Coculture of THP-1 Human Mononuclear Cells with *Candida albicans* Results in Pronounced Changes in Host Gene Expression

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Background. The host's first line of defense against bloodstream infection with *Candida albicans* involves the recognition and clearance of the fungus by neutrophils and monocytes/macrophages. The purpose of the present study was to examine changes in the monocytic cell gene-expression profile in response to *C. albicans* stimulation.

Methods. RNA was isolated from THP-1 cells 3 h after coculture with live *C. albicans* SC5314 cells. After hybridization to microarrays, genes differentially expressed by at least 2.0-fold were included in the final data set.

Results. As expected, *TNFA, IL8, CD83, MIP1A,* and *MIP1B* were among the genes up-regulated. This was confirmed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR), fluorescence-activated cell sorting analysis, and enzyme-linked immunosorbent assay. Furthermore, *RGS1, RGS2, RGS16, DSCR1, GROB, EGR3, FLT4,* and *TNFAIP6* were also up-regulated in response to *C. albicans,* whereas *CCR2* and *NCF2* were among the genes down-regulated in response to *C. albicans.* Differential expression of selected genes was confirmed at several time points by real-time RT-PCR.

Conclusions. This study defines the gene expression profile of an early response of human mononuclear cells to *C. albicans* and identifies genes not previously known to be responsive to this pathogen.

The human opportunistic pathogen *Candida albicans* causes superficial and disseminated disease in immunocompromised individuals. Superficial *C. albicans* infections occur most often in the oropharynx and vagina. Although not invasive or life threatening, oropharyngeal candidiasis is one of the most common infections in persons with HIV/AIDS. In contrast, disseminated candidiasis is deadly, accounting for the highest incidence of mortality (40%) of any cause of bloodstream infections [1], and it remains one of the leading causes of death in neutropenic patients with cancer [2].

Competent host response to disseminated candidiasis involves neutrophils and mononuclear phagocytes for recognition and clearing of fungal cells [3]. In addition to their role as phagocytic cells, both mononuclear phagocytes and neutrophils are capable of secreting immunomodulatory cytokines that influence the host immune response to fungal infection [4, 5]. *C. albicans*–stimulated monocytes, as well as stimulated $CD4^+$ and $CD8^+$ T cells and NK cells, produce macrophage inflammatory protein (MIP)–1α, MIP-1β, and RANTES, which are responsible for chemoattraction of activated $CD4^+$ Th1 T cells, dendritic cells (DCs), and monocytes to the site of infection [6]. Additionally, monocytes produce interleukin (IL)–1β, tumor necrosis factor (TNF)–α, and IL-10 in response to *C. albicans* hyphae and produce IL-12 in response to *C. albicans* unable to form hyphae [7–9].

C. albicans interacts with monocytes through Toll-like receptors (TLRs) 2 and 4 [10], the integrin CD11b/CD18 [11], and the β glucan receptor dectin-1 [12]. Intracellularly, signaling involves at least mitogen-activated protein kinase and protein kinase C pathways to induce expression of host factors [13]. It remains unresolved whether other pathways, such as extracel-

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lular-related kinases, are also involved in the production of factors such as chemokines in response to *C. albicans.*

The human monocytic cell line THP-1 affords a competent in vitro model of monocytes/macrophages during interaction with fungal cells. Previous studies have utilized THP-1 cells to examine human monocyte/macrophage chemokine production in response to whole fungal cells or fungal cell wall components [14, 15], phagocytosis of fungal cells [16], and differentiation and cell surface marker expression [17, 18]. THP-1 cells have proven advantageous in microarray analyses, since, in addition to their established usefulness as a monocyte/macrophage model, their homogeneous genetic background minimizes the amount of variability in the resulting gene expression profiles [19, 20]. Therefore, because of their established function as a model of peripheral blood mononuclear cells (PBMCs) and their attractiveness for use in microarray analysis, we chose to use THP-1 cells in an in vitro model of host monocyte–*C. albicans* interaction.

In the present study, to better explore the impact of *C. albicans* on host monocyte gene expression, we simultaneously examined the expression of ~18,400 human genes by use of microarray hybridization of RNA from THP-1 cells cocultured for 3 h with *C. albicans* strain SC5314. Further consideration was given to several genes with known involvement in the host response to *C. albicans,* by examining mRNA and protein expression during a span of 12 h in THP-1 cells cocultured with this fungus. In addition, several genes whose expression has never before been associated with the host response to *C. albicans* were examined during the time course by real-time reverse-transcriptase polymerase chain reaction (RT-PCR), to identify differential mRNA expression.

MATERIALS AND METHODS

Human cell line and* C. albicans *isolate. The THP-1 human monocytic cell line (American Type Culture Collection) was used in this study. Cells were maintained in culture medium (RPMI 1640 and 10% fetal calf serum) at 37°C in a humidified chamber containing 5% CO_2. SC5314 is a wild-type, virulent strain capable of producing hyphae. It was stored as a glycerol stock at −70°C and was grown in yeast nitrogen base broth containing 5% dextrose at 30°C in a shaking incubator.

Coculture conditions. Overnight fungal cultures were washed, resuspended in culture medium, and incubated in a shaking incubator for 3 h. THP-1 cells were also washed, counted using a hemacytometer, plated at 2×10^6 cells/well, and allowed to equilibrate at 37°C for 3 h. After incubation, fungal cultures were washed, counted using a hemacytometer, and plated with THP-1 cells at a fungus-monocyte ratio of 3:10. This ratio was determined (data not shown) to preserve cell viability while providing suitable host gene response to known response genes, such as *TNFA.* Cocultures were incubated at 37°C in a CO_2 incubator for 3 h (for microarray hybridization) or for 1–12 h (for subsequent analyses). After incubation, each coculture was examined by light microscopy; the majority of *C. albicans* cells had formed hyphae by 1 h, and many *C. albicans* cells were intracellular by 6 h. Viability of THP-1 cells was assessed by trypan blue exclusion (≥80% viability was observed), supernatants were collected, and RNA was isolated from THP-1 cells. Supernatants from cocultures were tested using an E-TOXATE kit (Sigma Chemical) and contained <0.06 EU/mL endotoxin. All experiments were performed in duplicate.

Total RNA isolation. Total RNA was isolated using Trizol reagent (Gibco/Invitrogen) in accordance with the manufacturer's instructions. RNA pellets were suspended in diethylpyrocarbonate-treated water and stored at −70°C. The integrity of RNA samples was assessed using an Agilent Bioanalyzer before microarray hybridization and by gel electrophoresis before real-time RT-PCR analysis.

Microarray hybridization and data analysis. Differential gene expression was measured by hybridizing Affymetrix U133A arrays and comparing normalized signals between THP-1 cells cultured in medium alone and those cultured with *C. albicans.* Two sets of hybridizations were performed using RNA samples generated from 2 independent coculture experiments. Ten micrograms of total RNA was subjected to first- and second-strand cRNA synthesis incorporating biotin-labeled nucleotides. cRNA was fragmented and subsequently hybridized overnight with microarray chips, using the manufacturer's hybridization buffer. Hybridized microarrays were washed and subjected to a signal-enhancement protocol consisting of an initial incubation with streptavidin–phycoerythrin (PE) conjugate, followed by staining with goat anti-streptavidin biotinylated antibody and a final staining with the streptavidin-PE conjugate. The microarrays were scanned using the GeneArray scanner with an argon ion laser excitation source, and emission was detected by a photomultiplier tube through a 570-nm longpass filter. Digitized image data were processed using GeneChip Operating Software (Affymetrix). Data normalization was performed as described elsewhere [21]. Genes were considered to be up-regulated if averaged normalized ratios were ≥2.0 and were considered to be down-regulated if averaged normalized ratios were ≤−2.0.

cDNA synthesis and real-time RT-PCR. First-strand cDNAs were synthesized from 2 μg of total RNA in a 21-μL reaction volume by use of the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Quantitative real-time PCRs were performed in duplicate using the 7000 Sequence Detection System (Applied Biosystems). Independent PCRs were performed in triplicate, using the same cDNA for both the gene of interest and 18S rRNA, by use of the SYBR Green PCR Master Mix (Applied Biosystems). Gene-specific primers were designed for the gene of interest and 18S rRNA by use of Primer

Express software (version 2.0; Applied Biosystems) and the Oligo Analysis & Plotting Tool (Qiagen) and are listed in table 1. The PCR conditions consisted of AmpliTaq Gold activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. To verify that a single product was amplified, a dissociation curve was generated at the end of each PCR cycle, by use of software provided with the 7000 Sequence Detection System. The change in fluorescence of SYBR Green I dye in every cycle was monitored by the system software, and the cycle threshold (CT) above background for each reaction was calculated. The CT value of 18S rRNA was subtracted from that of the gene of interest to obtain a ΔCT value. The ΔCT value of the least abundant sample at all time points for each gene was subtracted from the ΔCT value of each sample to obtain a ΔΔCT value. The gene expression level relative to the calibrator was expressed as $2^{-\Delta\Delta CT}$ [22].

ELISAs. MIP-1β, MIP-1α, IL-8, and TNF-α concentrations were determined by use of commercial ELISA kits (R&D Systems). Supernatants were stored at −70°C until assayed. Experiments yielding supernatants were performed independently in duplicate, and each supernatant was plated in duplicate in the ELISA. Optical densities were read at the appropriate wavelength on a microplate reader, and measurements were calculated as means ± SEs.

Fluorescence-activated cell sorting (FACS) analysis. *C. albicans*–THP-1 cell cocultures were performed as described above, except that the coculture incubation time was 6 h. Each culture was split into two 5-mL round-bottom tubes, and cells were collected briefly by centrifugation and washed twice in PBS. Cells were incubated with 20 μL of either an anti–human CD83 monoclonal antibody or isotype control (both labeled with PE from Pharmingen) at 4°C for 30 min, washed twice in PBS, and resuspended in 0.5 mL of 1% paraformaldehyde. All samples were kept on ice until analyzed. Cell surface expression of CD83 was assessed on a Becton Dickinson FACSCalibur flow cytometer, with $>1 \times 10^4$ events collected for each sample. Cells were gated according to light-scatter properties to exclude cellular debris. Gating for fluorescence intensity was determined by manually gating in the isotype control medium-cultured THP-1 cell sample and maintaining that gating for subsequent samples. Two replicate experiments were performed.

RESULTS

The comparison of the gene-expression profiles of *C. albicans*–stimulated and unstimulated THP-1 cells revealed 131 genes differentially expressed by at least 2.0-fold (table 2). Of these, 47 genes were up-regulated, and 84 genes were down-regulated. The up-regulated antipathogen-response genes included *MIP1B, MIP1A,* and *TNFA*. Signal-transduction genes found to be up-regulated included *DSCR1* (Down syndrome critical region 1), *EGR3* (early growth response 3), *RGS1* (regulator of G protein signaling), and *FLT4* (fms-related tyrosine kinase 4). Pol II transcription genes that were down-regulated in THP-1 cells in response to *C. albicans* stimulation included *LMYC* and *CEBPA* (CCAAT/enhancer binding protein α). Other genes of interest that were down-regulated in THP-1 cells in response to *C. albicans* stimulation were the IL-10 receptor antagonist *IL10RA* and the chemokine receptor *CCR2.*

Further analysis was performed on several antipathogen-response genes and their gene products that are known to be responsive to *C. albicans,* by following their expression over time in response to *C. albicans* stimulation. Real-time RT-PCR

Table 1. Sequences of primers used in real-time reverse-transcriptase polymerase chain reactions.

	Primer sequence	
Gene	Forward	Reverse
18S rRNA	5′-GCCCGAAGCGTTTACTTTGA-3′	5′-TCCATTATTCCTAGCTGCGGTATC-3′
CD83	5′-ACACGGTCTCCTGGGTCAAGT-3′	5′-TCCCCTGAGGTGGTCTTCCT-3′
IL8	5′-GTTTTTGAAGAGGGCTGAGAATTC-3′	5′-CATGAAGTGTTGAAGTAGATTTGCTTG-3′
MIP1A	5′-CTGCATCACTTGCTGCTGACA-3′	5′-CACTGGCTGCTCGTCTCAAAG-3′
MIP1B	5′-CAGCACCAATGGGCTCAGA-3′	5′-AAGCTTCCTCGCGGTGTAAG-3′
TNFA	5′-GCCCAGGCAGTCAGATCATC-3′	5′-GGGTTTGCTACAACATGGGCT-3′
GROB	5′-CTCAACCCCGCATCGC-3′	5′-GATTTGCCATTTTTCAGCATCTT-3′
CCR2	5′-GCTGTCCACATCTCGTTCTCG-3′	5′-TCTTCACCGCTCTCGTTGGTAT-3′
NCF2	5′-GGATGCCTTCAGTGCCGT-3′	5′-TGTCTCGGTTAATGCTTCTGGTAA-3′
TNFAIP6	5′-TTAGCCATCCATCCAGCAGC-3′	5′-CCATCTCGCAACTTACAAGCAG-3′
DSCR1	5′-CGAGTCAGAATAAACTTCAGCAACC-3′	5′-GGTGTGAGCTTCCTATGTGTAAGGT-3′
EGR3	5′-TTGGGAAAGTTCGCCTTCG-3′	5′-ATGATGTTGTCCTGGCACCA-3′
RGS2	5′-AAGATTGGAAGACCCGTTTGAG-3′	5′-TCCCAGGAGTAGAGGAATTTTGTAA-3′
RGS1	5′-CTTTCTGCTGCTGAAGTAATGC-3′	5′-TGACCAGTTTGGTTGGCAAG-3′
RGS16	5′-TCACACACCTGAGTCTCCACG-3′	5′-CAACCTCTCTTCCCGCTGG-3′
FLT4	5′-AAGAAGTTCCACCACCAAACAT-3′	5′-TGAAAATCCTGGCTCACAAGC-3′

Table 2. Differential gene expression of THP-1 cells exposed to *Candida albicans* strain SC5314 versus medium.

Gene function category, GenBank accession no.	Common name	Molecular function	Fold change in expression (SC5314 vs. medium)
Antipathogen response			
NM_002984	*MIP1B*	Cell motility and adhesion; inflammation	8.5
NM_002983	*MIP1A*	Chemokine	6.5
NM_000594	*TNFA*	Mediates proinflammatory response, apoptosis	3.8
NM_004120	*GBP2*	Induced by IFN-γ during macrophage activation	3.3
M57731	*GROB*	Produced by activated monocytes	3.1
NM_000584	*IL8*	Chemoattraction, activation of neutrophils	3.0
NM_004233	*CD83*	Antigen presentation and/or lymphocyte activation	2.6
AW188198	*TNFAIP6*	May be involved in cell-cell communication	2.2
NM_001565	*SCYB10*	Chemotactic for monocytes and T cells	2.1
NM_005384	*NFIL3*	Binds to promoters of *IFNG* and *IL3* genes	2.0
NM_001558	*IL10RA*	Binds IL-10 with high affinity	−2.2
NM_000647	*CCR2*	Receptor for MCP-1, MCP-3, and MCP-4	−2.4
BC001606	*NCF2*	Necessary for superoxide production	−2.4
Carbohydrate metabolism			
M55905	Malate enzyme	Malate dehydrogenase	2.0
M33197	*GAPDH*	Second step in glycolysis	−2.4
Cell cycle			
NM_005343	*HRAS*	Has intrinsic GTPase activity	−2.2
NM_001761	Cyclin F	Involved in control of cell cycle during S and G2 phases	−2.2
U17074	*CDKN2C*	Inhibits cell growth and proliferation	−2.3
BE439987	*GAS7*	Maturation, differentiation of cerebellar neurons	−2.3
Cell motility			
NM_006289	Talin 1	Connections of cytoskeleton to plasma membrane	−2.0
NM_001613	Actin α2	Structural component of cytoskeleton	−2.1
X00351	β actin	Structural component of cytoskeleton	−2.3
Cell proliferation			
NM_001328	*CTBP1*	Corepressor targeting diverse transcription factors	−2.9
NM_003550	*MAD1L1*	Mitotic arrest deficient, yeast, homolog	−3.4
Cell stress			
NM_005627	*SGK*	Serine/threonine kinase	3.1
BF575213	*SOD2*	Superoxide dismutase 2	2.0
DNA binding			
NM_002114	*HIVEP1*	May act in T cell activation	2.7
NM_003670	*BHLHB2*	Transcription factor	2.7
NM_003542	Histone H4	Nucleosome formation	2.5
NM_001674	*ATF3*	Binds the cAMP response element (cre)	2.4
AB002282	*EDF1*	Transcriptional activator	−2.0
NM_014977	*ACINUS*	Chromatin condensation after activation by *CASP3*	−2.1
L04282	*ZNF148*	Involved in transcriptional regulation	−2.1
AA972711	*ZNF292*	May function as a transcription factor	−2.1
NM_002945	*RPA1*	Required for simian virus 40 DNA replication in vitro	−2.2
BE675843	*FBXL11*	f-box and leucine-rich repeat protein 11	−2.9
BE795648	*SSRP1*	Recombination signal sequence recognition protein	−3.4
NM_019081	*KIAA0430*	Unknown	−3.7
DNA repair			
AF034956	*RAD51L3*	Meiotic recombination, repair of damaged DNA	2.0
NM_002434	*MPG*	Excises damaged DNA formed by alkylated lesions	−2.0
Lipid, fatty-acid, sterol metabolism			
BC005127	*ADFP*	Adipophilin	2.9
NM_000237	*LPL*	Hydrolyzes triacylglycerol component of lipoproteins	2.6
NM_016372	*TPRA40*	Unknown	−2.1
NM_012268	*PLD3*	Phospholipase D3	−3.2

(continued)

Table 2. ***(Continued.)***

Gene function category, GenBank accession no.	Common name	Molecular function	Fold change in expression (SC5314 vs. medium)
Other metabolism			
NM_000361	Thrombomodulin	Responsible for conversion of activated protein C	−2.9
NM_000101	*CYBA*	α subunit of cytochrome *b*	−3.2
Pol II transcription			
X99268	*TWIST*	Regulates the HAT activities of p300 and PCAF	2.3
AA349848	…	Unknown	−2.0
U62296	*NFYC*	Stimulates transcription by binding CCAAT	−2.0
M19720	*LMYC*	Transcription factor	−2.0
AL022398	*IRF6*	Transcription factor	−2.0
NM_002695	RNA pol II, E	Subunit of RNA polymerase II	−2.1
NM_003721	*RFXANK*	Activates transcription from class II MHC promoters	−2.1
NM_004364	*CEBPA*	CCAAT/enhancer binding protein α	−3.7
Protein degradation			
AL545523	*PSMC3*	Degradation of ubiquitinated proteins	−2.4
Protein folding			
AB034951	*hsp73*	Chaperone	−2.1
Protein modification			
AL541302	*SERPINE2*	Serine protease inhibitor	3.1
NM_001774	*CD37*	Leukocyte differentiation antigen	−2.0
NM_003801	*GPAA1*	Human GPI anchor attachment protein	−2.0
AA631254	*MAN1B1*	Maturation of Asn-linked oligosaccharides	−2.0
NM_006278	*SIAT4C*	Involved in amino acid glycosylation	−2.4
NM_014501	*E2EPF*	Keratinocyte ubiquitin carrier protein	−2.6
U17496	*PSMB8*	Generates class I binding peptides	−2.6
NM_000918	*P4HB*	Procollagen-proline, 2-oxoglutarate 4-dioxygenase	−3.3
Protein synthesis			
NM_002708	*PPP1CA*	Participates in regulation of protein synthesis	−2.6
AF104913	eIF-4G1	Recognition of mRNA cap	−2.6
BC001173	eIF-3η	Promotes binding of met-tRNAi and mRNA	−2.9
AA393940	eIF-5A	Promotes formation of first peptide bond	−3.0
RNA processing			
AA112507	*LSM4*	Binds to 3′-terminal u tract of u6 snRNA	−2.2
BG528818	*CDC40*	May function in second step of pre-mRNA splicing	−2.4
BC003629	snRNA U2	Spliceosome component	−2.8
AI925305	*S164*	Unknown	−3.8
Signal transduction			
NM_004414	*DSCR1*	May be involved in transcriptional regulation	5.9
NM_004430	*EGR3*	Putative transcription factor	4.9
NM_002923	*RGS2*	Negatively regulates G protein–coupled receptor signal	4.3
NM_002922	*RGS1*	Negatively regulates G protein–coupled receptor signal	4.0
BF304996	*RGS16*	Negatively regulates G protein–coupled receptor signal	3.5
NM_002664	Pleckstrin	Has an EF-hand calcium-binding motif	2.6
M31159	*IGFBP1*	Potentiates insulin-like growth factor activity	2.6
AI754416	*CEP3*	Cdc42 effector protein 3	2.4
NM_019903	Adducin 3	Binds to calmodulin	2.4
U58111	*FLT4*	Similar to vascular endothelial growth factor	2.2
U08015.1	*NFATc*	Nuclear factor of activated T cells	2.0
NM_002607	*PDGFA*	Member of VEGF and PDGF family	2.0
NM_000376	Vitamin D receptor	Nuclear hormone receptor	−2.0
NM_012120	*CD2AP*	Adapter protein between membrane proteins and actin cytoskeleton	−2.0
NM_003646	*DGKZ*	Eukaryotic diacylglycerol kinase family	−2.2
AI809341	*CD45*	T cell activation through the antigen receptor	−2.2
BF439282	*PDZGEF1*	Ras/Rap1A-associating guanine exchange factor	−2.3
U34074	*AKAP1*	Anchors PKA to mitochondrial outer membrane	−2.3

(continued)

Table 2. ***(Continued.)***

Gene function category, GenBank accession no.	Common name	Molecular function	Fold change in expression (SC5314 vs. medium)
NM_000565	IL6 receptor	IL-6 receptor complex subunit	−2.3
NM_014017	*MAPBPIP*	Modulator of MAPK kinase signaling	−2.6
BE675800	*RXRA*	Nuclear hormone receptor	−2.9
U88968	*MBP1*	myc-binding protein 1	−2.9
NM_002872	*RAC2*	Involved in regulation of NADPH oxidase	−3.4
NM_006472	*TXNIP*	Unknown	−3.6
Small molecule transport			
NM_002667	Phospholamban	Calcium channel regulator	2.9
NM_031213	*MGC5244*	Unknown	−2.0
AF145029	Importin 12	Nuclear transport receptor	−2.0
NM_021213	*PCTP*	Facilitates transfer of phosphatidylcholine	−2.0
AL566172	*ATP6V0D1*	Transport processes in vacuolar system	−2.4
AI718937	*KCTD12*	Unknown	−2.8
Unknown			
NM_013332	*HIG2*	Hypoxia-inducible protein	3.1
NM_018470	*HT009*	Unknown	2.7
NM_006096	*NDRG1*	Induced by nickel, homocysteine, tunicamycin	2.6
NM_024121	*FLJ20979*	Unknown	2.2
NM_018603	*PRO1496*	Unknown	2.2
BE858194	*FLJ37393*	Unknown	2.2
AI348009	…	Unknown	2.1
AF041410	*MAG*	Unknown	2.1
AL136842	*DKFZp434A0530*	Unknown	2.0
NM_001450	*FHL2*	Unknown	2.0
W84482	*RYBP*	Unknown	2.0
BF221673	*IDN3*	Unknown	−2.0
AK025608	*FLJ21955*	Unknown	−2.0
N80935	…	Unknown	−2.0
W19873	*THAP11*	Unknown	−2.0
AL136821	*KIAA0701*	Unknown	−2.0
NM_022372	*GBL*	Unknown	−2.0
AU151793	*FLJ12793*	Unknown	−2.1
BC004913	*PRCC*	Unknown	−2.1
NM_018133	*FLJ10546*	Unknown	−2.2
AA114166	*LOC157919*	Unknown	−2.2
BC001425	*DDA3*	Unknown	−2.2
AF067173	*MAGOH*	Unknown	−2.3
BE256479	*LOC344737*	Unknown	−2.3
AK022732	*FLJ12670*	Unknown	−2.5
BC002574	*FLJ10520*	Unknown	−2.7
NM_014887.1	*CG005*	Unknown	−2.7
NM_000918	*P4HB*	Unknown	−3.3
Vesicular transport			
AK001465	*SEC63*	Integral membrane protein	2.0

NOTE. GPI, glycosylphosphatidylinositol; HAT, histone acetyltransferase; IFN, interferon; IL, interleukin; MAPK, mitogen-activated protein kinase; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex; PCAF, p300/CREB binding protein–associated factor; PDGF, platelet-derived growth factor; PKA, protein kinase A; VEGF, vascular endothelial growth factor.

revealed early, maximal expression (by 3 h) of *TNFA, MIP1A, CD83,* and *MIP1B* mRNA in THP-1 cells cocultured with *C. albicans* (figure 1). In these same cells, *IL8* mRNA expression reached maximal expression levels by 9 h.

Supernatants from THP-1 cells cocultured with *C. albicans* or in medium alone were used to measure cytokine/chemokine levels by ELISA (figure 2). As expected, cells stimulated by *C. albicans* produced significantly more IL-8, TNF-α, MIP-1α, and MIP-1β protein than did cells cultured in medium alone. CD83 protein expression at 6 h was assessed by surface staining of

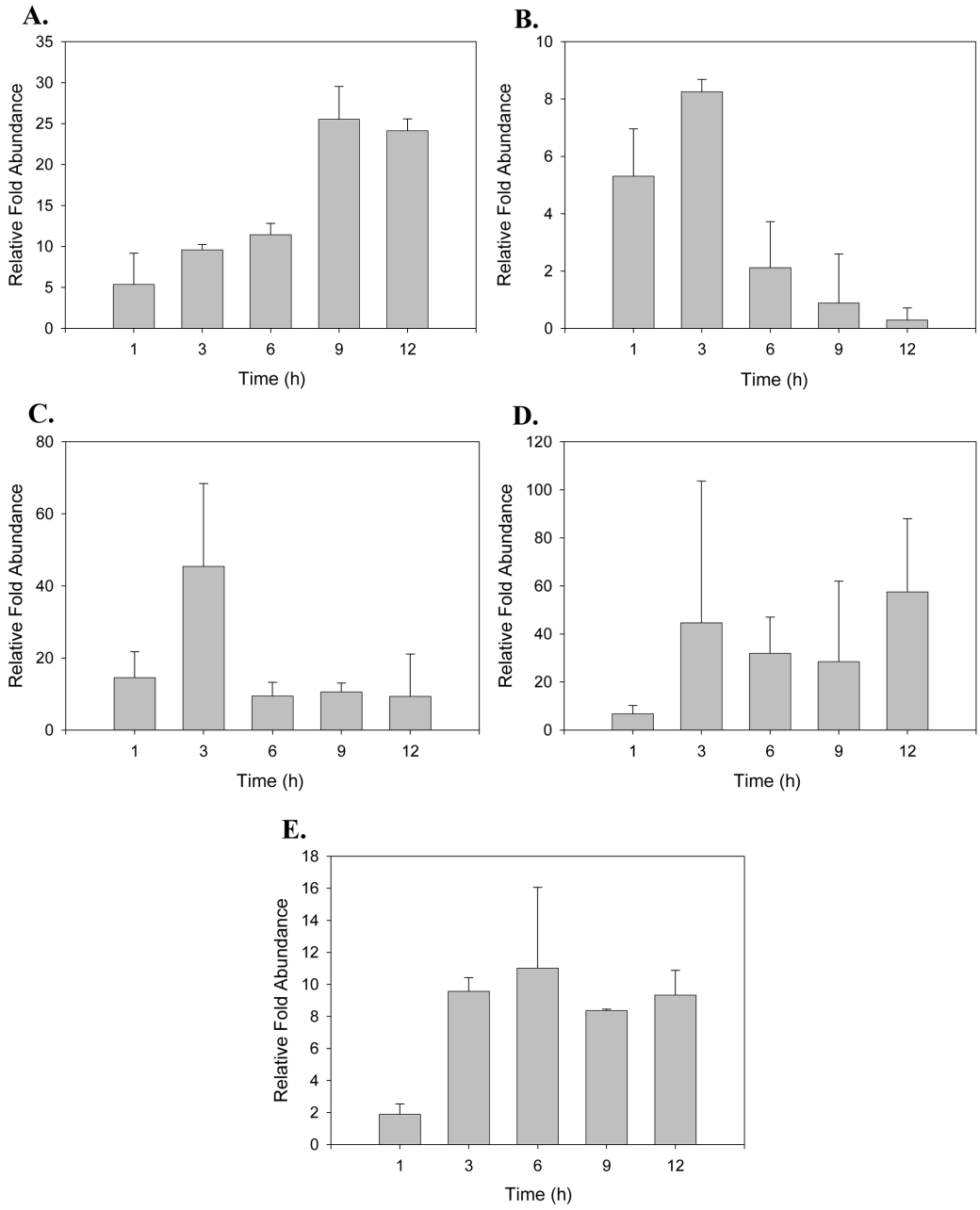

Figure 1. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of *IL8 (A), TNFA (B), MIP1A (C), MIP1B (D),* and *CD83 (E)* RNA expression in THP-1 cells in response to *Candida albicans.* Expression levels were normalized and compared with the sample with the least abundant signal, as described in Materials and Methods. Data represent the mean of 3 measurements of 2 real-time RT-PCR experiments.

stimulated and unstimulated THP-1 cells with PE-labeled anti–human CD83 monoclonal antibody and subsequent analysis by flow cytometry (figure 3). Although there was modest surface expression of CD83 on cells cultured in medium alone, there was an increase in the level of surface expression of CD83 on cells stimulated with *C. albicans*. Specifically, the mean channel on the FACS histogram shifted from 7.55 for medium-stimulated cells to 16.37 for *C. albicans*–stimulated cells, suggesting that each THP-1 cell analyzed by FACS increased the number of CD83 molecules on its surface. Surprisingly, *IL1B* failed to reach the minimum cutoff of 2-fold difference in expression in the microarray analysis. *IL1B* was therefore examined by real-time RT-PCR over time. *IL1B*, like *IL8*, also reached its maximal level of expression by 9 h (figure 4).

Several genes previously not known to be involved in the host response to *C. albicans* were also selected for further examination of mRNA expression by real-time RT-PCR (figure 4). These included *DSCR1, RGS1, RGS2, RGS16, GROB, EGR3,*

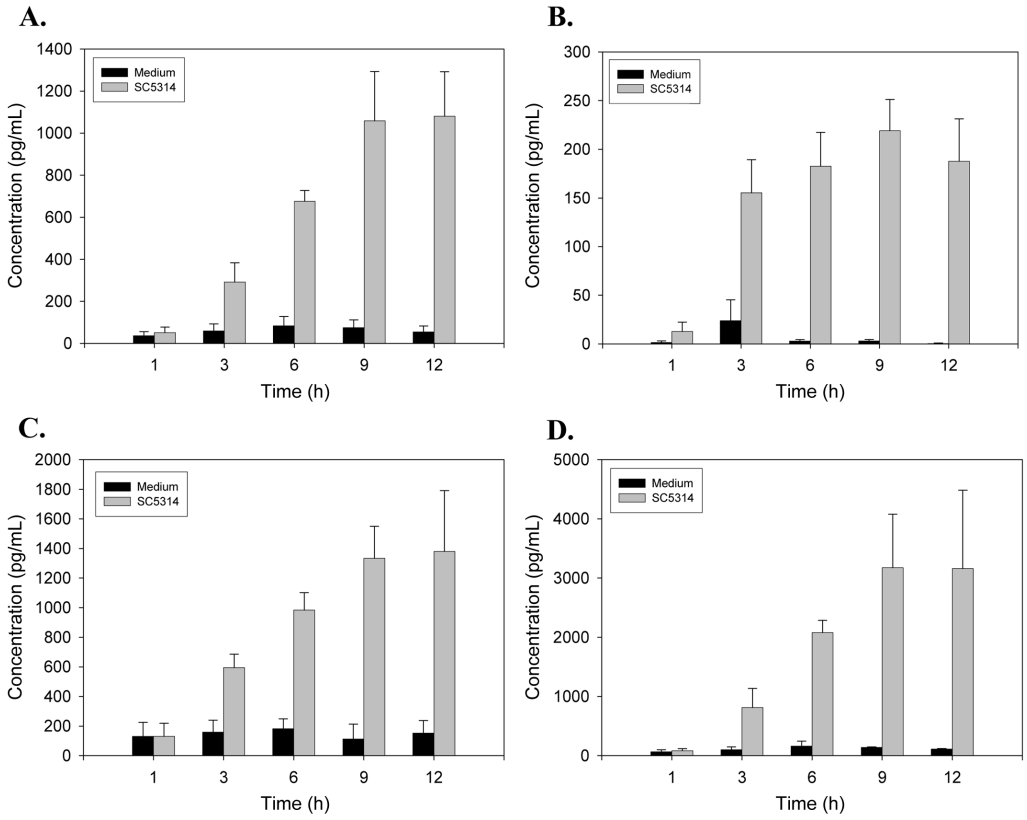

Figure 2. ELISAs measuring protein expression of interleukin (IL)–8 *(A),* tumor necrosis factor (TNF)–α *(B),* macrophage inflammatory protein (MIP)–1α *(C),* and MIP-1β *(D)* from THP-1 cells in response to *Candida albicans.* Data represent means ± SEs of duplicate samples from independent duplicate experiments.

FLT4, CCR2, TNFAIP6, and *NCF2. DSCR1, RGS2, GROB,* and *FLT4* had expression patterns similar to those of *TNFA, IL1B, MIP1A,* and *MIP1B. RGS1* exhibited an expression pattern similar to that of *IL8,* with a maximal expression of nearly 40-fold at the 6-h time point that was sustained for the remainder of the time course. *NCF2* and *CCR2* exhibited an expression pattern that was inverse to that of *RGS1* and *IL8,* with expression levels decreasing at least 2-fold by the 6-h time point. *RGS16* and *TNFAIP6* exhibited later maximal expression similar to that of *RGS1* and *IL8,* but their expression levels decreased at later time points.

DISCUSSION

Induction by* C. albicans *of expression of antipathogen response genes in THP-1 cells. Among the most highly represented up-regulated genes were those involved in the antipathogen response, with *MIP1A* and *MIP1B* the most up-regulated genes identified by microarray. Interestingly, *IL8* mRNA production was much greater at later time points than at 3 h, when RNA was harvested for microarray hybridization, suggesting that *IL8* may respond to factors produced earlier in the stimulation. Although it did not make the 2-fold cutoff for inclusion in the list of differentially expressed genes, with an average of 1.8-fold expression (data not shown), *IL1B* was examined by real-time RT-PCR time course analysis, since its expression in human leukocytes was previously associated with response to *C. albicans* infection [9]. The analysis indicated that *IL1B* levels were at least 2-fold higher at every time point in *C. albicans*–stimulated cells than in medium-cultured cells.

Some *C. albicans*–specific, antipathogen-response genes we did not see in our list of differentially expressed genes were *IL10, IL12A, IL12B,* and *SCYA5* (RANTES). *IL10* has been shown to be up-regulated in monocytes in response to filamentous *C. albicans. IL12A* and *IL12B,* genes that encode the p35 and p40 subunits of IL-12 p70, have been demonstrated to be up-regulated in response to yeast forms of *C. albicans.* RANTES has also been shown to be expressed in response to *C. albicans* stimulation. Because the experiments in the present study were performed with filamenting *C. albicans,* it was not surprising to not see up-regulation of *IL12A* or *IL12B.* One study reported detection of *IL10* mRNA in DCs in response to hyphae at 18 h after stimulation [23]. RANTES mRNA expression is reported in the literature to be increased slightly at 3 h and greatly increased at 20 h after stimulation in PBMCs; however, the

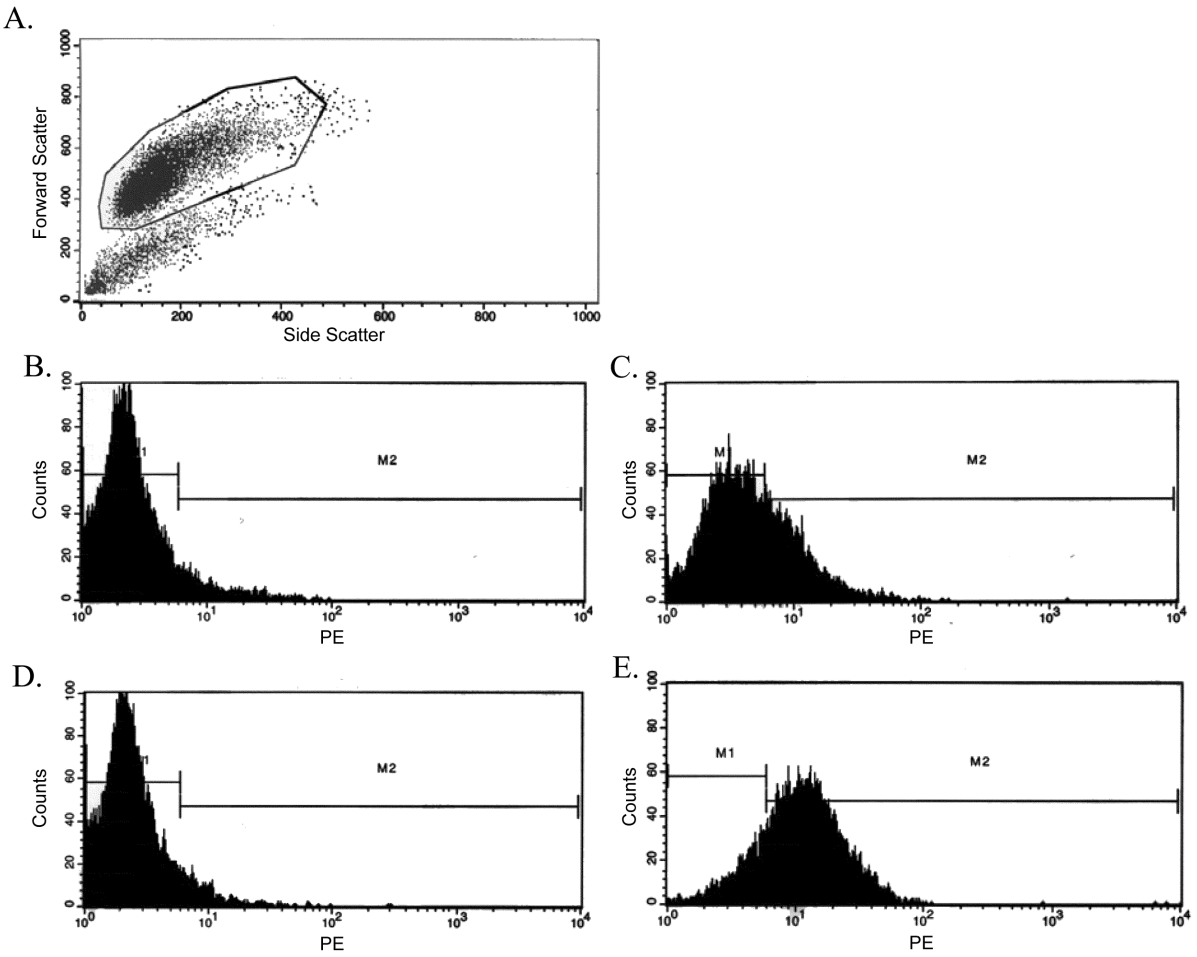

Figure 3. Fluorescence-activated cell sorting analysis of CD83 expression on THP-1 cells in response to *Candida albicans*. *A,* Scatter plot indicating the cell population analyzed in subsequent histograms. *B* and *C,* Histograms of THP-1 cells cultured in medium and stained with phycoerythrin (PE)–labeled antibodies to the isotype control *(B)* or human CD83 *(C). D* and *E,* Histograms of *C. albicans*–stimulated THP-1 cells stained with PE-labeled antibodies to the isotype control *(D)* or human CD83 *(E).* Histograms and scatter plot shown are representative of 2 experiments.

expression level is not quantified from the Northern hybridizations shown [6].

TNFA was also up-regulated in *C. albicans*–stimulated THP-1 cells. Several studies have described the increased expression of this cytokine in monocyte, granulocyte, or PBMC cultures with *C. albicans* [24–27]. Additionally, we observed that *TNFA* mRNA induction is at its highest level within 1 h of coculture and is virtually at its maximal protein level by 3 h. Such an early *TNFA* response may be critical and responsible for the induction of many of the other molecules in the gene list. For example, *ATF3* (activating transcription factor 3), *DSCR1,* and *RGS16* are inducible by TNF-α [28–30]. TNF-α also strengthens the function of monocyte-derived $CD83^+$ DCs by enhancing their proliferation in the presence of *C. albicans,* protecting their phagocytic ability, and enhancing their allogeneic T-cell stimulatory activity [31].

The up-regulation of *CD83* in THP-1 cells was somewhat surprising, since it is a marker on mature DCs. However, monocytes stimulated with *C. albicans* hyphae had increased expression of CD83, although they possessed characteristics atypical of DCs [25]. Although CD83 is primarily used as a cell surface determinant, studies designed to determine a potential role of the molecule in DCs have shown that soluble forms to be involved in modulating the immune response of T cells by inhibiting DC-driven allogeneic and peptide-specific T cell proliferation while inhibiting the maturation of DCs by causing the down-regulation of CD80 and CD83 on immature DCs [32].

TNFAIP6 (also known as TNF-stimulated gene 6, or *TSG6*) is expressed in mononuclear cells, among other cell types, in response to TNF-α and IL-1. It is thought to function as an anti-inflammatory molecule, as part of a negative feedback loop during inflammation [33]. It also acts to inhibit protease action during inflammation, by forming stable complexes with components of the serine protease inhibitor inter-α inhibitor (IαI),

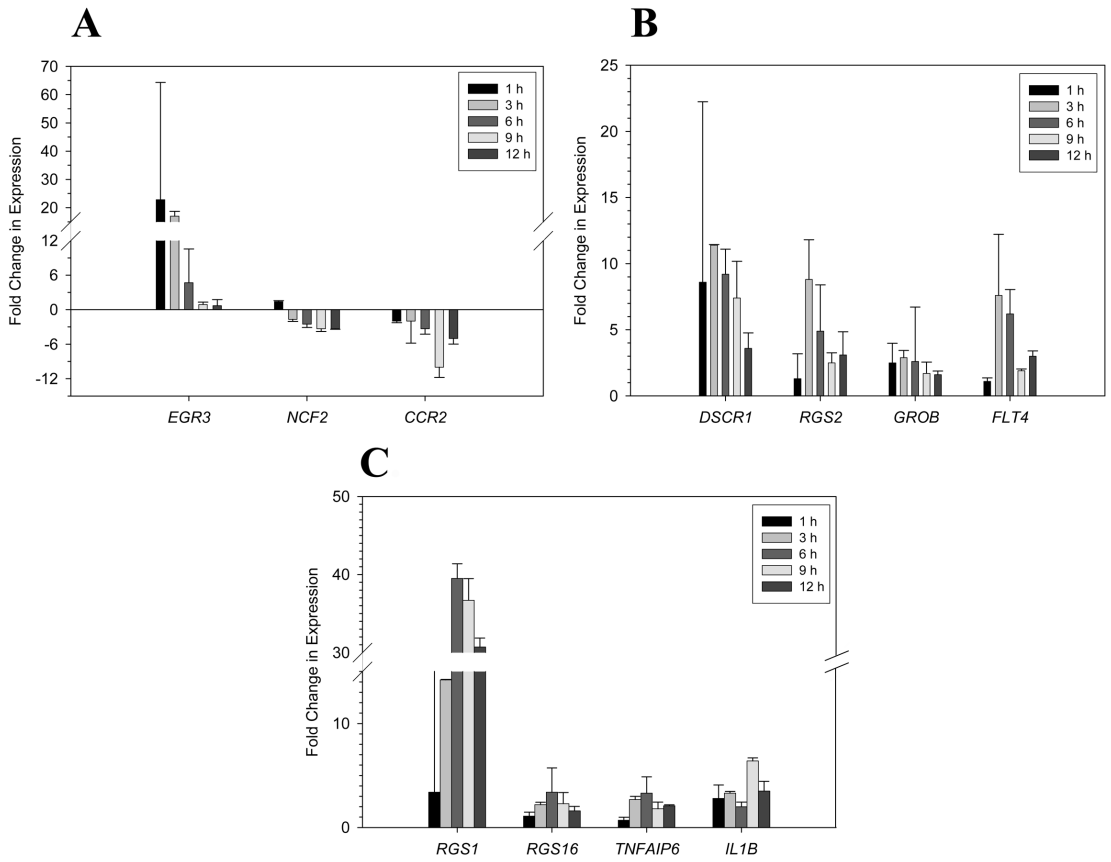

Figure 4. Results of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) on expression of selected other genes in THP-1 cells cultured with *Candida albicans,* compared with THP-1 cells cultured in medium alone. Genes are grouped according to highest expression level before 3 h *(A),* at 3 h *(B),* or at 6 h or later *(C).* Expression levels were normalized to 18S rRNA levels and compared with the level found in the corresponding medium control sample. Data represent the mean of 3 measurements of 2 real-time RT-PCR experiments.

which inhibits the protease activity of plasmin, important in the protease network associated with inflammation. The induction of *TNFAIP6* is consistent with the expression of *TNFA* in response to *C. albicans* stimulation.

CCR2, which is down-regulated 2.4-fold in response to *C. albicans,* is a G protein–coupled receptor for the chemokines monocyte chemotactic protein (MCP)–1, MCP-3, and MCP-4. Examination of a pulmonary *Cryptococcus neoformans* infection model in *CCR2* knockout mice revealed that these mice had a prolonged duration of disease and were less able to recruit macrophages and $CD8^+$ T cells into the lung [34]. These mice were found to have a Th2-type response, chronic pulmonary eosinophilia, and high serum IgE levels, suggesting that CCR2 is required for the development of a Th1 response to *C. neoformans.* Additionally, studies of the maturation of DCs revealed that expression of *CCR2* mRNA was down-regulated to nondetectable levels [35].

The protein encoded by *NCF2* (neutrophil cytosol factor 2, or $p67^{phox}$) is the limiting cofactor in the assembly of the NADPH oxidase enzyme complex in neutrophils. NADPH oxidase catalyzes the production of oxygen radicals that are essential in the defense against pathogens, and the *NCF2* gene product is involved in the final activation of the enzyme complex. Although TNF-α–treated monocytic cells have *NCF2* up-regulation [36], the present study indicates that *NCF2* is down-regulated in the presence of increased levels of TNF-α. It is possible that some other factor produced in response to *C. albicans* is overriding the effect of TNF-α in modulating the expression of *NCF2* in *C. albicans*–stimulated cells.

GROB (or *MIP2A*), up-regulated >3-fold in this study, is produced by activated monocytes and neutrophils at the site of inflammation. It enhances neutrophil function by increasing CD11b cell surface expression, superoxide production, chemotaxis, and enhancing killing [37]. *GROB* also enhances superoxide production in monocytes and has recently been shown to be produced by monocyte-derived DCs in response to bacterial flagellar proteins or lipopolysaccharide [38].

Differential expression of signal transduction molecules in* C. albicans*–stimulated THP-1 cells. *DSCR1* is a gene found in the chromosome 21 Down syndrome critical region. Recently, it was found to be involved in putative negative feedback regulation after vascular endothelial growth factor (VEGF)

stimulation in endothelial cells [29]. Similar to cyclosporin A, it is antagonistic to calcineurin signaling, resulting in down-regulation of several VEGF-responsive genes, such as *ESEL* (E-selectin). These genes have been shown to be up-regulated in endothelial cells upon stimulation with *C. albicans* [39]. It is possible that *DSCR1* up-regulation in *C. albicans*–stimulated THP-1 cells is involved with the normal negative regulation of monocyte-specific molecules during the inflammatory process.

The molecules *RGS1, RGS2,* and *RGS16* were up-regulated ~4-fold in response to SC5314. Each are involved with regulating GTPase activity of the Gα subunit of G protein–coupled receptors, diminishing the duration of downstream signaling that occurs. Previously, it was shown that disruption of *RGS1* in mice leads to abnormal trafficking of antibody-secreting cells, as well as to abnormalities in the spleen and Peyer patches [40]. Another study demonstrated that TLR signaling in human monocyte-derived DCs leads to increased *RGS1* and *RGS16* expression [41]. In short, it seems that the *RGS* gene products help to ensure normal responses of monocyte-derived DCs through TLRs and chemokine receptors.

EGR3 is a zinc-finger transcription factor and an immediate-early gene product. It was up-regulated nearly 5-fold in response to *C. albicans.* Expression of *EGR3* is inhibited by cyclosporin A and can be induced by a variety of external stimuli [42]. EGR3 activates transcription of many genes, including *FasL, TRAIL,* and *TNFA* [43].

FLT4, which is up-regulated >2-fold in response to *C. albicans,* is a VEGF receptor typically found on the surface of endothelial cells. A recent study reports FLT4 protein expression on the surface of immature DCs that were derived from $CD14^+$ monocytes cultured with granulocyte-macrophage colony-stimulating factor and IL-4 [44]. These immature DCs also expressed CD1a, HLA-DR, and CD86, as well as endothelial cell markers such as VE-cadherin and FLT1. However, as these cells were allowed to mature in the presence of TNF-α, they lost their expression of endothelial cell markers in favor of CD83 expression. The role of *FLT4* in THP-1 cells in response to *C. albicans* expression is unclear.

Down-regulation of protein-synthesis genes in response to* C. albicans *stimulation. Four protein-synthesis genes were down-regulated in cells cocultured with *C. albicans.* Since there are >30 genes involved in translation initiation, the down-regulation of these genes was probably not indicative of down-regulation of protein synthesis in general. In fact, one of these genes, *eIF5A,* has been demonstrated recently to be a regulator of p53 [45]. Up-regulation of *eIF5A* leads to p53 up-regulation and increased probability of apoptosis. Therefore, down-regulation of *eIF5A* in the present study may have contributed to the proliferation of THP-1 cells in response to *C. albicans* stimulation.

Conclusions. The present study provides important information about the gene-expression profile of human monocyte-like cells in response to *C. albicans.* Identification of newly identified genes provides insight into the regulation of the antipathogen response, while time course studies indicate the dynamics of the response. Future studies examining the role of the *RGS* genes, *DSCR1, EGR3,* and *FLT4* in the host response to *C. albicans,* especially pertaining to their interaction with TLRs or other *C. albicans*–interacting molecules, is warranted.

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