 6–8).

Of the total of about 12,000 genes studied, 2,471 genes demonstrated at least threefold changes after allergen administration (Table 1). There were genes that were upregulated or downregulated up to 25- or 28-fold, respectively, demonstrating a very active biological response on exposure to *Aspergillus* antigens. Among these, 1,362 genes were upregulated and 1,109 genes were downregulated. The genes that showed more-than-threefold changes were those involved in cytokine/chemokine expression, signal transduction pathways, and transmembrane receptor activities. On analysis of these genes using the gene ontology (GO) tools and DAVID, we identified 27 cytokine/chemokine-related genes, 130 genes related to signal transduction, and 87 genes with transmembrane receptor activity (Tables 1 and 2). The genes showing significant modulations are shown in Fig. 1, while genes representing arginine metabolism are shown in Table 3.

Twenty-seven genes belonging to cytokines, chemokines, and growth factors showed three- to eightfold increases in *Aspergillus*-challenged mice, and 9 showed suppression compared to controls (Table 2). Various extents of suppression were detected for tumor necrosis factor 1 alpha (TNF-1 α) receptor, CC chemokine ligands 4 and 12, transforming growth factor β (TGF- β), and gamma interferon (IFN- γ), while enhanced expression was shown by interleukin-5 (IL-5), IL-5 receptors, and CC chemokine receptors 4 and 5. The immunocytochemistry of the lung sections indicated a Th2 cytokine response, as evidenced by the presence of IL-4 and IL-5 but not IFN- γ .

Abnormal expression of 159 genes belonging to signal transduction and transmembrane receptors was demonstrable in antigen-exposed mice (Table 1). CD22, SDF receptor 2, CD44, Toll-like receptor 6 (TLR6), serotonin receptor, tyrosine kinase, prostaglandin receptors, and multiple histocompatibility

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TABLE 1. Signal transduction and transmembrane-associated genes

Affy ID ^a	Fold change ^b	Gene product name or function	Affy ID ^a	Fold change ^b	Gene product name or function
103003_i_at	13.2	CD44 antigen	100122_at	4.3	Guanine nucleotide binding protein, beta 5
101737_at	10.4	Follicle-stimulating hormone beta	95333_at	4.3	Interleukin-18 receptor accessory protein
92983_at	10.1	Protein tyrosine phosphatase, receptor type, J	102554_at	4.3	Olfactory receptor 16
102939_s_at	8.8	CD22 antigen	93902_at	4.2	Growth factor receptor bound protein 2-associated protein 1
103421_at	8.8	Stromal cell-derived factor receptor 2	98304_at	4.2	Toll-like receptor 6
100573_f_at	8.5	Glucose phosphate isomerase 1	100998_at	4.1	Histocompatibility 2, class II antigen A, beta 1
99435_at	7.1	Fibroblast growth factor 7	101014_at	4.1	Interferon (alpha and beta) receptor 2
160480_at	7.0	Protein tyrosine phosphatase, receptor type, S	92257_at	4.0	Circadian locomotor output cycles kaput
161609_at	6.8	Regulator of G protein signaling 16	92938_at	4.0	Gamma-aminobutyric acid (GABA-A) receptor, subunit alpha 1
99379_f_at	6.6	Histocompatibility 2, K region	93690_at	4.0	Growth factor receptor-bound protein 10
97174_r_at	6.6	Histocompatibility 2, Q region locus 10	100779_at	4.0	Interleukin-12b
98818_at	6.6	Nuclear receptor subfamily 3, group C, member 1	103957_at	4.0	Transferrin receptor
101312_at	6.4	Glutamate receptor, ionotropic, NMDA2B (epsilon 2)	93433_s_at	3.9	Asialoglycoprotein receptor 1
92529_s_at	6.3	Aryl hydrocarbon receptor nuclear translocator	92372_at	3.9	Bone morphogenetic protein 6
160763_at	6.3	Mitogen-activated protein kinase 13	102287_at	3.9	CD3 antigen, zeta polypeptide
102298_at	6.2	Nerve growth factor, beta	100716_at	3.9	Hyaluronan synthase 1
94155_at	6.2	Regulator of G protein signaling 4	102658_at	3.9	Interleukin-1 receptor, type II
97741_at	6.0	Thyroid-stimulating hormone, beta subunit	92248_at	3.9	Nuclear receptor subfamily 4, group A, member 2
97946_at	5.9	Prolactin-like protein F	102857_at	3.8	A kinase (PRKA) anchor protein 4
94712_at	5.9	Vascular endothelial growth factor C	161608_r_at	3.8	Hyaluronidase 2
161718_at	5.8	Braf transforming gene	99000_at	3.8	Mitogen-activated protein kinase 7
103258_at	5.7	Lymphocyte antigen 75	95313_at	3.7	Coagulation factor II (thrombin) receptor
93895_s_at	5.6	Inositol 1,4,5-triphosphate receptor 1	95390_at	3.7	Neuronal PAS domain protein 2
96766_s_at	5.6	TYRO3 protein tyrosine kinase 3	104601_at	3.7	Thrombomodulin
97509_f_at	5.5	Fibroblast growth factor receptor 1	93785_at	3.6	Folate receptor 1 (adult)
102281_at	5.4	Tumor necrosis factor receptor superfamily, member 7	102021_at	3.6	Interleukin-4 receptor, alpha
161863_r_at	5.3	CD3 antigen, delta polypeptide	104479_at	3.6	Purinergic receptor P2Y, G-protein-coupled 2
93294_at	5.3	Connective tissue growth factor	160836_at	3.6	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM), and short cytoplasmic domain, (semaphorin) 4D
160893_at	5.3	Guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	94571_at	3.6	Vomerolnasal 1 receptor, A1
161730_f_at	5.2	Integrin, alpha E, epithelial-associated	104012_at	3.5	Gonadotropin-releasing hormone receptor
98350_at	5.2	Somatostatin receptor 2	103266_at	3.5	Leukotriene B4 receptor 1
98375_at	5.2	Vasoactive intestinal peptide receptor 2	102573_at	3.5	Neurexophilin 1
161849_r_at	5.1	Insulin II	102932_at	3.5	Nuclear receptor subfamily 5, group A, member 2
95557_at	5.0	Bone morphogenetic protein 1	161309_r_at	3.5	Regulator of G-protein signaling 11
97658_f_at	5.0	Insulin I	97725_at	3.4	Bone morphogenetic protein receptor, type 1B
103256_at	5.0	Membrane-spanning 4 domains, subfamily A, member 4D	93714_f_at	3.4	Histocompatibility 2, Q region locus 7
99069_at	5.0	Neurotrophic tyrosine kinase, receptor, type 2	101144_at	3.4	Interleukin-18 receptor 1
97001_r_at	5.0	Olfactory receptor 157	99142_at	3.4	Membrane progesterin receptor alpha
102830_at	4.9	CD86 antigen	101776_at	3.3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
95319_at	4.9	Neurotensin receptor	101829_at	3.3	Cholinergic receptor, muscarinic 1, CNS
160771_r_at	4.9	Protein tyrosine phosphatase, receptor type, F interacting protein, binding protein 2	101209_at	3.3	Fc receptor, IgE, high-affinity I, alpha polypeptide
92578_at	4.9	Small inducible cytokine subfamily E, member 1	98863_at	3.3	Glutamate receptor, ionotropic, kainate 2 (beta 2)
95321_at	4.8	5-Hydroxytryptamine (serotonin) receptor 4	103949_at	3.3	Indian hedgehog
102692_s_at	4.8	Histocompatibility 2, T region locus 3	94792_at	3.3	Macrophage scavenger receptor 1
160854_at	4.8	Mitogen-activated protein kinase kinase 7	95016_at	3.3	Neuropilin
162261_f_at	4.8	Zona pellucida glycoprotein 2	101453_at	3.2	Melanoma inhibitory activity
93947_g_at	4.7	Estrogen receptor 1 (alpha)	99337_at	3.2	Brain-specific angiogenesis inhibitor 1-associated protein 2
104381_at	4.7	Nuclear receptor subfamily 1, group H, member 3	93393_at	3.2	Ectodysplasin-A
101828_at	4.7	Ret proto-oncogene	104672_at	3.2	Frizzled-related protein
95932_at	4.7	Zona pellucida glycoprotein 1	94779_f_at	3.2	Killer cell lectin-like receptor subfamily A, member 9
101779_at	4.6	Dopamine receptor 3	93696_at	3.2	Nuclear receptor subfamily 1, group I, member 2
97128_at	4.6	Interleukin 5 receptor, alpha	96557_at	3.2	Patched homolog 2
97774_at	4.6	Opioid receptor, mu	101765_at	3.1	Anti-Mullerian hormone
102921_s_at	4.6	Tumor necrosis factor receptor superfamily, member 6	94696_at	3.1	Chemokine (C-C motif) receptor 4
98840_at	4.5	BMX non-receptor tyrosine kinase	161968_f_at	3.1	Chemokine (C-C motif) receptor 5
99342_at	4.5	Gamma-aminobutyric acid (GABA-A) receptor, subunit delta	93412_at	3.1	Duffy blood group
94773_at	4.5	Nerve growth factor, alpha	102345_at	3.1	Endometrial bleeding-associated factor
103362_at	4.4	Prostaglandin E receptor 4 (subtype EP4)	94566_at	3.1	G protein-coupled receptor G2A
99894_at	4.4	Prostaglandin F2 receptor negative regulator	96420_at	3.1	Inositol 1,4,5-triphosphate receptor 2
			160220_at	3.1	Zinc finger protein 110
			100142_at	3.1	DiGeorge syndrome critical region gene 2

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TABLE 1—Continued

Affy ID ^a	Fold change ^b	Gene product name or function	Affy ID ^a	Fold change ^a	Gene product name or function
100972_s_at	3.0	Chemokine (C-C motif) ligand 27	161544_r_at	−3.6	Colony-stimulating factor 2 receptor, beta 2, low affinity (granulocyte-macrophage)
162171_f_at	3.0	Fibroblast growth factor receptor 2	104685_g_at	−3.6	Glutamate receptor, ionotropic, NMDA1 (zeta 1)
161598_at	3.0	Glial cell line-derived neurotrophic factor	98114_at	−3.6	Niemann Pick type C1
94285_at	3.0	Histocompatibility 2, class II antigen E beta	96588_at	−3.7	Prostaglandin E receptor 3 (subtype EP3)
92767_at	−3.0	Bone morphogenetic protein receptor, type 1A	162218_f_at	−3.8	Integrin beta 5
161377_at	−3.0	Epidermal growth factor-like module-containing, mucin-like, hormone receptor-like sequence 1	98806_s_at	−3.8	Tubby candidate gene
99991_at	−3.0	Interleukin-17 receptor	97541_f_at	−4.0	Histocompatibility 2, D region locus 1
92532_at	−3.1	Arginine vasopressin receptor 1A	161202_r_at	−4.0	Platelet-derived growth factor receptor, alpha polypeptide
95306_at	−3.1	G protein-coupled receptor 14	94764_at	−4.1	Integrin alpha L
98034_at	−3.1	Histocompatibility 2, class II, locus Mb1	94791_s_at	−4.4	Leukocyte tyrosine kinase
99811_at	−3.1	Neuropeptide Y receptor Y6	99896_at	−4.4	Melanocortin 5 receptor
98810_at	−3.2	5-Hydroxytryptamine (serotonin) receptor 5B	99387_at	−4.8	Formyl peptide receptor 1
162407_at	−3.2	TRAF-interacting protein	93254_at	−5.0	Mitogen-activated protein kinase 1
103289_at	−3.2	Low-density lipoprotein receptor-related protein 4	102274_at	−5.1	Histocompatibility 2, O region alpha locus
98841_at	−3.3	Activin receptor IIA	162371_r_at	−5.2	Eph receptor B6
101801_at	−3.3	Formyl peptide receptor, related sequence 3	98784_at	−5.8	G protein-coupled receptor 12
97793_at	−3.3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)	99804_at	−6.0	Adrenergic receptor, alpha 2c
92200_at	−3.5	Adenylate cyclase-activating polypeptide 1	92469_at	−6.7	Secreted frizzled-related sequence protein 4
104211_at	−3.5	Integrin alpha 3	95771_i_at	−9.0	Frizzled homolog 4 (<i>Drosophila</i>)
			161194_r_at	−9.9	Tumor necrosis factor receptor superfamily, member 1a

^a Unique Affymetrix identification number of each gene.^b Average fold change in gene expression in the *Aspergillus*-treated mice compared to the controls.

molecule-related genes with direct immunological and allergic relevance showed increased expression (Table 1). Other significant genes showing enhanced expression are fibroblast growth factor 1, integrin, TNF, leukocyte tyrosine kinase, granulocyte-macrophage colony-stimulating factor, and platelet-derived growth factor receptor alpha.

Innate immunity is directly linked to certain receptors such as

TABLE 2. Cytokine and chemokine-related molecules

Affy ID ^a	Fold change ^b	Gene product name or function
99435_at	7.1	Fibroblast growth factor 7
95557_at	5.1	Bone morphogenetic protein 1
92578_at	4.9	Small inducible cytokine subfamily E, member 1
97128_at	4.6	Interleukin-5 receptor, alpha
95333_at	4.3	Interleukin-18 receptor accessory protein
98304_at	4.2	Toll-like receptor 6
101014_at	4.1	Interferon (alpha and beta) receptor 2
92372_at	3.9	Bone morphogenetic protein 6
102658_at	3.9	Interleukin-1 receptor, type II
100779_at	3.9	Interleukin-12b
102021_at	3.6	Interleukin-4 receptor, alpha
101144_at	3.4	Interleukin-18 receptor 1
93393_at	3.2	Ectodysplasin-A
94696_at	3.1	Chemokine (C-C motif) receptor 4
161968_f_at	3.1	Chemokine (C-C motif) receptor 5
100972_s_at	3.0	Chemokine (C-C motif) ligand 27
102345_at	3.0	Endometrial bleeding-associated factor
161598_at	3.0	Glial cell line-derived neurotrophic factor
94146_at	−3.0	Chemokine (C-C motif) ligand 4
99991_at	−3.0	Interleukin-17 receptor
102559_at	−3.3	Bone morphogenetic protein 2
93717_at	−3.6	Chemokine (C-C motif) ligand 12
93455_at	−3.7	Bone morphogenetic protein 4
97734_at	−3.8	Thrombopoietin
102751_at	−4.2	Transforming growth factor, beta 3
101788_f_at	−4.5	Interferon alpha family, gene 1
161194_r_at	−9.9	Tumor necrosis factor receptor superfamily, member 1a

^a Unique Affymetrix identification number of each gene.^b Average fold change in gene expression in the *Aspergillus*-treated mice compared to the controls.

the TLRs. In *Aspergillus*-induced invasive disease, TLR2 plays a major role in imparting immunity. It is probable that the enhanced expression of TLR6 has a major role in the immunity of allergic aspergillosis. In the present study, we have demonstrated significant expression of several Th2-related genes such as the IL-4 and IL-5 receptor genes. In addition, there was also alteration in the expression of chemokine receptors such as CCR4 and CCR5 showing marked expression in *A. fumigatus*-exposed animals compared to control mice (11, 13). Another interesting observation is the augmentation of matrix metalloproteinase 9 in *A. fumigatus*-exposed mice. Previous studies indicate that this protein has a detrimental effect on the asthmatic responses by eliciting a Th2 type of response and in airway remodeling (12).

When genes involved in arginine metabolism were specifically analyzed, we found alteration of several of these genes (Table 3). Enhanced presence of arginase and NO has been reported in both human asthma and animal models of asthma (10, 17). Enzymes, arginase 1, ornithine transcarboxylase, and ornithine amino transferase are part of citric acid cycle of the respiratory metabolic pathway. Arginase catalyzes the conversion of L-arginine to urea and L-ornithine, depleting the availability of L-argi-

TABLE 3. Enzymes involved in arginine metabolism and significantly altered after *Aspergillus* treatment in the mouse

Fold change ^a	Gene product name or function
14.8	Ornithine aminotransferase
8.5	S-Adenosylmethionine decarboxylase 1
7.9	Arginase 1
7.6	Ornithine transcarbamylase
3.0	Nitric oxide synthase 1, neuronal
−3.2	Aldehyde dehydrogenase family 3, subfamily A2
−3.4	Glutamate oxaloacetate transaminase 2, mitochondrial

^a Average fold change in gene expression in the *Aspergillus*-treated mice compared to the controls.



FIG. 1. A hierarchical dendrogram produced by clustering of expression levels for two control and three *Aspergillus*-sensitized mice (columns) and 21 genes (rows). Color visualization is as follows: green, low expression; black, intermediate expression; and red, high expression to facilitate interpretation. The diagram was generated using JMP software (SAS Institute Inc., Cary, NC).

nine substrate for inducible nitric oxide synthase (iNOS). It is believed that arginase competes with iNOS for their substrate L-arginine and inhibits NO production, as seen in experimental trypanosomiasis (2). It is also known that Th1 cytokines induce iNOS, whereas Th2 cytokines induce arginase. Arginase inhibits NO production and favors the synthesis of polyamines by producing L-ornithine, which is metabolized by ornithine decarboxylase to polyamines. The eightfold upregulation of *S*-adenosylmethionine decarboxylase (AMD1) is very pertinent as it catalyzes polyamine synthesis (Table 3), although its role in allergic diseases is not known (16).

Of the genes expressed in mice, none of the recently identified genes associated with asthma in humans, such as ADAM33, ESE-3, or PhFII, could be identified in the present study (4, 14, 15). This may be due to their restricted presence in humans or due to the lack of expression in the murine model, which is not known at present. The expression and

suppression of the specific genes in the model of allergic aspergillosis may have significant implications as such information may provide better understanding of the disease mechanism as well as in developing therapeutic strategies.

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