Transcriptional Profiling of Candida glabrata during Phagocytosis by Neutrophils and in the Infected Mouse Spleen

Yuichi Fukuda,¹ Huei-Fung Tsai,¹ Timothy G. Myers,² John E. Bennett³

Clinical Mycology Section, Laboratory of Clinical Infectious Diseases,¹ and Genomic Technologies Section, Research Technologies Branch,² National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland, USA

Expression microarray analysis of Candida glabrata following phagocytosis by human neutrophils was performed, and results were compared with those from C. glabrata incubated under conditions of carbohydrate or nitrogen deprivation. Twenty genes were selected to represent the major cell processes altered by phagocytosis or nutrient deprivation. Quantitative real-time PCR (qRT-PCR) with TaqMan chemistry was used to assess expression of the same genes in spleens of mice infected intravenously with Candida glabrata. The results in spleen closely paralleled gene expression in neutrophils or following carbohydrate deprivation. Fungal cells responded by upregulating alternative energy sources through gluconeogenesis, glyoxylate cycle, and long-chain fatty acid metabolism. Autophagy was likely employed to conserve intracellular resources. Aspartyl protease upregulation occurred and may represent defense against attacks on cell wall integrity. Downregulated genes were in the pathways of protein and ergosterol synthesis. Upregulation of the sterol transport gene AUS1 suggested that murine cholesterol may have been used to replace ergosterol, as has been reported in vitro. C. glabrata isolates in spleens of gp91phox⁻/⁻ knockout mice with reduced oxidative phagocyte defenses were grossly similar although with a reduced level of response. These results are consistent with reported results of other fungi responding to phagocytosis, indicating that a rapid shift in metabolism is required for growth in a carbohydrate-limited intracellular environment.

Candida glabrata is second in frequency only to Candida albicans as a cause of hematogenously disseminated candidiasis and is associated with equivalent mortality, largely attributed to preexisting comorbidities (1). Studies of host defense against Candida glabrata have been limited. Experimental infections in mice have shown much less virulence than with C. albicans, which is lethal to mice with an intravenous inoculum of 10⁷ cells or less (2). Inocula of C. glabrata injected intravenously into C57BL/6J mice were not lethal, even at 5 × 10⁷ yeast cells (3). Organ burden after 10⁷ C. glabrata cells was higher in spleen than in kidney or liver and gradually fell with time from an initial 10⁶ cells/g at 24 h (see below). The cells responsible for this drop in fungal burden appear to be macrophages, which were abundant in these spleens; neutrophils were sparse (our unpublished data). An important role for oxidative killing by these macrophages can be inferred from the lethality and progressive organ burden found in knockout mice with defective phagocyte oxidative capacity (4). Downregulated genes were in the pathways of protein and ergosterol synthesis. Upregulation of the sterol transport gene AUS1 suggested that murine cholesterol may have been used to replace ergosterol, as has been reported in vitro. C. glabrata isolates in spleens of gp91phox⁻/⁻ knockout mice with reduced oxidative phagocyte defenses were grossly similar although with a reduced level of response. These results are consistent with reported results of other fungi responding to phagocytosis, indicating that a rapid shift in metabolism is required for growth in a carbohydrate-limited intracellular environment.

Transcriptional response of C. glabrata to phagocytic attack has been studied in vitro, using murine macrophage cell lines and microarray (5) as well as bone marrow-derived murine macrophages and fluorescent fusion proteins (6). Responses to human neutrophils in vitro and in the mouse spleen in vivo have not been reported.

Analyzing expression of fungal genes in infected tissue has the potential of improving understanding of pathogenesis. The technical problem is the high ratio of host mRNA to fungal mRNA. In order to improve the ratio, samples with high fungal burden have been selected, such as by excising C. albicans abscesses from rabbit kidneys (7), harvesting infected tissue from the liver surface of an animal infected intraperitoneally (8), or lavaging Cryptococcus neoformans (11) or Aspergillus fumigatus (12) from mouse lungs early after intratracheal inoculation. Usually, a second step, such as differential centrifugation, lysis of mammalian cells, or RNAse treatment, is required (9). As mentioned above, gene expression of single fungal cells has also been studied microscopically using fungi with fluorescent reporters inserted into open reading frames (ORFs) (14). The topic of measuring gene expression in vivo has been reviewed (15, 16).

A useful proxy has been to study fungi internalized by phagocytes in vitro, but the validity of this approach is open to conjecture. The approach used here was to study C. glabrata internalized by human neutrophils, selecting 20 genes from representative cellular processes and using the selectivity of the primer-probe com-
bination in TaqMan chemistry to measure fungal gene expression directly in spleens of infected mice. The concordance of in vitro and in vivo results encourages the use of this approach for measuring in vivo gene expression.

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MATERIALS AND METHODS

Strains, media, and growth conditions. Candida glabrata strain Cg29 is a clinical strain used in a prior study to infect mice (3). Cg29 cells were incubated in yeast extract-peptone-dextrose (YPD) broth (1% yeast extract, 2% peptone, 2% dextrose) (MP Biomedicals, Solon, OH) or synthetic complete (SC) medium (0.17% yeast nitrogen base [YNB] without amino acid and ammonium sulfate, 2% dextrose, 0.5% ammonium sulfate, amino acids, 20 mg/liter adenine sulfate, and 20 mg/liter uracil) as described previously (17) except the medium used in the starvation experiments, which was used as described below (18). The Candida cells were incubated at 37°C.

Incubation of C. glabrata with neutrophils. Human whole blood with preservative-free heparin was collected from healthy donors following informed consent under an institutional review board (IRB)-approved protocol. Neutrophils (PMN) (PMN) were purified using 3% dextran clinical grade (MP Biomedicals) and Percoll (GE Healthcare Bio-Sciences, Piscataway, NJ). This procedure yielded about 95% pure PMN. The number of PMN was counted using a hemocytometer. Cg29 cells were incubated overnight with shaking at 37°C in YPD broth. These cells were diluted into the same fresh medium to an OD600 of 0.5 and incubated at 37°C for 4 h to obtain SC medium with shaking at 37°C. Cultures were diluted into the same medium without PMN. Tubes were tumbled vertically for 30 and 60 min at 8 rpm at 37°C in a ProBlot hybridization oven (Labnet, Edison, NJ). This procedure yielded about 95% pure PMN. The number of PMN was counted using a hemocytometer. Cultures were centrifuged, resuspended with phosphate-buffered saline (pH 7.4), and counted with a hemocytometer. A total of 1.0 × 10⁸ Cg29 cells and 5.0 × 10⁷ PMN were inoculated into 15-ml conical tubes containing RPMI 1640 (Gibco, Grand Island, NY) with 25 mM HEPES (Mediatech, Herndon, VA) (pH 7.2) plus 5% fresh donor heparinized plasma for a total volume of 10 ml. For the control culture, 10⁸ Cg29 cells were incubated in the same culture medium without PMN. Tubes were tumbled vertically for 30 and 60 min at 8 rpm at 37°C in a ProBlot hybridization oven (Labnet, Edison, NJ). Tubes were immediately transferred into ice-cold water, and phagocytosis was confirmed microscopically. The phagocytic index was calculated as the mean number of internalized C. glabrata yeasts per PMN observed microscopically in 50 random fields (oil immersion objective, ×1,000). Cells were collected by centrifugation at 6,000 rpm for 5 min at 4°C, resuspended with 10 ml ice-cold water, and vortexed for 3 min. This procedure resulted in lysis of all neutrophils as determined by microscopic examination of the centrifuged sediment. Tubes were centrifuged at 6,000 rpm for 5 min at 4°C, and the pellets were immediately frozen on dry ice-ethanol after discarding the supernatant. Total RNA was prepared directly from the collected cells as described below (18). The Candida cells were incubated at 37°C.

qRT-PCR. To confirm the results of microarray of PMN experiments, quantitative real-time PCR (qRT-PCR) was performed on selected mRNA. The RNA samples were recovered from Cg29 cells with and without PMN of three selected PMN experiments, treated with the Turbo DNA-free kit (Applied Biosystems, Foster City, CA), and reverse transcribed using the High-Capacity cDNA archive kit (Applied Biosystems) according to the manufacturer’s instructions. TaqMan probe and primer sets were designed for the selected genes and the ACT1 gene of C. glabrata by using Primer Express version 3.0 (Applied Biosystems) (Table 1). The Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was also used to confirm that there were no homologs of human or mouse genomic DNA that can be amplified by these primers. Different concentrations of cDNA in 5 μl were added to 12.5 μl of TaqMan Universal PCR master mix (2X), 2.5 μl of 2.5 μM TaqMan probe (Applied Biosystems), and 2.5 μl of 9 μM forward and reverse primers (Invitrogen) each. The reaction was performed in triplicate in 96-well plates on an ABI Prism 7500 (Applied Biosystems). Data analysis was performed by a threshold cycle (ΔCT) method by following the manufacturer’s instructions (Applied Biosystems). The threshold cycles of the target were normalized to the CT of the ACT1 gene (ΔCT = CT Target - CT ACT1). The ΔΔCT (ΔCT with PMN - ΔCT without PMN) was calculated by the mean of the ΔCT values of different concentrations of cDNA from three biological replicates. Assays were also performed on different cDNA concentrations to ensure similar primer efficiencies among the various targets. Therefore, the negative ΔΔCT values are equivalent to log2 relative fold changes.

Because mouse mRNA was so much more abundant than fungal mRNA in the tissue, the amount of cDNA from spleen loaded into the qRT-PCR required 7.5 μg instead of the 100 ng used for C. glabrata grown in SC medium or incubated with neutrophils. Figure 1 compares the number of spleen CFU and the ΔCT values of ACT1, CDR1, and ERG11 in the same spleen following the intravenous injection of 10⁷ CFU of strain 84 into C57BL/6 mice. Each time point is the average from three mice.
This experiment utilized strain 84 (19), although numbers of CFU were comparable to those of Cg29 (3).

It should be noted that the background subtraction to obtain ΔΔCT differed between our human neutrophil and mouse spleen studies. The neutrophil ΔΔCT was obtained by subtracting the ΔCT of C. glabrata grown in RPMI 1640 with 5% fresh human plasma in the absence of neutrophils. For spleen, the ΔΔCT was obtained by subtracting the ΔCT of log-phase cells grown in SC, reflecting the metabolic state of C. glabrata inoculated into the mice. The issue of background control has been discussed by Wilson et al. (16), who point out the problem inherent in the use of background controls.

Systemic candidiasis murine model. Female immunocompetent C57BL/6J mice (stock number 002365) lacking phagocyte superoxide production were used (Jackson Laboratory, Bar Harbor, ME). Mice were housed in an AAALAC-accredited NIAID facility and used under a protocol approved by the NIAID Animal Care and Use Committee. C. glabrata Cg29 cells were cultured in YPD medium with shaking for 17 h at 37°C. Groups of three mice were inoculated via the tail vein with 4.8 × 10^6 C. glabrata cells grown in C57BL/6J or gp91phox−/− mice on a C57BL/6J background (strain B6.129S6–CdymM1D1/L, stock number 002365) lacking phagocyte superoxide production were used (Jackson Laboratory, Bar Harbor, ME). After 24 h observation, spleens were removed and immunoassay on January 10, 2021 by guest http://iai.asm.org/ Downloaded from

### RESULTS

Neutrophil-C. glabrata coculture system. The microscopic examination showed that 81% of Cg29 cells were engulfed by neutrophils after 30 min incubation. The average number of C. glabrata cells in 333 neutrophils (phagocytic index ± standard deviation) at 30 min was 3.2 ± 0.14 C. glabrata cells/neutrophil.

Differentially upregulated genes after 30 and 60 min incubation. A total of 519 genes were upregulated upon exposure to PMN for 30 min. An additional 6 LYS genes were upregulated after 60 min. Of upregulated genes, 339 genes were annotated. A general classification of annotated upregulated genes by function is shown in Fig. 2. Data were further organized into significantly overrepresented biological processes using the GO Term Finder program. Because the global transcriptional response of Candida albicans and Saccharomyces cerevisiae upon exposure to PMN was reported to resemble carbohydrate and amino acid deprivation (20, 21), we exposed Cg29 cells to these two conditions to compare the profile with that during phagocytosis by PMN. Further, expression data of C. glabrata exposed to a murine macrophage-like cell line for 2 or 6 h (8) and to carbohydrate depletion for 20 min (22) were extracted from published data to compare with our results (Fig. 3). The numbered categories in the heat map correspond to several genes in the number ontology (GO) diagram in Fig. 2. Uproregulated genes in the neutrophil closely paralleled those induced by carbohydrate depletion in nearly all listed categories of metabolic processes, including LYS genes. Methionine metabolic processes were notably induced in C. glabrata in the neutrophil and by nitrogen deprivation in C. glabrata. Two genes of the MET cluster were also upregulated: MET28 and MUP1. These genes are transiently expressed during S phase of the S. cerevisiae cell cycle.

### TABLE 1 Probes and primers for qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>ORF</th>
<th>Probe (5′–3′)</th>
<th>Forward primer (5′–3′)</th>
<th>Reverse primer (5′–3′)</th>
</tr>
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<tbody>
<tr>
<td>ACT1</td>
<td>CAGL0K12694</td>
<td>TCGAATATTATGCACCATCATGTCTGTGTTTAGCT AACGATTATTCAATTGCAACAACG CCTCACAATAAGGAAAGTCTGCG</td>
<td>TCGAGCCTGTGTCGTCGTCGACTTTAAGA TGGTCAAGGCCGCACTTC</td>
<td>TGGCAAACATGCCAAAACAAAGCGTTA</td>
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![FIG 1](Comparison of spleen CFU and ΔΔCT values of ACT1, CD1, and ERG11 in the same spleen following the intravenous injection of 10^7 CFU of strain 84 into C57BL/6J mice. Each time point shows the average from three mice.)
MET28 is a major transcriptional regulator of methionine biosynthesis and responds to low intracellular S-adenosylmethionine (23). MUP1 is a methionine transporter. Upregulated carbohydrate processes (category 6) included gluconeogenesis (FBP1, PCK1, TDH3, PYC1), the glyoxylate cycle (ICL1, MLS1), and utilization of extracellular trehalose (NTH1). In category 3, genes in transport, including genes related to amino acids (GAPI, succinate-fumarate (SFC1), methionine (MUP1), and acetate or ammonium (ADY2), were upregulated. In category 9, actin cytoskeleton organization includes upregulation of HUA1, which drives vesicle-mediated transport (category 2). PDR1, a transcriptional factor which regulates drug efflux transport (CDR1, PDH1), was induced in neutrophils and in carbohydrate depletion. Also upregulated were genes in categories of oxidative stress (GAD1, ASK10, CTA1, SOD2) as well as genes in the autophagy pathway and the YPS glycosphatidylinositol-linked aspartyl proteases. Figures 4 and 5 provide further information on autophagy, peroxisome, and YPS genes for comparison with reported results of *C. glabrata* in murine macrophages (7, 24).

**Differentially downregulated genes.** A total of 360 genes were downregulated upon exposure to PMN for 30 min (Fig. 6). Of downregulated genes, 331 genes were annotated. Results at 60 min were nearly identical (Fig. 6). A general classification of annotated downregulated genes by function is shown in Fig. 1. Data were further organized into significantly overrepresented biological processes as described above. The same congruence of neutrophil phagocytosis and glucose deprivation was found for downregulated genes as was found for upregulated genes. Downregulated genes included those involved in synthesis of proteins (categories 1 and 2), ribosomes (category 3), and membrane sterols (category 4). Genes controlling transport of sugars (HXT6) and proteins (DNP2, SEC63) (category 3) were repressed in neutrophils and carbohydrate depletion. In category 6, genes controlling cell wall mannans biosynthesis (PSA1, CIS3) and beta-glucan assembly (GSC2, EXGI, UTR2) were significantly repressed. We also observed the repression of the process involved in cell cycle M phase (MSC7, CS3). The repression of these genes is consistent with that with carbohydrate depletion. These results suggested that the Cg29 cells are trying to shut down the processes of protein synthesis and cell membrane and cell wall biosynthesis because of carbohydrate depletion.

**Comparison with published *C. glabrata* microarrays.** The published microarray data on *C. glabrata* strain ΔHTU (Δhis3 Δtrp1 Δura3) exposed to carbohydrate depletion for 20 min
closely parallel the data reported here, for both upregulated and downregulated genes (22). In contrast, the transcriptome of *C. glabrata* strain BG2 phagocytosed by the murine macrophage cell line J774A.1 for 2 and 6 h had no broad similarity to our results with strain Cg29 phagocytosed by human neutrophils (7). Among the similarities was upregulation of the catalase \( \text{CTA1} \), glyoxylate pathway genes \( \text{PCK1} \) and \( \text{ICL1} \), and six lysine biosynthesis genes. In regard to the LYS genes, deprivation of the essential amino acid lysine appears to have elicited this upregulation in neutrophils at 60 min and in murine macrophages at 2 and 6 h. Downregulation of the ergosterol pathway genes was seen in both macrophages and neutrophils. The dissimilarities between phagocytes are a reminder that *C. glabrata* has multiple strategies to survive within the host environment (25).

Selection of 20 genes from neutrophil experiments for qRT-PCR on infected mouse spleen. Data from the neutrophil expression experiments were used to select 20 genes for qRT-PCR studies of mouse spleen. \( \text{ACT1} \) was used as the loading control. Pathways represented by the genes are as follows: methionine biosynthesis, \( \text{STR3} \), \( \text{MET28} \), and \( \text{MET4} \); alternative carbohydrate sources, \( \text{PCK1} \), \( \text{ICL1} \), \( \text{IDP2} \) (tricarboxylic acid [TCA] cycle), \( \text{FAA2} \) (fatty acid degradation), \( \text{CDC19} \) (glycolysis), and \( \text{CAT8} \) (diauxic shift); drug efflux, \( \text{PDR1} \), \( \text{PDH1} \), and \( \text{CDR1} \); ergosterol biosynthesis, \( \text{ERG2} \), \( \text{ERG10} \), and \( \text{ERG11} \); sterol transport, \( \text{AUS1} \); protein and cell wall synthesis and cell wall integrity, \( \text{ASC1} \) (core component of the small ribosomal subunit of protein synthesis), \( \text{CIS3} \), and \( \text{SLT2} \); oxidative stress, \( \text{CTA1} \) (peroxisomal catalase). We validated our primer/probe selections by running qRT-PCR on cDNA from our neutrophil experiments and correlating the log2 values for the

![FIG 3](https://example.com/fig3.png) **Heat map to compare expressions of significantly upregulated genes in neutrophils and under carbohydrate and nitrogen deprivation.** Expression data of *C. glabrata* exposed to a murine macrophage-like cell line for 2 or 6 h (7) or to carbohydrate depletion for 20 min (22) were extracted from published data to compare with our results. The indicated color scale is based on log2 changes. Yellow indicates that a signal was not detected.

![FIG 4](https://example.com/fig4.png) **Peroxisome and autophagy gene expression in *Candida glabrata* in neutrophils.** Microarray results are expressed as log2 ratios, with growth in RPMI 1640 as a comparator.

![FIG 5](https://example.com/fig5.png) **Aspartyl protease (YPs) gene expression in *Candida glabrata* in neutrophils.** Microarray results are expressed as log2 ratios, with growth in RPMI 1640 as a comparator.
FIG 6 Heat map to compare expressions of significantly downregulated genes in neutrophils and under carbohydrate and nitrogen deprivation. Expression data of C. glabrata exposed to a murine macrophage-like cell line for 2 or 6 h (7) or to carbohydrate depletion for 20 min (22) were extracted from published data to compare with our results. The indicated color scale is based on log2 changes. Yellow indicates that a signal was not detected.

20 genes. Correlation was very high between microarray and qRT-PCR values (Spearman \( r^2 \) value = 0.94).

The 20 target genes we studied provided a mean CT of 10 \( \mu g \) of cDNA from 31.69 cycles (range, 27.33 to 35.95) in gp91phox mice and 35.29 (range, 31.22 to 39.26) in normal mice, sometimes approaching the limit of 40 cycles with 5 \( \mu g \) cDNA. Genes with low expression levels were not able to be measured. As another consideration, efficiency of the reaction fell as input cDNA increased above 7.5 \( \mu g \), causing nonlinearity of the dose-response curve and a lower \( r^2 \). Computing the slope of the line relating micrograms of cDNA and \( \Delta C_T \), using triplicate values for 5 and 7.5 \( \mu g \) in qRT-PCR, we found a slope that was consistent with the slope of C. glabrata grown in SC medium (−3.46) and in neutrophils (−3.67). However, the slope of the line between 7.5 and 10 \( \mu g \) input cDNA was higher than −3 in three genes from gp91phox mice and five genes in normal mice, indicating decreased efficiency of the reaction at 10 \( \mu g \) input cDNA. We therefore used 7.5 \( \mu g \) cDNA for our analysis. Another limitation of this approach is that it required an organism burden of at least \( 10^5 \) CFU/g tissue (Fig. 1). This can be achieved consistently only in the first 24 h of the self-resolving infection in wild-type mice. Although decreased efficiency of the qRT-PCR may have affected quantitation of mRNA abundance, the parallel between the results with neutrophils and those with mouse spleen was encouraging.

Comparison of gene expressions of C. glabrata in infected mouse spleens and neutrophils. Figure 7 compares the qRT-PCR results with C. glabrata in neutrophils and in spleens of wild-type and gp91phox−/− knockout mice with impaired oxidative killing of intracellular organisms. Expression of four genes was too low to measure in wild-type mice, and expression of one gene was too low to measure in knockout mice. A high level of concordance was seen between results in neutrophils and those in mouse spleens. ERG11 and CDR1 expression were not 2-fold upregulated or downregulated in any system (note the use of log2 notation). Up-regulation was seen in other genes with the exception of CDC19, a pyruvate kinase essential for glucose utilization. CDC19 was downregulated in neutrophils but not in mouse spleens. Nutrient depletion, particularly carbohydrate sources, was seen reflected in the mouse spleen, as in the neutrophils. Log, 5.94- and 5.48-fold (61- and 45-fold) upregulation of AUS1, a major sterol transporter, along with downregulation of ergosterol synthesis genes, may reflect the ability of C. glabrata to take up cholesterol when ergosterol synthesis is limited (26).

Effect of oxidative stress on gene expression in C. glabrata in mouse spleens. To examine the effect of oxidative stress on the gene expression in the mouse spleen, we also compared the log2 fold changes of 20 target genes in C57BL/6J mouse spleens and those in gp91phox−/− mouse spleens (Fig. 7). Results were generally similar for the transcriptional regulation of these 20 genes in C57BL/6J mice, gp91phox−/− mice, and neutrophils. However, there was a trend toward lower transcriptional regulation of C. glabrata in the spleens of gp91phox−/− mice compared to that in C57Bl/6J mice. Recalling that the comparator for mouse spleens was SCD culture medium, the better growth of C. glabrata in spleens of gp91phox−/− mice than in wild-type mice was also consistent with more-favorable growth conditions when oxidative killing was impaired.

DISCUSSION

We used the selectivity of TaqMan chemistry to study expression in whole-tissue homogenates without osmotic lysis or differential centrifugation. Description of the limitations of this technique may be of interest. TaqMan chemistry did allow selection of primers and probes for 20 genes and an ACT1 loading control which gave negligible signals with up to 10 \( \mu g \) cDNA from uninfected mouse spleen. We chose spleens of mice infected intravenously because of our experience that spleens gave higher colony counts than liver or kidney (3). In our prior report, using C. glabrata Gg29, spleens provided \( 10^8 \) to \( 10^9 \) CFU/gram with C57BL/6J mouse and about a log more in gp47phox−/− and gp91phox−/− mice. Over
FIG 7 Gene expression in *Candida glabrata* in spleens of infected C57BL/6J and gp91phox−/− mice and in neutrophils. qRT-PCR results from neutrophils are expressed as log2 relative fold changes compared with results for Cg29 cells incubated in RPMI 1640 without PMN. Spleen qRT-PCR results are compared with results for Cg29 cells grown in SC medium.
the 9 days after inoculation, fungal burden fell in wild-type mice and rose slowly in gp47phox−/− and gp91phox−/− mice. We did not routinely quantitate fungal burden in the experiments reported here, but when we did, the results paralleled those of our former experiments.

Incubation of C. glabrata with neutrophils found changes in gene expression consistent with severe carbohydrate deprivation, including upregulation of genes in the glyoxylate cycle, beta-oxidation of long-chain fatty acids, and gluconeogenesis. Genes in the autophagy and pexophagy pathways were also upregulated, likely providing mechanisms for sequestering resources in a nutrient-deprived environment (27). The role played by upregulation of the glycosphatidylinositol-linked aspartyl proteases is less clear but likely involved altering the fungal cell surface (7). Evidence of carbohydrate depletion has been found in many published systems of fungi incubated with neutrophils or macrophages (18, 20, 21). The glyoxylate cycle, utilized during glucose deprivation, was shown to be essential for virulence in C. albicans (28).

Induction of methionine pathway genes, as found in our neutrophil experiment, has been reported for C. albicans and S. cerevisiae strains incubated with human neutrophils (18). Our results with C. glabrata in neutrophils closely followed results with S. cerevisiae, including upregulation of MET genes, including STRE3 and MUP1, a high-affinity methionine permease (21). We also found a similar pattern between C. glabrata in neutrophils and Rubin-Bejerano et al.’s results with nitrogen deprivation in S. cerevisiae (21). This effect occurred despite the presence of serum as a potential nitrogen source in both our systems.

Autophagy pathway genes, particularly ATG11 and ATG17, were found to be important for survival of C. glabrata in murine macrophages (24). In that report, a decline in the peroxisome number during phagocytosis was consistent with pexophagy. In our neutrophil experiments, ATG11, ATG17, and five others in the autophagy pathway were upregulated, as were 7 of 8 peroxisome genes (Fig. 4). The recycling of nutrients through pexophagy and autophagic degradation has been reviewed recently (27, 29).

Gene expression in mouse spleen was consistent with that with severe carbohydrate deprivation. Results in gp91phox−/− mice were closer to growth in the SC medium control than to growth in the neutrophil, including SLT2, consistent with the better fungal growth in the spleens of these animals (Fig. 7). SLT2 is a mitogen-activated protein (MAP) kinase that is important for cell integrity in C. glabrata (30) and for regulation of selective autophagy of peroxisomes (pexophagy) in S. cerevisiae (31). Expression of CTA1, coding for a peroxisomal catalase, was upregulated in spleens of gp91phox−/− mice, as in wild-type mice and neutrophils. Catalase should be an important defense against oxidative attack. However, CTA1 has been reported as dispensable for C. glabrata pathogenicity in mice (32), consistent with the concept that C. glabrata has multiple pathways to permit growth in the inimical environment of the host.

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