Functional Genomics of Innate Host Defense Molecules in Normal Human Monocytes in Response to *Aspergillus fumigatus*†

Karoll J. Cortez,[1] Caron A. Lyman,[1] Shyam Kottilil,[2] Hee Sup Kim,[1] Emmanuel Roilides,[1,3]
Jun Yang,[4] Brandie Fullmer,[4] Richard Lempicki,[4] and Thomas J. Walsh[1]*

*Immunocompromised Host Section, Pediatric Oncology Branch, NCI, NIH, Bethesda, Maryland[1]; Laboratory of Immunoregulation,
NIAID, NIH, Bethesda, Maryland[2]; 3rd Department of Pediatrics, Aristotle University, Thessaloniki, Greece[3];
and SAIC—Frederick, Inc., Clinical Service Program, Frederick, Maryland[4]*

Aspergillus fumigatus induces the release of innate immune-related molecules from phagocytic cells early in the course of infection. Little is known, however, about the complex expression profiles of the multiple genes involved in this response. We therefore investigated the kinetics of early gene expression in human monocytes (HMCs) infected with conidia of *A. fumigatus* using DNA microarray analysis. Total RNA from HMCs at 0, 2, 4, and 6 h was extracted, linearly amplified, hybridized onto Affymetrix HG133 Plus 2.0 gene chips, and analyzed with an Affymetrix scanner. Changes in gene expression were calculated as a ratio of those expressed by infected versus control HMCs. *Aspergillus fumigatus* induced differential regulation of expression in 1,827 genes (*P* < 0.05). Genes encoding cytokines and chemokines involved in host defense against *A. fumigatus*, including interleukin-1β (IL-1β), IL-8, CXCL2, CCL4, CCL3, and CCL20, as well as the opsonin long pentraxin 3, were up-regulated during the first 2 to 6 h, coinciding with an increase in phagocytosis. Simultaneously, genes encoding CD14, ficolin1, and MARCO were down-regulated, and genes encoding IL-10 and matrix metalloproteinase 1 were up-regulated. Up-regulation of the genes encoding heat shock proteins 40 and 110 and connexins 26 and 30 may point to novel molecules whose role in the pathogenesis of aspergillosis has not been previously reported. Verification of the transcriptional profiling was obtained for selected genes by reverse transcription-PCR and enzyme immunoassay. Thus, *A. fumigatus* conidia induced a coordinated expression of genes important in host defense and immunomodulation.

Invasive aspergillosis is an important cause of morbidity and mortality among immunocompromised patients. Risk factors for developing aspergillosis include aplastic anemia (46), hematopoietic stem cell transplantation (32), solid organ transplantation (44), and phagocytic deficiencies, such as chronic granulomatous disease and human immunodeficiency virus infection (40, 43). *Aspergillus fumigatus* is the species most commonly identified as the cause of invasive aspergillosis.

Monocytes/macrophages play a pivotal role in normal host defense against *A. fumigatus*. The innate host response against this pathogen is mediated by release of immune-related molecules from phagocytic cells. However, little is known about the complex expression kinetic profiles of the multiple genes mediating the initial immune response to *A. fumigatus*. Thus, we hypothesized that conidia of *A. fumigatus* would induce reciprocal changes in expression of genes encoding products related to innate immunity that may provide insight into early host-parasite interaction.

In this study, we used microarray analysis to elucidate the initial kinetics of the transcriptional response of genes encoding innate host defense molecules in normal human monocytes after in vitro stimulation/infection with *A. fumigatus* conidia. We report herein the changes in expression of genes whose products are involved in the initial innate immune response, including proteins involved in phagocytosis, endocytosis, cytokine and chemokine signaling and their receptors; proteins involved in cell adhesion and cell communication; scavenger receptors, Toll-like receptors, and other immunological receptors; matrix metalloproteinases; pattern recognition receptors; and heat shock proteins. On a functional genomic level, these studies elucidate the intricate interaction between effector cells, their mediators, and the microorganism over the initial 6 h of infection with *A. fumigatus*.

MATERIALS AND METHODS

***Aspergillus fumigatus* preparation.** A well-characterized strain of *Aspergillus fumigatus* (NIH 4215, ATCC MYA-1163), originally isolated from a cancer patient with invasive aspergillosis, was used throughout these studies. The isolate was preserved on frozen potato dextrose agar slants at −70°C. Working stocks of *A. fumigatus* conidia were harvested from freshly prepared 5-day-old slants and suspended in phosphate-buffered saline as described previously (42). Conidia were stored at 4°C for up to 1 week.

Monocyte isolation. Human monocytes (HMCs) were isolated from five healthy volunteers by automated leukapheresis of blood and subsequently separated by counterflow centrifugal elutriation (model J-6 M centrifuge; Beckman Instruments, Fullerton, CA). Cells were washed in Hanks' balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} (HBSS−) and resuspended to a final concentration of 1×10^7 cells/ml in HBSS with Ca^{2+} and Mg^{2+} (HBSS+). Viability was assessed as greater than 98% by trypan blue exclusion, and the cell population isolated was >90% monocytes by Wright-Giemsa stain and/or nonspecific esterase stain.

Incubation of monocytes with *A. fumigatus* conidia. Monocytes from five different donors and conidia of *A. fumigatus* were incubated and processed in five separate experiments. For each donor, HMCs and *A. fumigatus* conidia were prepared in HBSS+ at concentrations of 1×10^7 cells/ml and 1×10^6 conidia/ml, respectively, for an E:T ratio of 10:1. Using a polystyrene six-well flat-bottomed

* Corresponding author. Mailing address: Pediatric Oncology Branch, National Cancer Institute, NIH, 10 Center Drive, CRC Rm. 1-5250, Bethesda, MD 20892-1882. Phone: (301) 402-0023. Fax: (301) 402-0575. E-mail: walsht@mail.nih.gov.
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plate (Becton Dickinson, Franklin Lakes, NJ), two wells were designated for cells alone (control HMCs) and four wells were set up for cells plus conidia (stimulated HMCs) for each time point. The wells containing control HMCs and two of the wells containing HMCs plus conidia were used for RNA extraction, while the remaining two wells with stimulated cells were used for phagocytosis and viability determinations.

Time points were established at 0, 2, 4, and 6 h. The plates were incubated at 37°C and 5% CO_2 on a plate shaker (Delfia 1296-004; Wallac, Turku, Finland) for gentle mixing. For the zero hour time point (T_0), HMCs and *A. fumigatus* conidia were maintained on ice and mixed immediately prior to centrifuging at 4°C, followed by RNA extraction (see below). Supernatants from aliquots from all time points were collected and frozen at −70°C for protein analysis.

Two of the wells containing cells plus conidia also contained 18-mm circular glass coverslips that were used to determine phagocytosis and viability of the HMCs at each time point. The coverglass slides were gently removed from the two designated wells at each time point. One of the slides was examined immediately using trypan blue exclusion to determine viability of HMCs. The second slide was allowed to dry at room temperature, fixed, and stained with a modified Wright-Giemsa stain. The percent phagocytosis was calculated by determining microscopically the percent HMCs containing organisms. The phagocytic index was calculated as the average number of conidia per phagocytosing cell (39).

RNA preparation and Affymetrix microarray analysis. Immediately following each time point, total RNA from 2×10^7 stimulated and unstimulated HMCs was extracted using the TRIzol method (Invitrogen, Carlsbad, CA). cRNA synthesis using 15 μg total RNA and hybridization to the HG-U133 Plus 2.0 GeneChip was performed following the manufacturer's (Affymetrix, Santa Clara, CA) recommended protocol. Affymetrix Microarray Analysis Suite (MAS) 5.0 was used for chip-to-chip normalization and gene expression value determination. For each of the five donors and four time points, a separate two-sample comparison between unstimulated control HMCs and *A. fumigatus* infected-HMCs was performed using GCOS software, and log₂ ratio data for treated/untreated cells were collected for statistical analysis.

Statistical determination of significant differential expression. Two-way analysis of variance (Partek Pro 5.1 Statistical Package, St. Charles, MO) was performed with donors set as a random effect, time as the main effect, and contrast set to compare time points only to T_0. Since the T_0 comparison is actually a technical replicate within donors (no treatment was applied in the treatment group), the data at T_0 were used to remove noisy probe sets by filtering out those probe sets that showed average changes among the five donors greater than 1.3-fold in either direction. Probe sets with mean expression values <40 for both comparison groups in the analysis of variance (near the limits of detection) were eliminated from further consideration. Differentially expressed genes were defined as those having a *P* value of ≤0.05 and an absolute change of greater than 1.5-fold for each time point compared to T_0. A 1.5-fold change previously has been shown to determine significance of differences in gene expression (3, 24, 28). Moreover, a number of reports have shown that change (fold) calculated from Affymetrix microarrays often underestimates the true change (fold) in expression (5, 6, 21, 37).

Genes were grouped by K-Means clustering (Spotfire DecisionSite, Somerville, MA) of log₂ ratio data. The data were plotted as the mean gene expression ratio of five healthy donors over time: for each of the five donors, the change (log₂ fold) for each time point relative to T_0 was determined with and without incubation with *Aspergillus*. Functional annotation of the genes was performed using DAVID version 2. (http://david.niaid.nih.gov/david/version2/index.htm).

Real-time PCR. HMCs from an independent group of three healthy donors were studied on three separate days. These cells were treated or untreated with *A. fumigatus* conidia for 0 and 6 h as described above. Total RNA was isolated from 2×10^7 cells using TRIzol, and 1 μg of total RNA was reverse transcribed with random hexamer primers using the TaqMan reverse transcription (RT) kit system (Applied Biosystems, Foster City, CA). For quantitative real-time PCR, one-eightieth of the first strand cDNA was used as a template for detection of each gene of interest. *GBJ2*, *PTGS2*, and *SOD2* were selected as target genes because each belonged to a different biological category and each displayed a magnitude of change in expression by microarray that was more likely to be detected by RT-PCR. *B2M* was used as a control gene. *GJB2*, *PTGS2*, *SOD2*, and *B2M* levels were detected using the Assay-on-Demand System (Applied Biosystems) and the TaqMan Universal Master Mix (Applied Biosystems). The fluorescence signals were measured in real time during the extension step using the iCycler iQ Real Time Detection System (Bio-Rad, Hercules, CA). The ratio change in target genes relative to the *B2M* control gene was determined within each sample by the $2^{-\Delta\Delta CT}$ method (27).
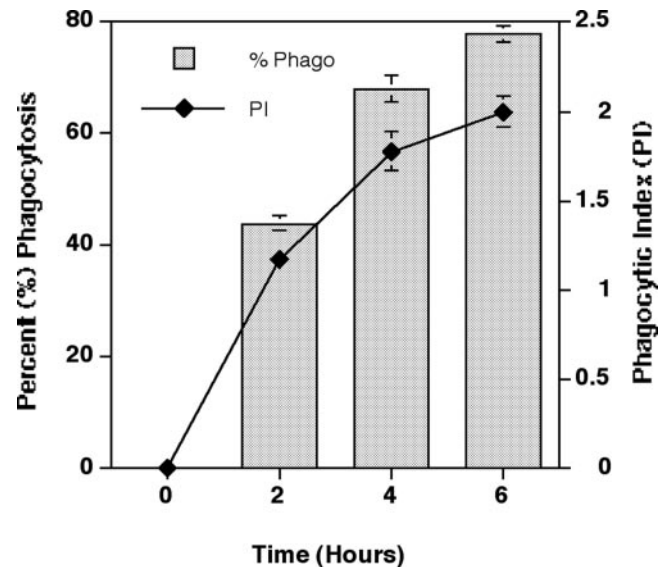

FIG. 1. Kinetics of phagocytosis of *A. fumigatus* conidia. Results are expressed as mean ± standard error % phagocytosis (number of HMCs ingesting ≥1 conidia of *A. fumigatus* per 100 counted cells) and as mean ± standard error phagocytic index (average number of conidia of *A. fumigatus* per phagocytosing HMC).

Determination of protein levels. One-milliliter aliquots of cell-free supernatants from HMCs alone and HMCs plus *A. fumigatus* conidia were collected from at least three separate experiments for each time point. Samples were stored at −70°C in curved-bottom cryotubes until processed. CXCL8/interleukin-8 (IL-8), CCL4/MIP1β, CCL5/RANTES, and MMP-1 protein levels were evaluated in duplicate serial dilutions of supernatants using commercially available quantitative sandwich enzyme immunoassay (EIA) technique (EIA kits) for CXCL8/IL-8, CCL4/MIP1β, CCL5/RANTES, and MMP-1 (R&D Systems, Inc., Minneapolis, MN), according to protocols specified by the manufacturer. The plates were read in an EIA reader (Titertek Multiskan MCC/340; Lab Systems, Finland) at a wavelength of 450 nm with wavelength correction set at 570 nm. The concentrations of cytokines in the samples were calculated from a standard curve of CXCL8/IL-8, CCL4/MIP1β, CCL5/RANTES, and MMP-1, respectively. Subsequently, the differences between protein levels in HMCs with *A. fumigatus* and control HMCs were obtained and plotted versus log₂ expression ratios of the genes over time.

RESULTS

The viability of HMCs was greater than 80% for all donors at all time points evaluated. The percent phagocytosis increased progressively over the 6-h time course from 0% to 80% (Fig. 1). The phagocytic index also increased progressively over time.

As expected, 99.9% of genes in normal HMCs were unchanged at the zero-hour time point. There were a total of 1,827 differentially expressed genes with signal/log ratio (log₂) of ≥1.5 measured over the 2- to 6-h time course (see Table S1 in the supplemental material). A heat map of the 1,827 differentially expressed genes arranged into seven distinct clusters according to the time of maximal up- or down-regulation is shown in Fig. 2. Cluster 1 represents a group of 95 genes that were maximally up-regulated at 2 h of incubation. Cluster 2 comprises 237 genes with maximal up-regulation at 4 h. Cluster 3 has a total of 295 genes with maximal up-regulation at 6 h of incubation. In contrast, cluster 4 displays 184 genes with maximal down-regulation at 2 h, while cluster 5 includes 335 genes with maximal down-regulation at 4 h, and cluster 6 has 500
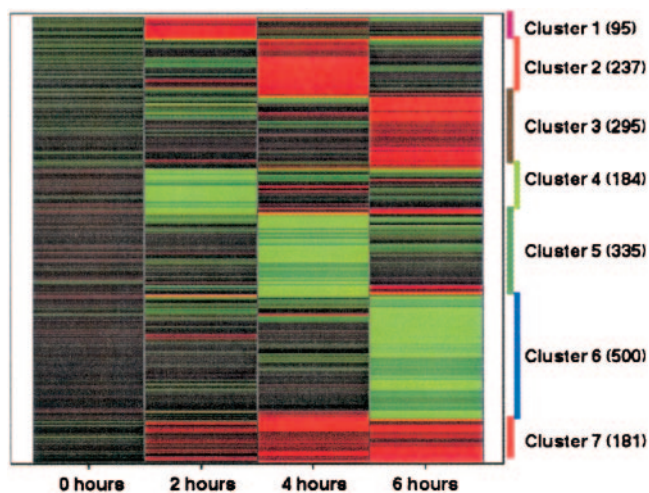
FIG. 2. Heat map of gene expression profile in HMCs exposed to conidia of *A. fumigatus*. The results show K-Means clustering of 1,827 genes significantly differentially expressed following the maximal up-regulation (red) or down-regulation (green) over time. Cluster 1, maximal up-regulation at 2 h; cluster 2, maximal up-regulation at 4 h; cluster 3, maximal up-regulation at 6 h; cluster 4, maximal down-regulation at 2 h; cluster 5, maximal down-regulation at 4 h; cluster 6, maximal down-regulation at 6 h; cluster 7, up-regulation over 2 to 6 h.

FIG. 3. Expression of phagocytosis- and endocytosis-related genes. (A) Mean expression ratio ($\log_2$) kinetics of genes involved in phagocytosis in HMCs in response to conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *MARCKS* (myristoylated alanine-rich protein kinase C substrate), *CDC42EP3* (Rho GTPase binding 3), *CD14* (cluster designation antigen 14), *CORO1A* (coronin 1A; also known as TACO); *FCN1* (M-ficolin precursor). (B) Mean expression ratio ($\log_2$) kinetics of genes involved in endocytosis in HMCs in response to conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *EHD1* (EH domain-containing 1), *SH3BP4* (SH3-domain binding protein 4), and *RAB20* (RAB20 member of RAS oncogene family).

genes displaying maximal down-regulation at 6 h. There was a group of 181 genes, cluster 7, that was up-regulated at 2 h and remained elevated throughout the 6-h time course with peak elevation at approximately 4 h. Of the 1,827 genes differentially expressed, 239 were related to previously documented or suspected immune functions according to Gene Ontology consortium and its databases GO Biological, GO Molecular Function, and GO Cellular Component (http://www.geneontology.org/) and the database SwissProt (http://www.pir.uniprot.org/) keyword. Of these 239 genes with suspected or previously documented immune-related function, 125 were up-regulated and 114 were down-regulated.

Genes encoding molecules related to phagocytic function of monocytes were examined in response to *A. fumigatus*. Figure 3A shows the kinetic profiles of those genes that were differentially expressed in response to the conidia. The phagocytosis-related genes that were up-regulated in response to *A. fumigatus* conidia included *MARCKS* (myristoylated alanine-rich protein kinase C substrate, a protein thought to be involved in cell motility, phagocytosis, membrane trafficking, and mitogenesis) and *CDC42EP3* (Rho GTPase binding 3). The kinetics of gene expression of *MARCKS* and *CDC42EP3* showed steep upward trends, peaking at 6 h.

Among the genes related to phagocytosis, *CD14*, *CORO1A*, and *FCN1* were down-regulated in response to *A. fumigatus* conidia. All of these displayed similar kinetic profiles (Fig. 3A). *CD14* (a glycosylphosphatidyl inositol-linked glycoprotein present on mononuclear and polymorphonuclear phagocytes), *CORO1A* (the gene encoding an actin binding protein involved in the fusion of phagosomes and lysosomes), and *FCN1* (the gene encoding a precursor of M-ficolin) were all significantly down-regulated by 6 h in response to *A. fumigatus*.

Genes encoding proteins involved in endocytosis also were examined. The kinetic profiles of those genes found to be
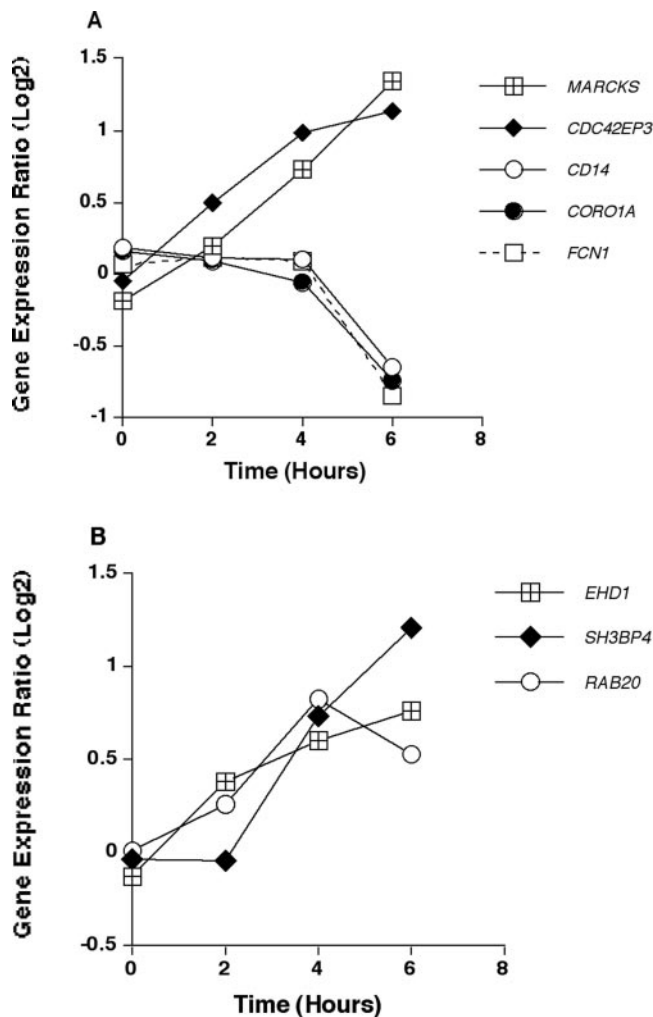
significantly differentially expressed in response to *A. fumigatus* are shown in Fig. 3B. *EHD1*, *SH3BP4*, and *RAB20* all encode proteins involved in endocytosis of particles by human monocytes. The protein encoded by *EHD1* is thought to play a role in the endocytosis of IGF1 receptors and to reside in the endocytic recycling compartment. *SH3BP4* homologies and consensus sequence sites indicate that it may be involved in a newly identified cascade of proteins involved in endocytosis, intracellular sorting, and the cell cycle. The protein encoded by *RAB20* is involved in apical endocytosis/recycling. The three genes followed similar kinetic profiles, with significant up-reg-
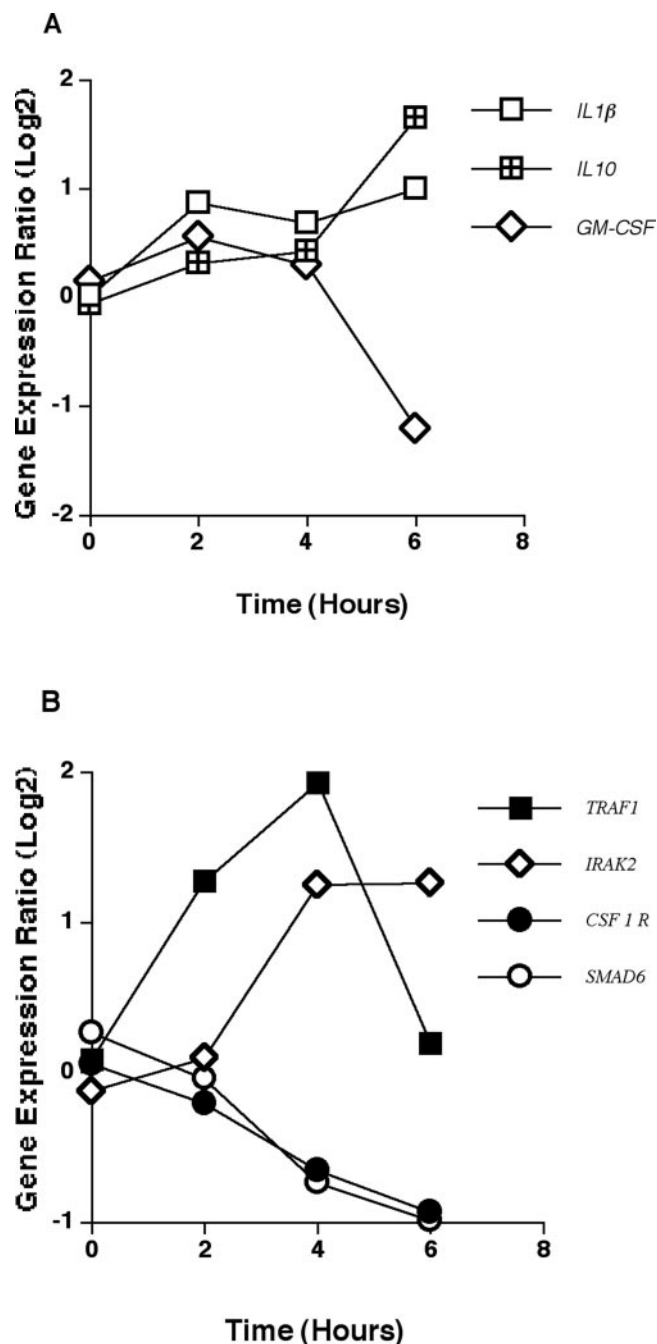
FIG. 4. Expression of cytokine- and cytokine receptor-encoding genes. (A) Mean expression ratio ($\log_2$) kinetics of genes encoding cytokines in HMCs in response to *A. fumigatus* conidia ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *IL1β* (interleukin-1β), *IL10* (interleukin-10), *CSF2* (granulocyte-macrophage colony-stimulator factor). (B) Mean expression ratio ($\log_2$) kinetics of genes encoding cytokine receptors in HMCs in response to conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *TRAF1* (tumor necrosis factor receptor-associated factor 1), *IRAK2* (interleukin-1 receptor-associated kinase 2), *CSF1R* (colony-stimulating factor 1 receptor; formerly McDonough feline sarcoma viral [v-fms] oncogene homolog; CD115), and *SMAD6* (SMAD; mothers against DPP homolog 6 [*Drosophila*]).

ulation in gene expression by 4 h and peak expression occurring between 4 and 6 h.

Cytokine-encoding genes that were significantly differentially expressed in response to *A. fumigatus* are shown in Fig. 4A. The genes encoding interleukin-1β (*IL1β*) and interleukin-10 (*IL10*) were up-regulated, beginning at 2 h and peaking at 6 h. In contrast, expression of the gene encoding granulocyte-macrophage colony-stimulating factor (*CSF2*) was not significantly changed until 6 h, when there was a significant down-regulation compared to baseline. Genes encoding other molecules with cytokine activity that were significantly differentially expressed in response to *A. fumigatus* are shown in Table 1.

Cytokine receptor-associated genes also were evaluated. The kinetic profiles of those genes that were differentially expressed in HMCs in response to *A. fumigatus* conidia are shown in Fig. 4B. Genes encoding TRAF1 (tumor necrosis factor receptor[TNFR]-associated factor 1; TRAF proteins associate with and mediate the signal transduction from various receptors of the TNFR superfamily) and IRAK2 (interleukin-1 receptor-associated kinase 2; IRAK2 is reported to participate in the IL-1-induced up-regulation of NF-κB) were significantly up-regulated by 4 h. Up-regulation of *IRAK2* was sustained at 6 h, while expression of *TRAF1* returned to baseline levels by 6 h. In contrast, expression of *CSF1R* (the gene encoding the colony-stimulating factor 1 receptor), and *SMAD6* (the gene encoding a protein that acts as a second messenger distal to the transforming growth factor-β [TGF-β] family of receptors) were significantly down-regulated by 4 h with down-regulation persisting at 6 h.

Chemokine and chemokine receptor-encoding genes that were significantly differentially expressed in response to *A. fumigatus* conidia are shown in Fig. 5. There were several distinct expression profiles observed among these genes. Expression of the genes *CXCL8/IL8*, *CXCL2/Groβ*, *CCL4/MIP1β*, and *CCL20/MIP3α* was significantly up-regulated by 2 h in response to *A. fumigatus*. Expression of *CCL4/MIP1β* and *CXCL2/Groβ* continued to be up-regulated over the 6-h time course, while expression of *CCL20/MIP3α* peaked at 2 h and expression of *CXCL8/IL8* peaked at 4 h. Expression of the gene encoding CCL3/MIP1α was initially down-regulated at 2 h but significantly up-regulated at 4 to 6 h. *CCL5/RANTES* was the only chemokine-

TABLE 1. Kinetic profiles of expression of genes encoding molecules with cytokine activity

| Gene expression and product	Gene	Mean $\log_2$ expression ratio at[a]:			
		0 h	2 h	4 h	6 h
Up-regulated					
TNF-α-induced protein 6	*TNFAIP6*	−0.27	0.38	0.35	0.96
TNF-α-induced protein 3	*TNFAIP3*	−0.03	0.43	0.73	0.80
Oncostatin M	*OSM*	0.12	0.44	0.67	0.58
Inhibin β A (activin)	*INHBA*	0.11	0.45	1.43	0.97
Amphiregulin	*AREG*	−0.09	0.41	0.75	0.82
Down-regulated					
TNF (ligand) superfamily member 8	*TNFSF8*	−0.02	0.11	0.15	−1.46
TNF (ligand) superfamily member 13	*TNFSF13*	0.14	0.11	−0.08	−0.61

[a] Quantitative expression of genes.
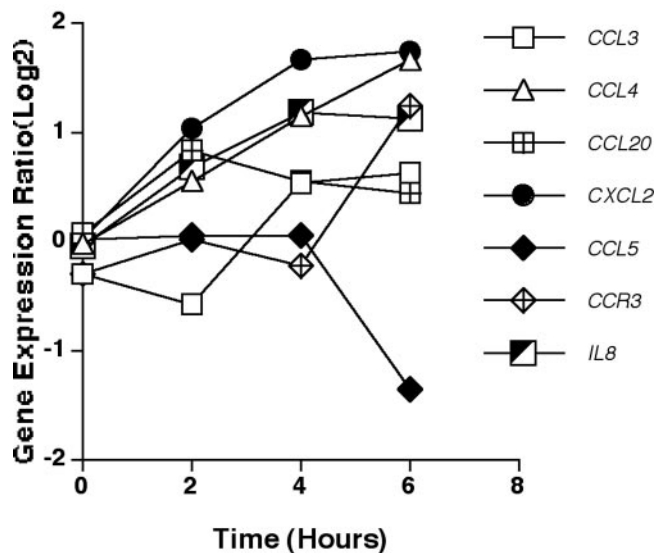
FIG. 5. Expression of chemokine and chemokine receptor genes. Mean expression ratio (log$_2$) kinetics of genes encoding chemokines and chemokine receptors in HMCs in response to conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *CCL3/MIP1α* (chemokine C-C motif ligand 3), *CCL4/MIP1β* (chemokine C-C motif ligand 4), *CCL20/MIP3α* (chemokine C-C motif ligand 20), *CXCL2/Gro*β (chemokine C-X-C motif ligand 2), *CCL5/RANTES* (regulated upon activation, normally T-cell expressed and secreted), *CCR3* (chemokine C-C motif receptor 3), and *IL8* (interleukin-8).

FIG. 6. Expression of prostaglandin-related genes. Mean expression ratio (log$_2$) kinetics of genes encoding prostaglandin synthesis molecules and receptors in HMCs in response to *A. fumigatus* conidia ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *COX2* (prostaglandin G/H synthase and cyclooxygenase), *PTGER1* (prostaglandin E receptor 1), *PTGER2* (prostaglandin E receptor 2), and *PTGER4* (prostaglandin E receptor 4).

encoding gene whose expression was significantly down-regulated in response to *A. fumigatus*, with maximal down-regulation at 6 h (Fig. 5).

The only chemokine receptor-encoding gene found to be significantly differentially expressed in response to *A. fumigatus* conidia was *CCR3*. As shown in Fig. 5, there was little change in gene expression over the first 4 h but a significant up-regulation in expression at 6 h.

The kinetic expression profiles of genes encoding prostaglandin-related molecules that significantly changed in response to conidia of *A. fumigatus* are shown in Fig. 6. *COX2* (the gene encoding the inducible cyclooxygenase) was up-regulated significantly by 2 h with increased expression over the 6-h time course. The genes encoding prostaglandin E2 receptors 2 and 4 (*PTGER2* and *PTGER4*) also were up-regulated. By comparison, expression of the gene encoding receptor 1 (*PTGER1*) was up-regulated significantly at 2 h, but returned to baseline by 4 h.

Figure 7 illustrates the expression kinetic profiles of genes involved in the oxidative response of HMCs to *A. fumigatus*. There was concomitant up-regulation of the gene encoding superoxide dismutase (*SOD2*) and down-regulation of the gene encoding catalase (*CAT*), both maximally changed at 6 h. Additionally, other genes encoding oxidative stress and response molecules, including *GPX3*, *PRDX5*, and *TXNRD2*, were down-regulated, with maximal down-regulation at 6 h. The gene encoding dual phosphatase 1 (*DUSP1*), also involved in the response to oxidative stress, was found to be maximally up-regulated at 6 h in response to *A. fumigatus*. Other genes involved in the oxidative burst, including those encoding
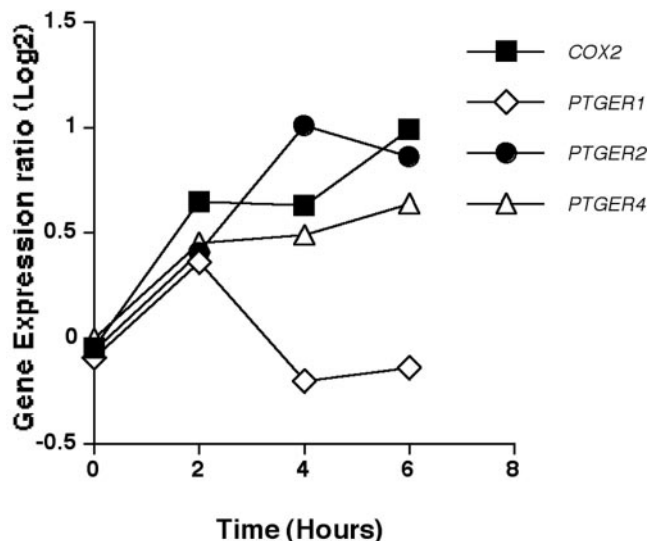
NADPH oxidase and myeloperoxidase, were not significantly differentially expressed in response to conidia of *A. fumigatus*.

Among genes encoding for pattern recognition receptors, only *PTX3* (long pentraxin 3) had a sustained up-regulation in response to *A. fumigatus* throughout the 6-h time course, peaking at 4 h (Fig. 8A). Conversely, genes encoding other lectins, such as galectin 1 (*LGALS1*) and galectin 9 (*LGALS9*), were down-regulated. There were several genes encoding non-lectin
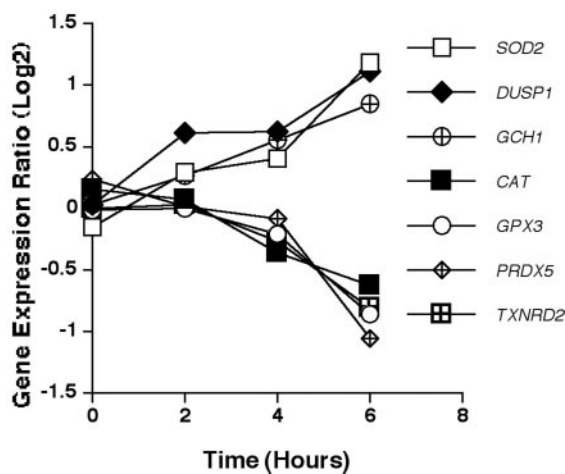
FIG. 7. Expression of oxidative response genes. Mean expression ratio (log$_2$) kinetics of genes encoding oxidative stress response molecules in HMCs in response to conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *SOD2* (superoxide dismutase 2), *DUSP1* (dual-specificity phosphatase 1), *GCH1* (GTP cyclohydrolase 1), *CAT* (catalase), *GPX3* (glutathione peroxidase 3), *PRDX5* (peroxiredoxin 5), and *TXNRD2* (thioredoxin reductase 2).

A

B

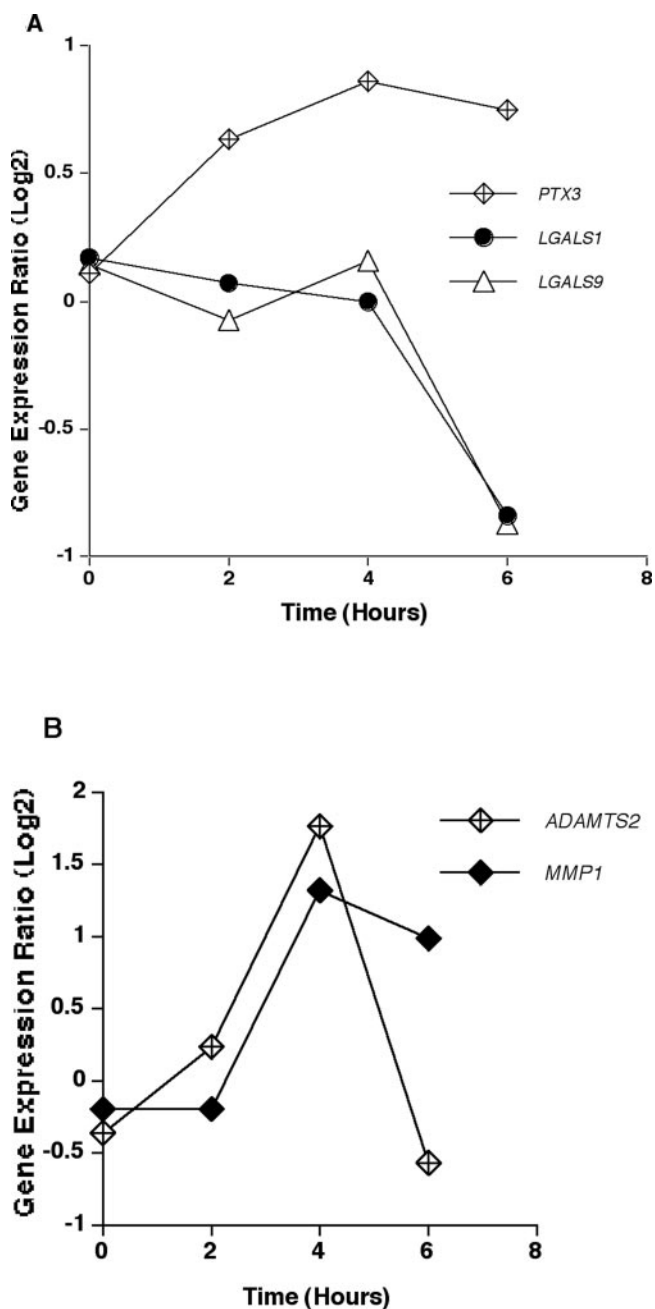FIG. 8. Expression of genes encoding pattern recognition receptors and matrix metalloproteinases. (A) Mean expression ratio (log$_2$) kinetics of genes encoding pattern recognition receptors in HMCs infected with conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *PTX3* (long pentraxin 3), *LGALS1* (galectin 1), and *LGALS9* (galectin 9). (B) Mean expression ratio (log$_2$) kinetics of genes encoding matrix metalloproteinases in HMCs infected with conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *ADAMTS2* (a desintegrin-like metalloproteinase with thrombospondin type 1 motif 2) and *MMP1* (matrix metalloproteinase 1; also known as interstitial collagenase).

cell adhesion molecules that also were significantly differentially expressed in HMCs in response to *A. fumigatus* (Table 2).

Genes encoding other immune receptors, including Toll-like receptors (TLRs) and scavenger receptors, also were examined

(Table 3). Among genes encoding the TLRs, only *TLR1* was significantly changed, with down-regulation being maximal at 6 h. Among the scavenger receptors, only *MARCO* (macrophage receptor with collagenous structure) was significantly differentially expressed in response to *A. fumigatus*, with maximal down-regulation at 6 h.

Of genes encoding metalloproteinases, *MMP1* (matrix metalloproteinase 1, interstitial collagenase) and *ADAMTS2* (a desintegrin-like metalloproteinase with thrombospondin type 1 motif 2) were significantly differentially expressed in response to conidia of *A. fumigatus*. As shown in Fig. 8B, *MMP1* and *ADAMTS2* were up-regulated, with peak up-regulation occurring at 4 h. By 6 h, *ADAMTS2* expression returned to baseline, whereas, *MMP1* expression remained significantly elevated over baseline.

As cell-cell communication is a critical function of cells involved in the immune response, expression of genes encoding connexins in HMCs in response to conidia of *A. fumigatus* was explored. As shown in Fig. 9A, genes encoding connexins 26 (*GJB2*) and 30 (*GJB6*) were significantly differentially expressed in response to *A. fumigatus*. *GJB2* expression increased continuously over the 6-h time course, while expression of *GJB6* peaked at 4 h with expression declining thereafter. As shown in Fig. 9B, genes encoding heat shock proteins Hsp40 and Hsp110 in HMCs were significantly differentially expressed in response to *A. fumigatus*, with maximal up-regulation occurring at 6 h.

Verification of microarray data in selected up-regulated genes was performed by real-time reverse transcription-PCR (Table 4). Increases in mRNA levels by RT-PCR were consistent with increased expression found using microarray analysis.

End product protein levels were determined for selected representative up- and down-regulated genes by EIAs. Kinetics of the mean protein levels (pg/ml) of CXCL8/IL-8, CCL4/MIP1β, CCL5/RANTES, and MMP-1 and their respective gene expression ratios (log$_2$) over time are shown in Fig. 10. The release of these protein products paralleled the expression of their respective genes.

DISCUSSION

To our knowledge, this is the first study to investigate the kinetic profile of the coordinated gene expression of innate host defense molecules of normal human monocytes in response to *A. fumigatus*. The functional genomic profile elicited in HMCs upon exposure to conidia of *A. fumigatus* demonstrated several important processes related to the pathogenesis of human aspergillosis. First, *A. fumigatus* conidia induced in HMCs the differential expression of genes encoding products known to be involved in the innate immune response, including cytokines, chemokines, cell adhesion molecules, and immune receptors. Additionally, several genes differentially expressed in HMCs in response to conidia of *A. fumigatus* highlighted potential mechanisms through which this organism initially may circumvent the host immune system. Genes regulating the inflammatory response also were differentially expressed. Moreover, expression of genes not previously appreciated to be involved in monocyte-mediated host response to *A. fumigatus*, such as those coding for heat shock proteins (Hsp40 and Hsp110) and connexins (connexins 26 and 30), also were sig-

TABLE 2. Kinetic profiles of expression of genes encoding molecules with cell adhesion capability

Gene expression and product	Gene	Mean log$_2$ expression ratio at[a]:			
		0 h	2 h	4 h	6 h
Up-regulated					
CD47 antigen immunoglobulin	*CD47*	−0.12	2.25	0.15	0.32
SF, member 4	*IGSF4*	−0.34	−0.48	0.96	−0.01
Protocadherin 11 X-linked	*PCDH11X*	−0.3	−1.43	2.01	−0.83
Cell adhesion molecule 1	*CEACAM1*	−0.23	−0.72	0.23	1.5
Catenin (cadherin-associated protein)	*CTNNB1*	−0.06	0.24	0.10	0.66
Metallopeptidase M8 family	*LMLN*	−0.03	−1	−0.82	1.22
Protocadherin 1 (cadherin-like 1)	*PCDH1*	−0.03	−0.48	−0.12	1.43
Immunoglobulin SF member 1	*IGSF1*	−0.35	0.24	1.29	0.94
Lymphocyte antigen 9	*LY9*	0.21	0.4	0.84	1.38
Thrombospondin 1	*THBS1*	−0.07	0.36	1.81	0.89
Down-regulated					
G-protein-coupled receptor 4	*EDG4*	−0.13	−0.93	−0.12	−0.32
Hyaluronan and proteoglycan link protein 1	*CRTL1*	−0.1	−1.08	0.03	−0.40
Platelet/endothelial cell adhesion molecule (CD31 antigen)	*PECAM1*	0.13	−0.14	−0.76	−0.24
Sialic acid binding Ig-like lectin 7	*SIGLEC7*	−0.03	−0.55	−1.32	−0.87
Rho GDP dissociation inhibitor (GDI) β	*ARHGDIB*	0.15	0.07	−0.83	−0.21
CD151 antigen	*CD151*	0.18	−0.11	0.14	−1.01
Flotillin 2	*FLOT2*	0.2	0.23	−0.24	−0.59
Paired Ig-like type 2 receptor α	*PILRA*	0.11	0.01	−0.10	−0.59
Selectin P ligand	*SELPLG*	0.12	−0.09	−0.21	−0.76

[a] Quantitative expression of genes.

nificantly affected. The data presented herein support the original hypothesis that the majority of genes that were enriched (up- or down-regulated in expression) were clustered in biological categories that are recognized to have or potentially have a role in innate immunity (see Table S2 in the supplemental material).

Aspergillus fumigatus induced significant changes in the expression of several genes in HMCs involved in phagocytosis. In this regard, there was significant up-regulation of the gene encoding CDC42EP3, a Rho GTPase involved in the regulation of the formation of F-actin-containing structures through its interaction with downstream effector proteins that are known to induce pseudopodia formation (18). Similarly, the three genes encoding endocytosis-related molecules that were differentially expressed in HMCs in response to *A. fumigatus* (*EHD1*, *SH3BP4*, and *RAB20*), were up-regulated by 4 h, suggesting an early attempt by the cells to ingest the invading organisms.

Concomitantly, there was down-regulation of other genes possibly involved in phagocytosis: *CD14*, *CORO1A*, and *FCN1*. Down-regulation of the gene encoding CD14, a surface protein expressed on monocytes and macrophages, has been reported previously in human monocytes in response to the obligatory intracellular parasite *Ehrlichia chaffeensis* (25). *E. chaffeensis* down-regulated mRNA expression of *TLR2*, *TLR4*, and *CD14*, genes coding for key pattern recognition receptors. These authors suggest that down-regulation of these receptors allows for survival of *E. chaffeensis* in the host cells. Down-regulation of *CD14* in response to *A. fumigatus* also may represent a survival mechanism triggered by the organism. Down-regulation of *CORO1A*, the gene encoding the actin-binding protein coronin, also may represent a potential escape mechanism for *Aspergillus*. Coronin is a phagosome-coating protein (13) that has previously been shown to be required for efficient phagocytosis in *Dictyostelium* (30). There was 70% reduction in yeast uptake demonstrated with deletion of this protein.

TABLE 3. Kinetic profiles of expression of genes encoding leukocyte antigens and receptors

Gene product[a]	Gene	Mean log$_2$ expression ratio at[b]:			
		0 h	2 h	4 h	6 h
Toll-like receptor 1	*TLR1*	0.10	−0.29	−0.84	−0.26
Macrophage receptor with collagenous structure	*MARCO*	0.37	−0.20	−0.30	−1.38
CD56 (Campath-1 Ag)	*CDW52*	0.18	0.03	−0.59	0.08
CD14	*CD14*	0.19	0.12	0.10	−0.65
Leukocyte immunoglobulin receptor subfamily B (with TM and ITM domains), member 1	*LILRB1*	0.15	0.08	−0.16	−0.76
Lymphocyte-specific protein 1	*LSP1*	0.15	0.02	0.17	−0.85

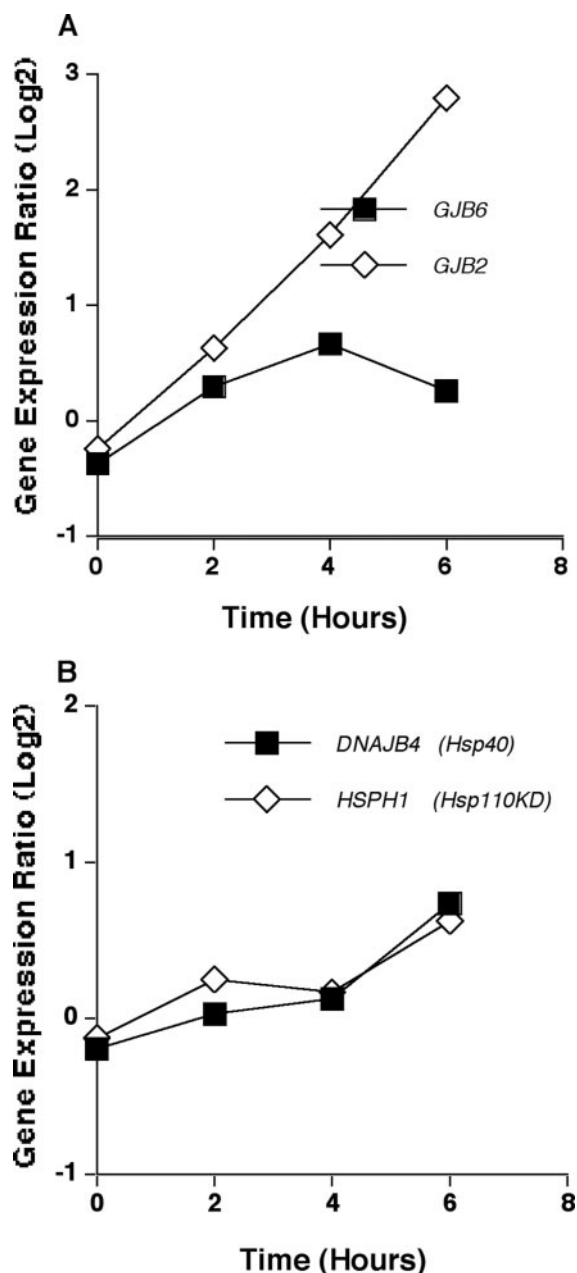[a] All are down-regulated.
[b] Quantitative expression of genes.

FIG. 9. Expression of genes encoding connexins and heat shock proteins. (A) Mean expression ratio ($\log_2$) kinetics of genes encoding connexins in HMCs infected with conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *GJB6* (gap junction protein B6, connexin 30) and *GJB2* (gap junction protein B2, connexin 26). (B) Mean expression ratio ($\log_2$) kinetics of genes encoding heat shock proteins in HMCs infected with conidia of *A. fumigatus* ($P \leq 0.05$ for peak change compared to T_0). Results for the following genes are shown: *DNAJB4* (heat shock protein 40 kDa). *HSPH1* (heat shock protein 110 kDa).

Ficolins are a family of proteins serving as receptors, opsonins, and complement activators (19). The protein encoded by *FCN1* is the precursor of M-ficolin, a phagocytic receptor expressed on the surface of HMCs. Down-regulation of *FCN1* in HMCs in the initial 6 h of interaction with *A. fumigatus* also

may represent an escape mechanism induced by the fungus, similar to the down-regulation of *CD14* and *CORO1A*.

There were a limited number of genes encoding cytokines or cytokine-related molecules that were significantly differentially expressed within the first 6 h in response to conidia of *A. fumigatus*. The gene encoding interleukin-1β (*IL1β*) was significantly up-regulated. IL-1β is an important proinflammatory cytokine involved in a variety of cellular activities, including phagocyte activation, cell proliferation, differentiation, and apoptosis (47). With similar kinetics, the gene encoding IL-10, a cytokine known to be important in the control of the inflammatory response, also was up-regulated in HMCs early in response to conidia of *A. fumigatus*. Our laboratory has previously shown that although IL-10 suppresses HMC-mediated hyphal damage of *A. fumigatus*, it enhances phagocytosis of conidia but with a net negative effect on host response to *A. fumigatus* (39). In a recent murine model, Netea et al. have proposed that IL-10 production may be a host defense evasion mechanism utilized by *Candida albicans* (35). Del Sero et al. have shown that IL-10 depletion leads to increased protection against invasive aspergillosis and candidiasis in C57b mice and that normalization of IL-10 levels suppresses host response to these infections (8). Consistent with this suppression of host response, increased levels of circulating IL-10 in neutropenic patients with invasive aspergillosis also are associated with progressive infection (41). Brouard et al. found that increased levels of IL-10 were associated with increased risk of aspergillosis in patients with cystic fibrosis (4). Furthermore, IL-10 is known to inhibit the synthesis of TNF-α, IL-6, IL-8, IL-12, and granulocyte-macrophage colony-stimulating factor induced by gamma interferon (IFN-γ) or lipopolysaccharide and down-regulate production of reactive oxygen and nitrogen intermediates (11). Consistent with these observations, we observed that expression of the *TNFα*, *IL6*, and *IL12* genes remained unchanged and that *CSF2* was down-regulated during the initial 6 h of interaction between HMCs and conidia of *A. fumigatus*.

Expression of genes encoding cytokine-receptor-associated proteins in HMCs in response to *A. fumigatus* also was evaluated. *TRAF1*, the gene encoding a member of the TRAF protein family, was up-regulated by 4 h. TRAF proteins associate with and mediate the signal transduction from various receptors of the TNFR superfamily (9). This protein, along with TRAF2, is required for TNF-α-mediated activation of MAPK8/JNK and NF-κB. IRAK2, whose gene expression also was up-regulated early in response to *A. fumigatus*, has been shown to be a proximal mediator of IL-1, a component of the IL-1

TABLE 4. Reverse transcriptase analysis of selected genes at peak 6 h

Gene product	Gene	$\log_2$ fold change[a]
Gap junction protein, beta-2 (connexin 26)	*GJB2*	3.7 ± 0.74
Prostaglandin-endoperoxide synthase 2	*PTGS2*	2.07 ± 0.73
Superoxide dismutase 2	*SOD2*	2.61 ± 0.63

[a] Data are expressed as mean ± standard error of the mean $\log_2$ change of gene expression for three separate donors displayed.
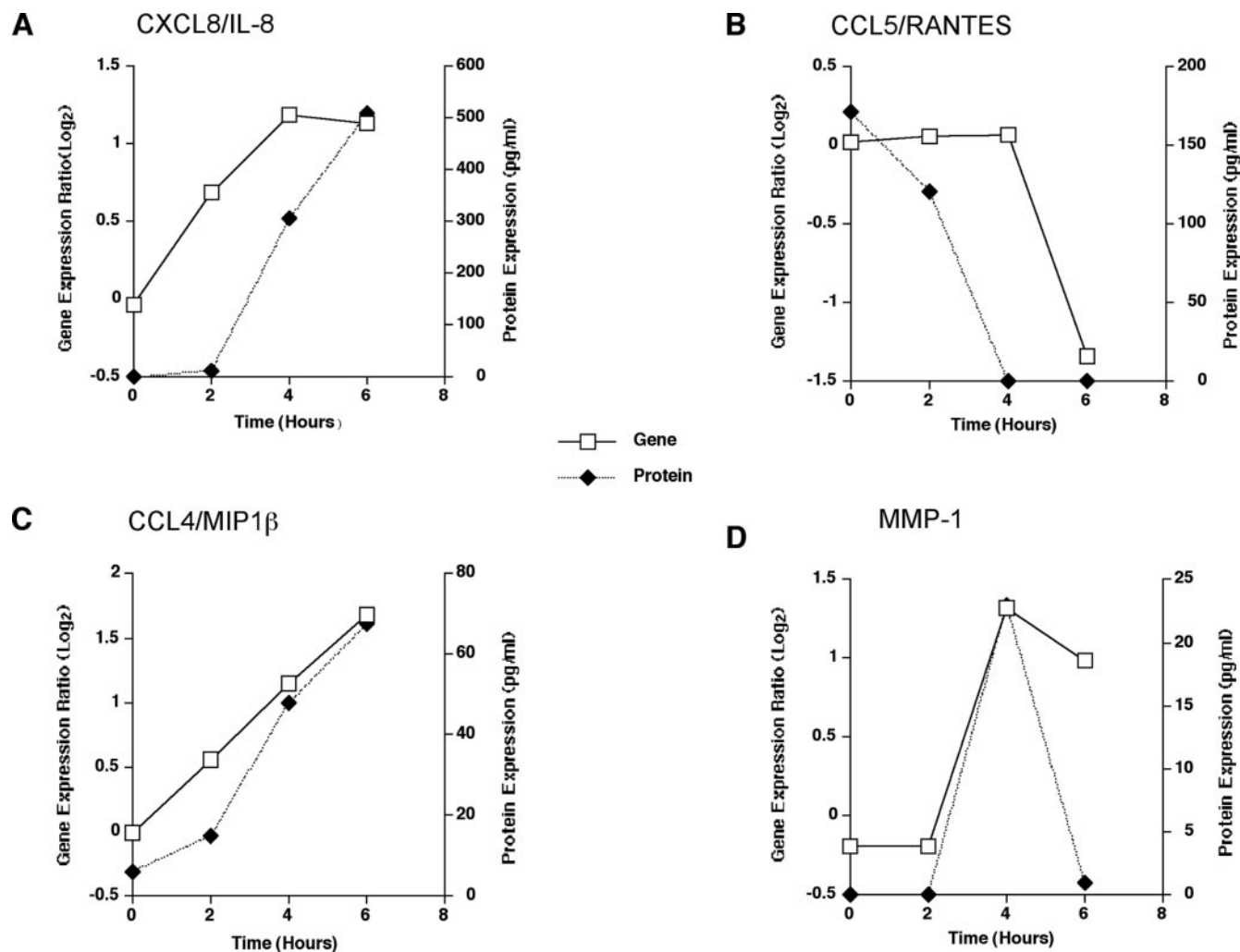
FIG. 10. Genomic expression and protein secretion of selected genes in HMCs in response to *A. fumigatus* conidia. (A) Mean expression ratio (log$_2$) kinetics of genes encoding CXCL8/IL-8 and simultaneous determination of IL-8 (end product) in supernatants of HMCs infected with conidia of *A. fumigatus*. (B) Mean expression ratio (log$_2$) kinetics of genes encoding CCL5/RANTES and simultaneous determination of RANTES protein in supernatants of HMCs infected with conidia of *A. fumigatus*. (C) Mean expression ratio (log$_2$) kinetics of genes encoding CCL4/MIP1β and simultaneous determination of CCL4/MIP1β (end product) in supernatants of HMCs infected with conidia of *A. fumigatus*. (D) Mean expression ratio (log$_2$) kinetics of genes encoding matrix metalloproteinase-1 (MMP-1) and simultaneous determination of MMP-1 (end product) in supernatants of HMCs infected with conidia of *A. fumigatus*.

receptor signaling complex, and like TRAF1 is involved in activation of NF-κB (34).

The protein encoded by *CSF1R* is the receptor for colony-stimulating factor 1, a cytokine involved in the production, differentiation, and activation of macrophages. *SMAD6* encodes a protein that acts as second messenger distal to the TGF-β family of receptors. These two genes were significantly down-regulated during the 6-h time course, suggesting a balance between activation and suppression of the inflammatory response, possibly to control damage inflicted to the host by the inflammatory response.

Genes encoding several chemokines associated with the recruitment and activation of phagocytes were significantly differentially expressed in HMCs in response to conidia of *A. fumigatus*. Our data showed significantly increased expression of *CCL3/MIP1α*, *CCL4/MIP1β*, and *CCL20/MIP3α* during the 6-h time course. These chemokines have previously been shown to be important in the early immune response to the yeast phase of *C. albicans* (23). The CXC chemokines, *CXCL2/Groβ* and CXCL8/*IL8*, which also were up-regulated, play a role in attracting neutrophils and monocytes to the site of an acute inflammatory response.

CXCL8/IL-8 appears to be an important immunoregulatory cytokine in response to *A. fumigatus*. Richardson et al. found that CXCL8/IL-8 enhanced the phagocytosis of conidia by polymorphonuclear neutrophils (38). Winn et al. also demonstrated that IL-15-stimulated polymorphonuclear leukocytes exhibit enhanced release of CXCL8/IL-8 in response to the hyphae of *A. fumigatus* (49). This current study further contributes to our understanding of IL-8 in the phagocytic response of HMCs to conidia of *A. fumigatus*. Expression of *IL8* was significantly up-regulated in parallel with release of its protein during HMC phagocytosis of *Aspergillus* conidia. While this interaction may augment the initial inflammatory response

to *A. fumigatus* by recruitment of polymorphonuclear leukocytes, there also may be a deleterious component to this response. Studies of tumor cell migration reveal that IL-8 increases transcriptional expression of matrix metalloproteinases and promotes tumor invasion (22, 29). Similarly, matrix metalloproteinases appear to be up-regulated in HMCs, which may contribute to enhanced invasion by *A. fumigatus*. This relationship is further discussed below in regulation of genes encoding matrix metalloproteinases.

In contrast, the gene encoding CCL5/RANTES was down-regulated during this early phase of infection with *A. fumigatus* conidia. CCL5/RANTES is a potent chemotaxin and is considered to be a type 1 T-cell chemokine (12). In a recent study, Marques et al. reported that IL-10 inhibited the mRNA expression of CCL5/RANTES in microglia infected with herpes simplex virus type 1 (31). Similarly, the increase in IL-10 induced by *A. fumigatus* in our studies may be responsible for the decreased expression of CCL5. The significance of down-regulation of CCL5/RANTES in response to *A. fumigatus* warrants further investigation as another potential mechanism of organism-mediated suppression of innate host responses.

CCR3 is a known receptor used by CCL5/RANTES (12). Of the genes encoding chemokine receptors, only *CCR3* was up-regulated in HMCs in response to *A. fumigatus* conidia. The up-regulation of this receptor may be a paracrine response to the down-regulation of CCL5/RANTES, as they both occur at the 6-h time point. Furthermore, this up-regulation of CCR3 may allow for selective amplification of a Th2-type response to *A. fumigatus*.

Toll-like receptors are important pattern-recognition receptors involved in sensing pathogenic microorganisms (45). Recognition of *A. fumigatus* conidia by HMCs involves both TLR2 and TLR4 (36). During the early 6-h time course, we found significant down-regulation in *TLR1*, with no significant change in expression of the genes encoding TLR2 and TLR4. Changes in expression of *TLR2* and *TLR4* most likely occur following longer exposure to the organism, or these results may differ due to variation in the experimental design. Further research is warranted to clarify the significance of TLR1 in response to *A. fumigatus*.

The gene encoding the macrophage scavenger receptor MARCO also was significantly down-regulated in HMCs in response to *A. fumigatus*. MARCO is a scavenger receptor that has an important role in clearance of inert particles and in mounting an efficient and appropriately regulated innate immune response against *Streptococcus pneumoniae* (1). That MARCO is down-regulated in the presence of conidia of *A. fumigatus* may be yet another potential mechanism utilized by *A. fumigatus* to evade innate immunity or of the host to modulate the initial inflammatory response.

HMCs also may recognize conidia of *A. fumigatus* through the use of soluble receptors such as collectins. This group of receptors is involved in cell adhesion, opsonization, complement activation, and apoptosis (14). The gene encoding the long pentraxin 3 (PTX3) was up-regulated at all time points. This early up-regulation of *PTX3* is consistent with its role in innate pulmonary host defense specific to *A. fumigatus* (15). PTX3 opsonizes *A. fumigatus* conidia and contributes significantly to improved survival in experimental pulmonary aspergillosis (16). Down-regulation of expression of genes en-

coding other lectins, galectin-1 and galectin-9, may represent the cell's attempt at prolonging its own survival, since both galectins are known to induce apoptosis (26).

Non-lectin cell adhesion molecules also may be important in early host response to fungi. Expression of *CD47*, a gene encoding a membrane protein involved in the increase of intracellular calcium concentrations that occur upon cell adhesion to extracellular matrices, was up-regulated in response to *A. fumigatus*. The early influx of Ca^{2+} through this membrane protein may lead to early cell activation.

The cell adhesion molecule LMLN has been shown to suppress cell-mediated immune responses through its ability to down-regulate IL-12 production, and thus inhibit classical activation of macrophages (33). In our experiments, MSP/LMLN was significantly up-regulated, a response that may constitute another potential mechanism utilized by *A. fumigatus* to evade host defense or to dampen the initial inflammatory response.

Aspergillus fumigatus also may deleteriously alter host interaction through up-regulation of host matrix metalloproteinases, which may facilitate subsequent fungal invasion. Among the genes encoding matrix metalloproteinases, *ADAMTS2* and *MMP1* were significantly differentially expressed in response to *A. fumigatus*. *ADAMTS2* encodes a desintegrin-like metalloproteinase with thrombospondin type 1 motif 2, while *MMP1* encodes matrix metalloproteinase 1, also known as collagenase 1. This increased expression of metalloproteinases may provide a mechanism by which *A. fumigatus* induces lysis of the extracellular matrix and in this way facilitates local invasion in connective tissue, parenchyma, and blood vessels. These observations are consistent with those observed in the pathogenesis of *Coccidioides posadasii* (48). *Coccidioides* induces expression of matrix metalloproteinase 9 (MMP-9) in rabbits with experimental coccidioidal meningitis. Production of MMP-9 is considered related to the inflammatory vasculitis associated with this infection. Additionally, in an in vitro model of oral epithelial cells and basement membrane, *C. albicans* induces the production of MMP-9 and matrix metalloproteinase 2 (7). There also is a striking analogy to tumor cell invasion, wherein malignant cells induce expression of *MMP1* from fibroblasts (2). This increased expression of *MMP1* promotes invasion by neoplastic epithelial cells into surrounding stroma.

Production of reactive oxygen species, such as superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radicals, is an important component of the oxidative host response in phagocytes. While killing invasive microorganisms is the primary function of these reactive oxygen species, their production and release are coordinately controlled to prevent cell and tissue damage. In this regard, while genes encoding superoxide dismutase 2 (*SOD2*) and dual phosphatase 1 (*DUSP1*) were significantly up-regulated, a concomitant and similar down-regulation occurred in the group of genes encoding catalase (*CAT*), glutathione peroxidase 3 (*GPX3*), and peroxiredoxin 5 (*PRDX5*). The up-regulation of *SOD2* and the down regulation of *CAT* may result in the persistence of H_2O_2 in the extracellular environment. This persistence may enhance fungicidal activity as well as serve as a second messenger (17). Thus, there appears to be a well-polarized and coordinated gene expression in oxidative response genes induced by the interaction of HMCs with *A. fumigatus* conidia in order to eliminate the organisms while simultaneously preserving the cell from excessive inflammatory damage.
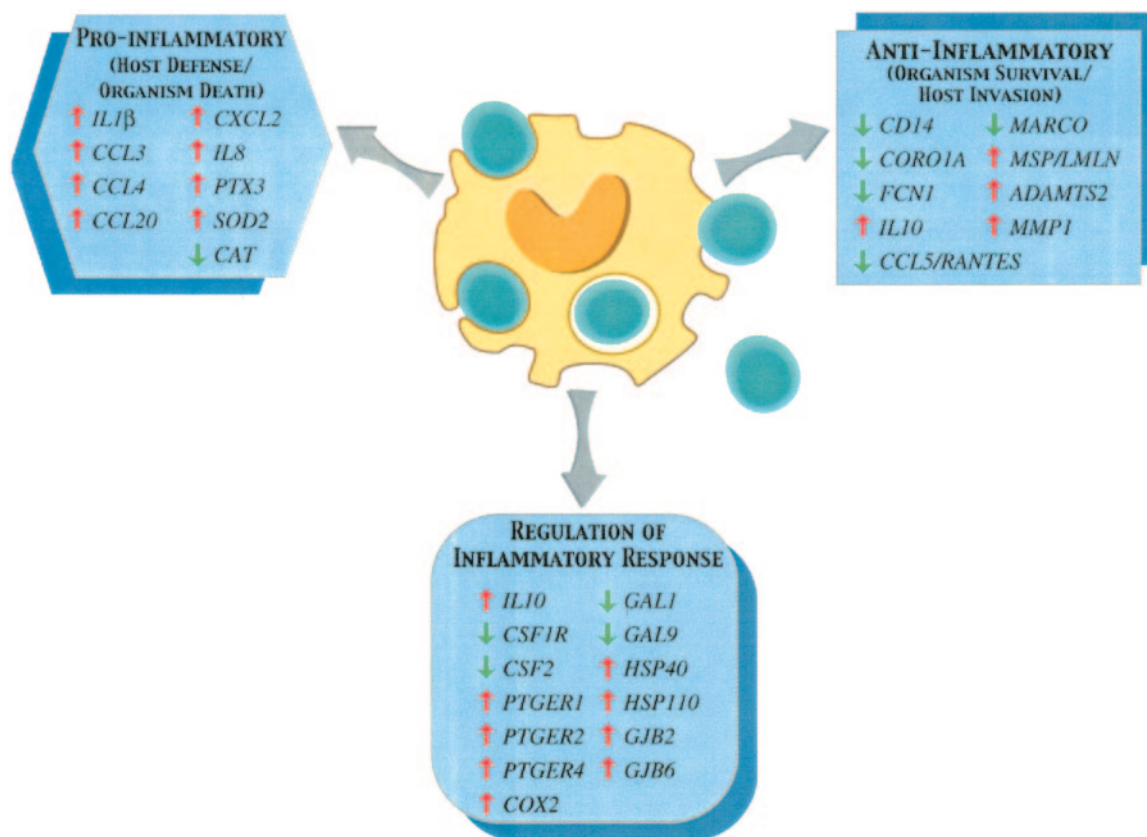
FIG. 11. Model of early gene expression of innate host defense molecules in normal human monocytes in response to conidia of *A. fumigatus*. The functional genomic response of normal human monocytes against *A. fumigatus* may fit a dichotomous model that is modulated by genes encoding immunoregulatory proteins. The dichotomous response involves genes coding for proteins mediating host defense and organism death or organism survival and host invasion. The latter may be induced by the organism or may be the result of immunomodulation causing down-regulation of host response. The third arm depicted in this model represents the complex and multifaceted immunoregulatory component of monocytes that may affect both arms of the reciprocal response elicited by the organism. The outcome of survival or death of *A. fumigatus* may be determined by specific host derangements that alter this finely regulated response. Results for the following genes are shown: *IL1β* (interleukin-1β); *CCL3*, *CCL4*, and *CCL20* (C-C chemokine ligands 3, 4, and 20); *CXCL2* (C-X-C chemokine ligand 2); *IL8* (interleukin-8); *PTX3* (pentraxin 3); *SOD2* (dismutase 2); *CAT* (catalase); *CD14* (cluster designation 14); *CORO1A* (coronin 1A); *FCN1* (ficolin 1 precursor); *IL10* (interleukin-10); *CCL5/RANTES* (C-C chemokine ligand 5); *MARCO* (macrophage receptor with collagenous structure); *LMLN* (leishmanolysin-like [metallopeptidase M8 family]); *ADAMTS2* (desintegrin-like and metalloprotease [reprolysin type] with thrombospondin type 1 motif 2); *MMP1* (matrix metalloproteinase 1); *CSF1R* (colony-stimulating factor receptor 1); *PTGER1*, *PTGER2*, and *PTGER4* (prostaglandin E receptors 1, 2, and 4), *COX2* (inducible cyclooxygenase 2); *GAL1* and *GAL9* (galectins 1 and 9); *HSP40* and *HSP110* (heat shock proteins 40 and 110 kDa); and *GJB2* and *GJB6* (connexins 26 and 30).

HMCs may coordinate their response to *A. fumigatus* through intercellular communication via gap junctions. Osteoclasts, which are derived from monocytes, coordinate their function through hemi-channel gap junctions (20). Vertebrate gap junctions are made up of a multigene family called connexins. Gap junctions are assumed to modulate various biological processes through mediating the massive movement of ions and second messengers, allowing for a form of "quorum sensing." However, little is known about their role in innate host defenses. We found that connexin-26 and connexin-30 were significantly differentially expressed in our study. Kim et al. also observed this pattern of up-regulation of connexins where genes encoding connexin-26 and connexin-31 were up-regulated in monocytes in response to *C. albicans* (23).

Eicosanoids are synthesized from arachidonic acid by enzymes called cyclooxygenases (COX), which exist in two isoforms, a constitutive enzyme, type 1 (COX-1), and type 2

(COX-2), which is induced by inflammatory mediators, mainly cytokines. Of the genes encoding prostaglandin-related molecules, the gene encoding prostaglandin endoperoxidase H synthase (*PTGS2/COX2*), the inducible isoform involved in the regulation of inflammation, was significantly up-regulated. *Candida albicans* induces *PTGS2/COX2* through TLR2 and TLR4 followed by phosphokinase C, and/or NF-κB (10). While the role of *COX2* expression in response to *A. fumigatus* is unknown, it may serve as a potent immunoregulator in the tracheobronchial tree.

These data demonstrate the utility of the microarray as a powerful tool for assessing kinetic changes in expression of innate host response genes in monocytes induced by *A. fumigatus*. A potential limitation in microarray analysis, however, is that expression of mRNA may not coincide with extracellular protein release either quantitatively or qualitatively. In our studies, gene expression patterns for three selected genes did in fact

mirror protein release. Another potential limitation of this study is the use of HMCs as opposed to pulmonary alveolar macrophages in determining the initial host response to conidia of *A. fumigatus*. However, given the lack of availability of human pulmonary alveolar macrophages, circulating HMCs that are precursors to tissue macrophages offer insight into the genetic profile of the initial response to *Aspergillus*.

These microarray data reveal a reciprocal regulatory control and coordinated kinetic expression pattern of multiple genes involved in the early host response to *A. fumigatus* conidia. As illustrated in Fig. 11, genes whose products are involved in organism death and injury are simultaneously regulated along with genes whose products may facilitate host invasion by dampening the inflammatory response. Furthermore, these microarray data reveal the coordinated temporal response of genes heretofore not known to be involved in the immunopathogenesis of human aspergillosis. Thus, this early kinetic profile of gene expression in response to *A. fumigatus* offers a greater understanding of the early host-pathogen interaction of this medically important mycosis.

ACKNOWLEDGMENTS

REFERENCES

1. **Arredouani, M., Z. Yang, Y. Ning, G. Qin, R. Soininen, K. Tryggvason, and L. Kobzik.** 2004. The scavenger receptor MARCO is required for lung defense against pneumococcal pneumonia and inhaled particles. J. Exp. Med. **200:**267–272.
2. **Arribas, J.** 2005. Matrix metalloproteases and tumor invasion. N. Engl. J. Med. **352:**2020–2021.
3. **Brocke-Heidrich, K., A. K. Kretzschmar, G. Pfeifer, C. Henze, D. Löffler, D. Koczan, H. J. Thiesen, R. Burger, M. Gramatzki, and F. Horn.** 2004. Interleukin-6-dependent gene expression profiles in multiple myeloma INA-6 cells reveal a Bcl-2 family-independent survival pathway closely associated with Stat3 activation. Blood **103:**242–251.
4. **Brouard, J., N. Knauer, P. Y. Boelle, H. Corvol, A. Henrion-Caude, C. Flamant, F. Bremont, B. Delaisi, J. F. Duhamel, C. Marguet, M. Roussey, M. C. Miesch, K. Chadelat, M. Boule, B. Fauroux, F. Ratjen, H. Grasemann, and A. Clement.** 2005. Influence of interleukin-10 on Aspergillus fumigatus infection in patients with cystic fibrosis. J. Infect. Dis. **191:**1988–1991.
5. **Choe, S. E., M. Boutros, A. M. Michelson, G. M. Church, and M. S. Halfon.** 2005. Preferred analysis methods for Affymetrix GeneChips revealed by a wholly defined control dataset. Genome Biol. **6:**R16. [Online.] http://genomebiology.com/2005/6/2/R16.
6. **Chudin, E., R. Walker, A. Kosaka, S. X. Wu, D. Rabert, T. K. Chang, and D. E. Kreder.** 2002. Assessment of the relationship between signal intensities and transcript concentration for Affymetrix GeneChip arrays. Genome Biol. **3:**RESEARCH0005. [Online.] http://genomebiology.com/2001/3/1/RESEARCH/0005.
7. **Claveau, I., Y. Mostefaoui, and M. Rouabhia.** 2004. Basement membrane protein and matrix metalloproteinase deregulation in engineered human oral mucosa following infection with Candida albicans. Matrix Biol. **23:**477–486.
8. **Del Sero, G., A. Mencacci, E. Cenci, C. F. d'Ostiani, C. Montagnoli, A. Bacci, P. Mosci, M. Kopf, and L. Romani.** 1999. Antifungal type 1 responses are upregulated in IL-10-deficient mice. Microbes Infect. **1:**1169–1180.
9. **Dempsey, P. W., S. E. Doyle, J. Q. He, and G. Cheng.** 2003. The signaling adaptors and pathways activated by TNF superfamily. Cytokine Growth Factor Rev. **14:**193–209.
10. **Deva, R., P. Shankaranarayanan, R. Ciccoli, and S. Nigam.** 2003. Candida albicans induces selectively transcriptional activation of cyclooxygenase-2 in HeLa cells: pivotal roles of Toll-like receptors, p38 mitogen-activated protein kinase, and NF-kappa B. J. Immunol. **171:**3047–3055.
11. **Donnelly, R. P., H. Dickensheets, and D. S. Finbloom.** 1999. The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. J. Interferon Cytokine Res. **19:**563–573.
12. **Fernandez, E. J., and E. Lolis.** 2002. Structure, function, and inhibition of chemokines. Annu. Rev. Pharmacol. Toxicol. **42:**469–499.
13. **Ferrari, G., H. Langen, M. Naito, and J. Pieters.** 1999. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. Cell **97:**435–447.
14. **Garlanda, C., B. Bottazzi, A. Bastone, and A. Mantovani.** 2005. Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annu. Rev. Immunol. **23:**337–366.
15. **Garlanda, C., E. Hirsch, S. Bozza, A. Salustri, M. De Acetis, R. Nota, A. Maccagno, F. Riva, B. Bottazzi, G. Peri, A. Doni, L. Vago, M. Botto, R. De Santis, P. Carminati, G. Siracusa, F. Altruda, A. Vecchi, L. Romani, and A. Mantovani.** 2002. Non-redundant role of the long pentraxin PTX3 in antifungal innate immune response. Nature **420:**182–186.
16. **Gaziano, R., S. Bozza, S. Bellocchio, K. Perruccio, C. Montagnoli, L. Pitzurra, G. Salvatori, R. De Santis, P. Carminati, A. Mantovani, and L. Romani.** 2004. Anti-Aspergillus fumigatus efficacy of pentraxin 3 alone and in combination with antifungals. Antimicrob. Agents Chemother. **48:**4414–4421.
17. **Giron-Calle, J., and H. J. Forman.** 2000. Phospholipase D and priming of the respiratory burst by H_2O_2 in NR8383 alveolar macrophages. Am. J. Respir. Cell Mol. Biol. **23:**748–754.
18. **Hirsch, D. S., D. M. Pirone, and P. D. Burbelo.** 2001. A new family of Cdc42 effector proteins, CEPs, function in fibroblast and epithelial cell shape changes. J. Biol. Chem. **276:**875–883.
19. **Holmskov, U., S. Thiel, and J. C. Jensenius.** 2003. Collectins and ficolins: humoral lectins of the innate immune defense. Annu. Rev. Immunol. **21:**547–578.
20. **Ilvesaro, J., and J. Tuukkanen.** 2003. Gap-junctional regulation of osteoclast function. Crit. Rev. Eukaryot. Gene Expr. **13:**133–146.
21. **Irizarry, R. A., B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf, and T. P. Speed.** 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics **4:**249–264.
22. **Janowska-Wieczorek, A., L. A. Marquez, J. M. Nabholtz, M. L. Cabuhat, J. Montano, H. Chang, J. Rozmus, J. A. Russell, D. R. Edwards, and A. R. Turner.** 1999. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34$^+$ cells and their transmigration through reconstituted basement membrane. Blood **93:**3379–3390.
23. **Kim, H. S., E. H. Choi, J. Khan, E. Roilides, A. Francesconi, M. Kasai, T. Sein, R. L. Schaufele, K. Sakurai, C. G. Son, B. T. Greer, S. Chanock, C. A. Lyman, and T. J. Walsh.** 2005. Expression of genes encoding innate host defense molecules in normal human monocytes in response to *Candida albicans*. Infect. Immun. **73:**3714–3724.
24. **Kogel, D., R. Schomburg, E. Copanaki, and J. H. Prehn.** 2005. Regulation of gene expression by the amyloid precursor protein: inhibition of the JNK/c-Jun pathway. Cell Death Differ. **12:**1–9.
25. **Lin, M., and Y. Rikihisa.** 2004. Ehrlichia chaffeensis downregulates surface Toll-like receptors 2/4, CD14 and transcription factors PU.1 and inhibits lipopolysaccharide activation of NF-kappa B, ERK 1/2 and p38 MAPK in host monocytes. Cell Microbiol. **6:**175–186.
26. **Liu, F. T.** 2000. Galectins: a new family of regulators of inflammation. Clin. Immunol. **97:**79–88.
27. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\text{Delta Delta } C(T)}$ method. Methods **25:**402–408.
28. **Lu, T., Y. Pan, S. Y. Kao, C. Li, I. Kohane, J. Chan, and B. A. Yankner.** 2004. Gene regulation and DNA damage in the ageing human brain. Nature **429:**883–891.
29. **Luca, M., S. Huang, J. E. Gershenwald, R. K. Singh, R. Reich, and M. Bar-Eli.** 1997. Expression of interleukin-8 by human melanoma cells upregulates MMP-2 activity and increases tumor growth and metastasis. Am. J. Pathol. **151:**1105–1113.
30. **Maniak, M., R. Rauchenberger, R. Albrecht, J. Murphy, and G. Gerisch.** 1995. Coronin involved in phagocytosis: dynamics of particle-induced relocalization visualized by a green fluorescent protein Tag. Cell **83:**915–924.
31. **Marques, C. P., S. Hu, W. Sheng, M. C. Cheeran, D. Cox, and J. R. Lokensgard.** 2004. Interleukin-10 attenuates production of HSV-induced inflammatory mediators by human microglia. Glia **47:**358–366.
32. **Marr, K. A., R. A. Carter, F. Crippa, A. Wald, and L. Corey.** 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin. Infect. Dis. **34:**909–917.
33. **Morrison, A. C., C. B. Wilson, M. Ray, and P. H. Correll.** 2004. Macrophage-stimulating protein, the ligand for the stem cell-derived tyrosine kinase/RON receptor tyrosine kinase, inhibits IL-12 production by primary peritoneal macrophages stimulated with IFN-gamma and lipopolysaccharide. J. Immunol. **172:**1825–1832.
34. **Muzio, M., J. Ni, P. Feng, and V. M. Dixit.** 1997. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. Science **278:**1612–1615.
35. **Netea, M. G., R. Sutmuller, C. Hermann, C. A. Van der Graaf, J. W. Van der Meer, J. H. van Krieken, T. Hartung, G. Adema, and B. J. Kullberg.** 2004. Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J. Immunol. **172:**3712–3718.
36. **Netea, M. G., A. Warris, J. W. Van der Meer, M. J. Fenton, T. J. Verver-Janssen, L. E. Jacobs, T. Andresen, P. E. Verweij, and B. J. Kullberg.** 2003. Aspergillus fumigatus evades immune recognition during germination through loss of toll-like receptor-4-mediated signal transduction. J. Infect. Dis. **188:**320–326.

37. **Rajagopalan, D.** 2003. A comparison of statistical methods for analysis of high density oligonucleotide array data. Bioinformatics **19:**1469–1476.

38. **Richardson, M. D., and M. Patel.** 1995. Stimulation of neutrophil phagocytosis of Aspergillus fumigatus conidia by interleukin-8 and N-formylmethionyl-leucylphenylalanine. J. Med. Vet. Mycol. **33:**99–104.

39. **Roilides, E., A. Dimitriadou, I. Kadiltsoglou, T. Sein, J. Karpouzas, P. A. Pizzo, and T. J. Walsh.** 1997. IL-10 exerts suppressive and enhancing effects on antifungal activity of mononuclear phagocytes against Aspergillus fumigatus. J. Immunol. **158:**322–329.

40. **Roilides, E., A. Holmes, C. Blake, P. A. Pizzo, and T. J. Walsh.** 1993. Impairment of neutrophil antifungal activity against hyphae of Aspergillus fumigatus in children infected with human immunodeficiency virus. J. Infect. Dis. **167:**905–911.

41. **Roilides, E., T. Sein, M. Roden, R. L. Schaufele, and T. J. Walsh.** 2001. Elevated serum concentrations of interleukin-10 in nonneutropenic patients with invasive aspergillosis. J. Infect. Dis. **183:**518–520.

42. **Roilides, E., K. Uhlig, D. Venzon, P. A. Pizzo, and T. J. Walsh.** 1993. Enhancement of oxidative response and damage caused by human neutrophils to *Aspergillus fumigatus* hyphae by granulocyte colony-stimulating factor and gamma interferon. Infect. Immun. **61:**1185–1193.

43. **Rosenzweig, S. D., and S. M. Holland.** 2004. Phagocyte immunodeficiencies and their infections. J. Allergy Clin. Immunol. **113:**620–626.

44. **Singh, N.** 2003. Fungal infections in the recipients of solid organ transplantation. Infect. Dis. Clin. N. Am. **17:**113–134, viii.

45. **Staros, E. B.** 2005. Innate immunity: new approaches to understanding its clinical significance. Am. J. Clin. Pathol. **123:**305–312.

46. **Torres, H. A., G. P. Bodey, K. V. Rolston, H. M. Kantarjian, I. I. Raad, and D. P. Kontoyiannis.** 2003. Infections in patients with aplastic anemia: experience at a tertiary care cancer center. Cancer **98:**86–93.

47. **Wewers, M. D.** 2004. IL-1beta: an endosomal exit. Proc. Natl. Acad. Sci. USA **101:**10241–10242.

48. **Williams, P. L., S. L. Leib, P. Kamberi, D. Leppert, R. A. Sobel, Y. D. Bifrare, K. V. Clemons, and D. A. Stevens.** 2002. Levels of matrix metalloproteinase-9 within cerebrospinal fluid in a rabbit model of coccidioidal meningitis and vasculitis. J. Infect. Dis. **186:**1692–1695.

49. **Winn, R. M., C. Gil-Lamaignere, E. Roilides, M. Simitsopoulou, C. A. Lyman, A. Maloukou, and T. J. Walsh.** 2003. Selective effects of interleukin (IL)-15 on antifungal activity and IL-8 release by polymorphonuclear leukocytes in response to hyphae of Aspergillus species. J. Infect. Dis. **188:**585–590.

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