Gene profiling and signaling pathways of Candida albicans keratitis

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**Purpose:** To compare the global gene expression patterns in uninfected and fungus-infected mouse corneas at the onset of Candida albicans keratitis.

**Methods:** Fungal keratitis was generated by scarifying the corneal epithelium of BALB/c mice followed by topical inoculation with Candida albicans. Corneal infection was allowed to progress for one day, and total RNA was then extracted from excised corneas. Microarray was performed to detect 45,102 murine genes and processed to identify genetic regulation of signaling pathways. Selected genes encoding interleukins (IL), chemokine ligands, and other cytokines were confirmed by quantitative real-time reverse transcriptase polymerase chain reaction (RT–PCR).

**Results:** Compared to mock-inoculated control eyes, genetic microarray analysis of Candida albicans keratitis showed that 3,977 genes (8.8%) changed at least twofold and 1,672 genes (3.7%) changed at least fourfold. Hierarchical clustering identified that upregulated genes affected immune and inflammatory responses, intercellular signaling, and cellular proliferation. Pathways having more than 20% of their genes significantly upregulated signaled leukocyte extravasation, increased interleukin production, and affected toll-like receptors. Upregulated transcript levels for IL-1β and IL-6 were confirmed by real-time RT–PCR.

**Conclusions:** Host gene expression during the initial stage of Candida albicans keratitis involves pathways contributing to acute inflammation mediated by interleukins and other signals of leukocyte recruitment. This murine study confirms the involvement of innate immunity in the cornea during the initiation of Candida albicans keratitis.

Fungal keratitis is a vision-threatening disease [1]. Often due to filamentous fungi in the tropics, corneal infection due to Candida albicans has a worldwide distribution [2]. Although linked to environmental exposure, especially in the setting of injury or keratopathy [3], the pathogenesis of fungal keratitis is not fully understood. Fungal virulence factors allow growth and invasion into the corneal stroma [4,5], but host responses also contribute to the pathophysiology of corneal disease. Studies have recently shown that matrix metalloproteinases [6,7] and other inflammatory mediators have key roles in the progression and outcome of keratomycosis. To gain further insight into the molecular processes of keratomycosis, this study investigated the global genetic expression pattern at the onset of Candida albicans keratitis.

Corneal infection by Candida albicans triggers an inflammatory response that leads to visual loss in more than half of affected eyes despite therapy [2]. The prompt inflammatory response consists predominantly of neutrophils [8], although lymphocytes and macrophages are also involved [9]. Our studies suggest that proinflammatory mediators play important roles at the onset of Candida albicans keratitis, but the early molecular events that are involved in the corneal and immune responses remain unclear. Therefore, we examined the early transcriptional profile of the fungus-infected cornea in comparison to controls. Observed changes in the corneal transcriptome were categorized to suggest potentially important pathways contributing to corneal inflammation during fungal keratitis.

Gene microarray methodology has evolved as an important technique to investigate the mechanisms for many ocular disorders including microbial keratitis [10,11]. In this study, we systematically evaluated the host gene expression during the early stage of Candida albicans keratitis in a mouse model. To identify molecular cascades of potential importance in pathogenesis, we compared corneas of mice that developed fungal keratitis after epithelial scarification to uninfected, scarified controls. Results were validated for selected genes encoding interleukins and other cytokines.

**METHODS**

**Experimental fungal keratitis:** A human isolate of Candida albicans strain SC5314 [12] was grown on Sabouraud dextrose agar (Difco, Detroit, MI) for three days at 25 °C. Yeasts were harvested and diluted in sterile phosphate-buffered saline (PBS) to yield 2×10⁵ colony-forming units (CFU)/μl [12]. Thirty adult, female BALB/c mice six- to eight-weeks old (Harlan Sprague-Dawley, Houston, TX) were anesthetized intraperitoneally with rodent combination anesthesia, and the corneas of right eyes were superficially scarified [13]. A 5 μl inoculum (1×10⁵ CFU) of Candida albicans was applied to the scarified cornea to induce experimental keratitis. An equivalent volume of sterile PBS diluent was applied to mock-infected controls. Animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and protocols.
Institutional Animal Care and Use Committee. The severity then adjusted and analyzed with BioConductor software using Affymetrix GCOS software version 1.4 (Affymetrix).

RNA extraction: Mouse corneas were dissected at 24 h p.i., and surrounding tissues were removed. Corneas were pooled in sets of five for total RNA extraction with triplicate groups and surrounding tissues were removed. Corneas were pooled to yield adjusted p values [15]. The criteria for significance of differentially regulated genes were established as greater than or equal to a twofold change with an adjusted p value of less than or equal to 0.05. Pathways were analyzed (Ingenuity Systems, Redwood City, CA) to determine the ratio of known genes within each pathway that were significantly upregulated during *Candida albicans* keratitis relative to the total number of known genes.

Reverse transcription of RNA and quantitative real-time RT-PCR: Total RNA isolated from corneas one day after inoculation was spectrophotometrically quantified at 260 nm. The first-strand cDNA was synthesized from 0.4 μg of total RNA with Ready-To-Go You-Prime First-Strand Beads (GE Healthcare, Princeton, NJ) and random hexamers (Applied Biosystems, Foster City, CA). Real-time polymerase chain reaction (RT-PCR) was performed using TaqMan Assays (Table 1) and TaqMan Gene Expression Master Mix and Assays (Applied Biosystems). Primers specific for interleukins, *IL-1β*, *IL-6*, and *IL-23α*; chemokine ligands, *CCL4* and *CCL7*; chemokine receptors, *CCR3* and *CCR5*; and transforming growth factor-beta 2 (*TGF-β2*) gene transcripts (Applied Biosystems) were used to quantify gene expression levels. The threshold cycle (Ct) for each target mRNA was calculated from normalized CT results, and mean results were calculated normalized Ct results, and mean results were used to determine relative fold changes between experimental groups. Two-group comparisons were analyzed with Student’s t-test. A p value less than or equal to 0.05 was considered statistically significant.

### RESULTS

**Experimental post-traumatic keratomycosis:** All animals infected with *Candida albicans* strain SC5314 developed signs of keratitis by 24 h p.i. Mean-standard deviation (SD) disease severity scores of three independent *Candida albicans*-infected groups (five per group) were 8.0±0.7, 8.8±0.8, and 8.2±0.8. Based upon one-way ANOVA, no significant difference occurred among the three groups (p=0.27), and the overall mean ocular disease severity was

| Gene (Symbol) | Assay ID | Amplicon length |
|---------------|----------|-----------------|
| Interleukin 1 beta (*IL-1β*) | Mm00433228_m1 | 90 |
| Interleukin 6 (*IL-6*) | Mm00446191_m1 | 124 |
| Chemokine (C-C) ligand 4 (*CCL4*) | Mm00443111_m1 | 70 |
| Chemokine (C-C) ligand 7 (*CCL7*) | Mm00443113_m1 | 122 |
| Chemokine receptor 3 (*CCR3*) | Mm01216172_m1 | 98 |
| Chemokine receptor 5 (*CCR5*) | Mm01216171_m1 | 78 |
| Interleukin 23 alpha (*IL-23α*) | Mm00518984_m1 | 61 |
| Transforming growth factor beta 2 (*TGF-β2*) | Mm01321739_ml | 109 |
| Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) | Mm99999915_gl | 107 |

The assay ID is from Applied Biosystems.
No clinical disease or obvious signs of infection was found in any eyes of the mock-infected control groups.

Corneal gene expression: Triplicate samples of RNA from five-cornea pools of infected and control, mock-infected groups met criteria for microarray analysis (Table 2). Overall, the scaling factor was less than or equal to 3 or within two SD around the mean, and the average background level was less than 100 for all samples. All three probes were present for both housekeeping genes coding for β-actin and GAPDH, and as expected, two probes were present for the spike-in probe sets, BioB, BioC, BioD, and CreX. RNA degradation plots demonstrated similar patterns for all chips (data not shown).

Among the total of 45,102 genes detected by the Affymetrix GeneChip 430.2 microarray, 3,977 genes (8.82%) in Candida albicans-infected corneas were significantly (p<0.05) and differentially (≥2.0 fold change) regulated compared to mock-infected control corneas. Of these, 1,987 genes were upregulated, and 1,990 were down-regulated. With a criterion of at least 4.0 fold significant change in the expression level, 1,672 genes (3.71%) were differentially expressed with 1,075 upregulated and 597 down-regulated. Thirty different genes were significantly upregulated more than 100 fold (Table 3). The frequency of significant gene expression followed a bell-shaped distribution (Figure 1).

Gene expression levels were assigned to general categories based upon known functions of gene products and the number of genes expressing greater than or equal to twofold significant difference (p<0.05). While functional categories included some down-regulated genes, the number of upregulated genes was consistently more prevalent (Figure 2). Canonical analysis showed that the ratio of significantly upregulated genes within the pathways ranged from 0.13 to 0.34 (Table 4). Several signaling pathways included genes that were upregulated fourfold or more (Appendix 1).

8.3±0.4. No clinical disease or obvious signs of infection was found in any eyes of the mock-infected control groups.

**Figure 1.** Distribution of differentially regulated corneal genes. The microarray results compare the number of corneal genes in mouse corneas from Candida albicans-infected to the number of corneal genes in mock-infected control mouse corneas. The results are categorized by expression-level differences in twofold increments. A minimum of significant (p<0.05), twofold upregulation or significant (p≤0.05) twofold down-regulation was required to be charted.

**Figure 2.** Differential upregulation and down-regulation of corneal genes. Microarray results compare gene expression levels in mouse corneas from Candida albicans-infected compared to corneas from mock-infected controls. The results were assigned to general categories based upon the known functions of the gene products. The number of genes expressing a twofold or greater significant difference (p<0.05) is plotted for the listed categories. Red bars represent the number of genes significantly upregulated, and black bars represent the number of genes significantly down-regulated.

**TABLE 2. RESULTS OF RNA QUALITY CONTROL EVALUATION.**

| Experimental group | Scaling factor | Average background | PCall % | β-Actin 3′ to 5′ ratio | GAPDH 3′ to 5′ ratio |
|--------------------|----------------|---------------------|---------|-----------------------|---------------------|
| Infected 1         | 2.5            | 64.5                | 56.9    | 2.6                   | 2.7                 |
| Infected 2         | 2.5            | 54.1                | 54      | 2                     | 2.1                 |
| Infected 3         | 2.4            | 55.8                | 52.4    | 1.8                   | 1.9                 |
| Control 1          | 2.4            | 60.6                | 52.9    | 2.8                   | 2.6                 |
| Control 2          | 2.4            | 54.9                | 53.7    | 3                     | 2.6                 |
| Control 3          | 4.6            | 53.7                | 47.2    | 3.1                   | 2.8                 |

Triplicate samples of five-cornea pools were used from Candida albicans-infected or mock-infected control animals. PCall % is the percent of probe sets that are called “present” by Affymetrix’s algorithm. The 3′ to 5′ ratio is the 3′-end to 5′-end probe intensity ratio. The number of “present” probes for housekeeping probe sets (β-actin and GAPDH) was 3, and the number of spike-in probe sets (BioB, BioC, BioD, and CreX) was 2.

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results were found for selected genes detected by microarray and real-time reverse transcriptase (RT–PCR) analysis (Table 5), although CCR3 expression differed between the infected and mock-infected corneas.

**DISCUSSION**

Fungal keratitis comprises a dynamic interaction between microorganisms and host [16]. Because most mechanisms that mediate the pathogenesis of fungal keratitis have not been elucidated, we explored the global gene expression during Candida albicans corneal infection with the use of genetic microarray. To control for the effects of corneal trauma [17,18], we compared corneas having post-traumatic fungal keratitis with scarified controls. At 24 h p.i., approximately 9% of genes were significantly expressed twofold or more and nearly 4% were differentially altered at least fourfold.

Our findings complement previous studies of microbial keratitis. Huang and Hazlett [11] found that approximately 10% of genes changed significantly 24 h after the onset of Pseudomonas aeruginosa keratitis in BALB/c and B6 mice. Wang and colleagues [10] exposed heat-killed spores of Aspergillus fumigatus to excised pieces of BALB/c mouse cornea and noted that several host genes were substantially upregulated including those encoding interleukin cytokines such as IL-3. These gene expression studies imply that microbial keratitis involves synchronized host processes that affect several aspects of inflammatory and immune responses, intercellular communication, and cellular metabolism.

Toll-like receptors (TLRs) may carry out the initial recognition of fungi at the corneal surface [19]. After binding microbial pathogens [20,21], TLRs can induce intracellular cascades that give rise to inflammatory cytokines. Several members of the TLR family that are expressed on the cornea respond to fungal infection [22,23], and our preliminary analysis suggests that TLR pathways are involved in Candida albicans keratitis.

The recruitment of acute inflammatory cells is a key event that immediately follows fungal adherence. Among genes detected by microarray, a quarter of them are involved in chemotactic and other signaling cascades [22] with the nuclear factor-κB pathway having an important role. This transcription factor regulates immune responses to infection and is increased during keratitis [24].

Several mediators of inflammation and wound healing such as metalloproteinases and interleukins are active in the initial events of corneal infection. Genes encoding IL-6 and IL-1β were expressed more than 300 fold during fungal keratitis. Corneal injury results in the upregulation of IL-1β [18], and infection further increases its expression [25]. IL-6

| Table 3. Categories of all genes significantly upregulated more than 100 fold. |
|---|
| **Category** | **Symbol** | **Description** | **GenBank accession** | **Relative upregulation** | **p value** |
| Chemokines | PPBP | Chemokine (C-X-C) ligand 7 | AE042817 | 834.4 | 0.01 |
| | CCL3 | chemokine (C-C) ligand 3 | AA95994 | 374.3 | 0.04 |
| | CXCL3 | chemokine (C-X-C) ligand 3 | AK144158 | 291.7 | 0.04 |
| | CCL4 | chemokine (C-C) ligand 4 | AF128218 | 145.2 | 0.011 |
| | CXCL5 | chemokine (C-X-C) ligand 5 | AK144397 | 132.4 | 0.05 |
| Metalloproteinases | MMP13 | matrix metallopeptidase 13 | AK150728 | 375.4 | 0.001 |
| | ADAM8 | a disintegrin and metallopeptidase domain 8 | AK089086 | 108 | 0.01 |
| | MMP9 | matrix metallopeptidase 8 | AK089234 | 102.5 | 0.0003 |
| Interleukin cytokines | IL-6 | interleukin 6 | AK089780 | 245.4 | 0.009 |
| | IL-1β | interleukin 1 beta | AK156396 | 136.8 | 0.03 |
| | OSM | oncostatin M | AK087945 | 122.7 | 0.006 |
| Leukocyte chemotaxis | FPR1 | formyl peptide receptor 1 | AK137714 | 145.2 | 0.01 |
| | FPR-RS2 | formyl peptide receptor, related sequence 2 | AF071180 | 132.8 | 0.05 |
| | SEL | selectin, lymphocyte | AK137390 | 114.3 | 0.01 |
| Leukocyte surface molecules | CLEC4D | C-type lectin domain family 4, member d | AF061272 | 118.2 | 0.04 |
| | TREM1 | triggering receptor expressed on myeloid cells 1 | AF241219 | 136.4 | 0.0063 |
| Cell proliferation molecules | CSF3 | colony stimulating factor 3 (granulocyte) | AK145177 | 114.3 | 0.001 |
| | HMGA2 | high mobility group AT-hook 2 | AC153362 | 106.8 | 0.002 |
| | G0S2 | G0/G1 switch gene 2 | AK03165 | 104.7 | 0.01 |
| Neuro-hormone mediators or receptors | IRG1 | immunoresponsive gene 1 | AK036446 | 328.9 | 0.03 |
| | NPPB | natriuretic peptide precursor type B | AB059044 | 204.1 | 0.000002 |
| | MRGPR42 | MAS-related GPR, member A2 | AK156316 | 202.6 | 0.0088 |
| Histamine synthesis | HDC | histidine decarboxylase | AB039880 | 251.5 | 0.03 |
| Prostaglandin synthesis | PTGS2 | prostaglandin-endoperoxide synthase 2 | AC114655 | 106.2 | 0.004 |
| Intracellular molecules | CYP4F18 | cytochrome P450, family 4, subfamily f, polypeptide 18 | AF233647 | 164.8 | 0.01 |
| | SAMS2 | SAM domain, SH3 domain and nuclear localization signals, 1 | AC166831 | 145.7 | 0.002 |
| | PLEK | pleckstrin | AF073294 | 142.6 | 0.003 |
| Protein transport | SLC15A3 | solute carrier family 15, member 3 | AF121080 | 114.2 | 0.02 |
and IL-1β activate neutrophils during Candida albicans infection [26]. By validating our microarray results for selected genes that had variable expression patterns, we showed that the early stage of fungal keratitis involves upregulation of proinflammatory interleukins in a pattern similar to that during bacterial keratitis [11]. We also confirmed that TGF-β does not appear to be altered during early fungal infection [27]. While injury induces interleukins and related cytokines, infection enhances the production of selected mediators of acute inflammation.

Our studies of fungal keratitis are consistent with other models of Candida albicans infection. Expression patterns of genes coding for IL-6 and chemokine receptors markedly increase soon after the onset of Candida albicans exposure [27], and this increase likely contributes to the recruitment and influx of leukocytes into the infected cornea. Histopathological evidence also indicates that corneal inflammation during the incipient stage of Candida albicans keratitis is a manifestation of the innate immune response [13].

In parallel with pathways promoting ulcerative keratitis, the cornea has anti-inflammatory mechanisms for inhibiting NF-κβ activity and dampening the effects of inflammatory mediators. Although increased during bacterial keratitis, IL-10 was not significantly altered 24 h after corneal inoculation with yeasts, possibly because of a different kinetic profile. However, approximately one-third of genes involved in IL-10 signaling were significantly upregulated during Candida albicans keratitis. Other inflammatory suppressors

| TABLE 4. UPREGULATED PATHWAYS IN CANDIDA ALBICANS KERATITIS COMPARED WITH MOCK-INFECTED CONTROLS. |
|-----------------|-----|-------|-------|-----|
| Signaling pathway | Log (p value) | Number of genes involved | Number upregulated genes | Ratio |
| Leukocyte extravasation | 16 | 191 | 44 | 0.23 |
| NF-κβ | 15.5 | 141 | 38 | 0.27 |
| Interleukin 10 | 13.2 | 68 | 23 | 0.34 |
| Interleukin 6 | 12.5 | 90 | 27 | 0.3 |
| Acute-phase response | 11.3 | 171 | 36 | 0.21 |
| B cell receptor | 6.7 | 144 | 26 | 0.18 |
| Toll-like receptor | 6.6 | 50 | 14 | 0.28 |
| Integrin | 6.1 | 188 | 30 | 0.16 |
| Natural killer cell | 6 | 112 | 19 | 0.17 |
| Granulocyte-macrophage colony-stimulating factor | 5.8 | 63 | 15 | 0.24 |
| Transforming growth factor beta | 4.9 | 84 | 16 | 0.19 |
| Chemokine | 4.7 | 75 | 15 | 0.2 |
| Interleukin 2 | 4.5 | 52 | 12 | 0.23 |
| Insulin-like growth factor 1 | 3.8 | 94 | 15 | 0.16 |
| T cell receptor | 3.5 | 100 | 15 | 0.15 |
| Epidermal growth factor | 3 | 47 | 9 | 0.19 |
| Vascular endothelial growth factor | 3 | 93 | 13 | 0.14 |
| Interleukin 4 | 3 | 69 | 11 | 0.16 |
| JAK/STAT | 2.6 | 112 | 19 | 0.17 |
| Apoptosis | 2.5 | 62 | 8 | 0.13 |

| TABLE 5. REAL-TIME RT-PCR CONFIRMATION OF MICROARRAY. |
|-----------------|-------|----------------|-------|
| Gene (Symbol) | Microarray | Real-time RT-PCR | p value |
| Interleukin 1 beta (IL-1β) | 342.4±477.5 | 378.0±317.4 | 0.92 |
| Interleukin 6 (IL-6) | 376.0±295.4 | 388.1±318.9 | 0.96 |
| Chemokine (C-C) ligand 4 (CCL4) | 228.7±175.5 | 200.9±172.2 | 0.85 |
| Chemokine (C-C) ligand 7 (CCL7) | 60.9±51.7 | 14.9±9.8 | 0.21 |
| Chemokine receptor 3 (CCR3) | -6.0±3.5 | 14.5±4.3 | 0.004 |
| Chemokine receptor 5 (CCR5) | 1.1±1.1 | 2.3±0.9 | 0.20 |
| Interleukin 23 alpha (IL-23α) | 9.1±3.3 | 14.2±9.9 | 0.44 |
| Transforming growth factor beta 2 (TGFβ2) | 1.8±1.0 | -1.8±3.7 | 0.19 |

Mean fold change ± standard deviation, comparing infected to mock-inoculated control corneas.
such as suppressor of cytokine signaling protein-3 were also upregulated.

Pathways of immune recognition may also be activated by fungal infection. We confirmed that IL-23 increases during *Candida albicans* keratitis [27]. Expression of this T-cell regulatory molecule supports that adaptive immunity may be involved in candidiasis, although to a lesser degree than innate immune reactions [28].

Our study provides evidence for a likely scenario of molecular events during early fungal keratitis. Soon after corneal surface injury and exposure to viable fungi, fungal components are recognized by host receptors such as lectins and toll-like receptors. Binding by ligands induces intracellular cascades that activate NF-κB, which, in turn, upregulates genes responsible for inflammatory responses. Corneal levels of interleukins and other cytokines rapidly increase. Neutrophils and macrophages are recruited toward the site of infection, helping to kill fungi but releasing chemokines that contribute to corneal ulceration and neovascularization.

In summary, gene microarray analysis is a useful means for understanding the molecular biology of ocular inflammatory disease. *Candida albicans* keratitis involves the induction of genes encoding several interrelated pathways, and this investigation provides insight into the pivotal reactions during the onset of fungal keratitis. Characterizing this coordinated sequence of events can provide insight into the pathogenesis of fungal keratitis that will open up new opportunities for therapeutic interventions.

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**REFERENCES**

1. Srinivasan M. Fungal keratitis. Curr Opin Ophthalmol 2004; 15:321-7. [PMID: 15232472]
2. Sun RL, Jones DB, Wilhelmus KR. Clinical characteristics and outcome of *Candida* keratitis. Am J Ophthalmol 2007; 143:1043-5. [PMID: 17524775]
3. Thomas PA. Fungal infections of the cornea. Eye 2003; 17:852-62. [PMID: 14631389]
4. Jackson BE, Mitchell BM, Wilhelmus KR. Corneal virulence of *Candida albicans* strains deficient in Tup1-regulated genes. Invest Ophthalmol Vis Sci 2007; 48:2535-9. [PMID: 17525181]
5. Jackson BE, Wilhelmus KR, Hube B. The role of secreted aspartyl proteinases in *Candida albicans* keratitis. Invest Ophthalmol Vis Sci 2007; 48:3559-65. [PMID: 17652724]
6. Rohini G, Murugeswari P, Prajna NV, Lalitha P, Muthukkaruppan V. Matrix metalloproteinases (MMP-8, MMP-9) and the tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2) in patients with fungal keratitis. Cornea 2007; 26:207-11. [PMID: 17251814]
7. Mitchell BM, Wu TG, Chong EM, Pate JC, Wilhelmus KR. Expression of matrix metalloproteinases 2 and 9 in experimental corneal injury and fungal keratitis. Cornea 2007; 26:589-93. [PMID: 17525657]
8. Mühäuser J, Wildfeuer A, Meister H, Dybas L, Haferkamp O. Experimental *Candida* keratitis. histological, immunological and electron microscopic studies. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1975; 195:251-62. [PMID: 1098511]
9. Isobe Y. The effect of topical steroid medication on experimental *Candida* keratitis. Nippon Ganka Gakkai Zasshi 1991; 95:45-51. [PMID: 2042529]
10. Wang Y, Liu T, Gong H, Zhou Q, Sun S, Xie L. Gene profiling in murine corneas challenged with *Aspergillus fumigatus*. Mol Vis 2007; 13:1226-33. [PMID: 17679939]
11. Huang X, Hazlett LD. Analysis of *Pseudomonas aeruginosa* corneal infection using an oligonucleotide microarray. Invest Ophthalmol Vis Sci 2003; 44:3409-16. [PMID: 12882789]
12. Mitchell BM, Wu TG, Jackson BE, Wilhelmus KR. *Candida albicans* strain-dependent virulence and Rim13p-mediated filamentation in experimental keratomycosis. Invest Ophthalmol Vis Sci 2007; 48:774-80. [PMID: 17251477]
13. Wu TG, Wilhelmus KR, Mitchell BM. Experimental keratomycosis in a mouse model. Invest Ophthalmol Vis Sci 2003; 44:210-6. [PMID: 12506077]
14. Carvalho B, Bengtsson H, Speed TP, Irizarry RA. Exploration, normalization, and genotype calls of high-density oligonucleotide SNP array data. Biostatistics 2007; 8:485-99. [PMID: 17189563]
15. Storey JD, Dai JY, Leek JT. The optimal discovery procedure for large-scale significance testing, with applications to comparative microarray experiments. Biostatistics 2007; 8:414-32. [PMID: 16928955]
16. Rozell B, Ljungdahl PO, Martinez P. Host-pathogen interactions and the pathological consequences of acute systemic *Candida albicans* infections in mice. Curr Drug Targets 2006; 7:483-94. [PMID: 16611036]
17. Varela JC, Goldstein MH, Baker HV, Schultz GS. Microarray analysis of gene expression patterns during healing of rat corneas after excimer laser photorefractive keratometry. Invest Ophthalmol Vis Sci 2002; 43:1772-82. [PMID: 12036978]
18. Cao Z, Wu HK, Bruce A, Wollenberg K, Panjwani N. Detection of differentially expressed genes in healing mouse corneas, using cDNA microarrays. Invest Ophthalmol Vis Sci 2002; 43:2897-904. [PMID: 12202508]
19. Jin X, Qin Q, Lin Z, Chen W, Qu J. Expression of toll-like receptors in the *Fusarium solani* infected cornea. Curr Eye Res 2008; 33:319-24. [PMID: 18398706]
20. Jin X, Qin Q, Tu L, Zhou X, Lin Y, Qu J. Toll-like receptors (TLRs) expression and function in response to inactivate hyphae of *Fusarium solani* in immortalized human corneal epithelial cells. Mol Vis 2007; 13:1953-61. [PMID: 17982419]
21. Zhao J, Wu XY. *Aspergillus fumigatus* antigens activate immortalized human corneal epithelial cells via toll-like receptors 2 and 4. Curr Eye Res 2008; 33:447-54. [PMID: 18568882]
22. Müller V, Viemann D, Schmidt M, Endres N, Ludwig S, Leverkus M, Roth J, Goebeler M. *Candida albicans* triggers
activation of distinct signaling pathways to establish a proinflammatory gene expression program in primary human endothelial cells. J Immunol 2007; 179:8435-45. [PMID: 18056390]

23. Sun CC, Su Pang JH, Cheng CY, Cheng HF, Lee YS, Ku WC, Hsiao CH, Chen JK, Yang CM. Interleukin-1 receptor antagonist (IL-1RA) prevents apoptosis in ex vivo expansion of human limbal epithelial cells cultivated on human amniotic membrane. Stem Cells 2006; 24:2130-9. [PMID: 16741227]

24. Wu XY, Han SP, Ren MY, Chang Y, Yu FX. The role of NF-kappaB activation in lipopolysaccharide induced keratitis in rats. Chin Med J (Engl) 2005; 118:1893-9. [PMID: 16313844]

25. Khan S, Cole N, Hume EB, Garthwaite L, Conibear TC, Miles DH, Aliwaga Y, Krockenberger MB, Willcox MD. The role of CXC chemokine receptor 2 in Pseudomonas aeruginosa corneal infection. J Leukoc Biol 2007; 81:315-8. [PMID: 17028201]

26. van Enckevort FH, Netea MG, Hermes AR, Sweep CG, Meis JF, Van der Meer JW, Kullberg BJ. Increased susceptibility to systemic candidiasis in interleukin-6 deficient mice. Med Mycol 1999; 37:419-26. [PMID: 10647123]

27. Kim HS, Choi EH, Khan J, Roilides E, Francesconi A, Kasai M, Sein T, Schaufele RL, Sakurai K, Son CG, Greer BT, Chanock S, Lyman CA, Walsh TJ. Expression of genes encoding innate host defense molecules in normal human monocytes in response to Candida albicans. Infect Immun 2005; 73:3714-24. [PMID: 15908401]

28. Ashman RB, Farah CS, Wanasaengsakul S, Hu Y, Pang G, Clancy RL. Innate versus adaptive immunity in Candida albicans infection. Immunol Cell Biol 2004; 82:196-204. [PMID: 15061774]

Appendix 1. Upregulated signaling pathways in Candida albicans keratitis compared with mock controls.

To access the data, click or select the words “Appendix 1”. This will initiate the download of a (pdf) archive that contains the file.