RESEARCH ARTICLE

Massive induction of innate immune response to *Candida albicans* in the kidney in a murine intravenous challenge model

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Abstract

In the experimental *Candida albicans* intravenous challenge model, the kidney is one of the main organs involved in disease. In progressive infection, fungal burdens are found to increase over time, with rapid increases occurring from 24 h postinfection. Renal transcriptional responses were analyzed at this time in the kidneys of mice infected by either a virulent or an attenuated *C. albicans* strain, allowing comparison of host responses in progressive and nonprogressive infection. The results of this study demonstrate that both infections share a common transcriptional response, consisting of functions associated with the acute-phase reaction. In addition, challenge with the virulent strain led to a massively increased expression of cytokine genes, other innate response genes and genes suggestive of initiation of the adaptive immune response. This immune response to *C. albicans* infection, which occurs only in progressive infection, may contribute to development of sepsis and, ultimately, host death.

Introduction

Systemic fungal infections remain a significant cause of morbidity and mortality, with *Candida albicans* being the most common causative agent (Odds et al., 2007; Bougnoux et al., 2008; Caggiano et al., 2008; Playford et al., 2008).

Intravenous *C. albicans* challenge in mice is a well-characterized model of severe clinical disseminated infection (MacCallum & Odds, 2005; Spellberg et al., 2005), used to investigate fungal virulence factors (Navarro-Garcia et al., 2001; Brand et al., 2004) and host immune responses (Ashman, 2008; Romani, 2008; MacCallum et al., 2009). In this model, within minutes of entering the bloodstream, *C. albicans* is detectable in all major organs (MacCallum & Odds, 2005), with the kidneys and brain being the main target organs (Louria et al., 1963; MacCallum & Odds, 2005), reflecting the situation in the human host (Parker et al., 1976; Odds, 1988). In infection initiated by the virulent strain SC5314, renal fungal burdens increase from the time of infection, with rapid increases occurring from 24 h postinfection. Although the kidneys are the most heavily colonized organs, and some loss of renal function has been shown (Leunk & Moon, 1979; Spellberg et al., 2005), renal failure is not the main cause of death. Host deterioration, and eventually death, is due to a progressive sepsis, reflecting the clinical situation (Spellberg et al., 2005).

Because of similarities to severe human disseminated infection, the mouse intravenous challenge model is ideal for studying the transcriptional response of the kidney to infection by *C. albicans*. DNA microarrays have been used previously to investigate the transcriptional responses of host cells (Prigneau et al., 2003; Ishibashi et al., 2004; Mullick et al., 2004a; Barker et al., 2005, 2008; Kim et al., 2005b; Fradin et al., 2006; Muller et al., 2007) and fungal cells (Fradin et al., 2003, 2005; Lorenz et al., 2004; Sandsowsky-Losica et al., 2006; Sohn et al., 2006; Fernandez-Arenas et al., 2007; Thewes et al., 2007; Zakikhany et al., 2007; Walker et al., 2009) during their interaction. Research has focused mainly on examining the responses of single host cell types, such as macrophages (Prigneau et al., 2003; Lorenz et al., 2004; Barker et al., 2005; Kim et al., 2005b), neutrophils (Fradin et al., 2005, 2006) and endothelial cells (Muller et al., 2007; Barker et al., 2008). The aim of this study was to investigate the transcriptional responses of the
whole kidney to infection by \textit{C. albicans}, and to examine how these responses may relate to disease development, including progression of sepsis.

\section*{Materials and methods}

\subsection*{Candida albicans strains and growth conditions}

\textit{Candida albicans} strains NGY152 (wild-type CA14 + Clp10, Brand et al., 2004) and NGY355 (\textit{pmr1A} null mutant, Bates et al., 2005) were maintained routinely on Sabouraud agar at 4°C, and stored long term in glycerol stocks at −80°C. For the mouse intravenous challenge, inocula were prepared as described previously (Bates et al., 2005).

\subsection*{Experimental infection model}

Female BALB/c mice (Harlan, UK) (average weight 20 g) were infected intravenously with an inoculum of $4.6 \times 10^4$ CFU g$^{-1}$ body weight. This inoculum level leads to a survival time of approximately 5 days for mice infected with the virulent strain NGY152 (MacCallum & Odds, 2005), but all animals infected with the attenuated strain NGY355 survived to the end of the 28-day experiment (Bates et al., 2005). Groups of mice (N = 3) were infected with \textit{C. albicans} NGY152 (virulent), NGY355 (attenuated) or saline (controls). Actual inocula levels were determined by viable counts.

Mice were humanely terminated at 24 h. Kidneys were dissected and processed to provide portions for RNA extraction (flash-frozen in liquid nitrogen), histological analysis (formalin-fixed) and burden determination (homogenized in saline). Other organs (lung, liver, spleen, and brain) were sampled and processed for organ burden determination as described previously (MacCallum & Odds, 2005). All work involving experimental animals was performed under UK Home Office licenses and regulations.

\subsection*{RNA extraction}

Frozen kidney pieces (approximately 50 mg) were ground to a fine powder in a Mikro-dismembrator U homogenizer (Sartorius Ltd., UK). The powder was resuspended in 1 mL TRIzol (Invitrogen, UK) and RNA extracted as per the manufacturer’s instructions. RNA samples were DNase treated and purified using RNeasy mini cleanup columns (Qiagen, UK); the concentration was determined by spectrophotometry (Nanodrop ND-1000; Labtech International, UK) and the quality of the RNA was analyzed by a Bioanalyzer (Agilent Technologies, UK).

\subsection*{Microarrays}

For microarray analysis, pools of RNA for each condition were produced. Briefly, equal quantities (8 µg) of the three independent samples were mixed and made up to 24 µL. Each pool was then divided into three aliquots. For each treatment, two aliquots were used in the production of biotin-labeled cRNA. Standard Affymetrix protocols and kits (one-cycle cDNA synthesis, first strand synthesis, second strand synthesis, double-stranded DNA clean-up, GeneChip IVT synthesis and clean-up of biotin-labeled cRNA) were used to produce the labeled cRNA samples (http://www.affymetrix.com). From each cRNA sample, 20 µg was fragmented (1 x fragmentation buffer, 94°C for 35 min), and 15 µg of this was used to produce a hybridization cocktail. Control noneukaryotic biotinylated and fragmented cRNAs (bioB, C and D from \textit{Escherichia coli} and cre from bacteriophage PI) were added to the hybridization cocktail.

Cocktails for all six samples were hybridized to Affymetrix mouse genome 430A 2.0 arrays according to the manufacturer’s instructions in an Affymetrix GeneChip hybridization oven 640. Arrays were washed in an Affymetrix GeneChip fluidics station and scanned with an Affymetrix GeneChip scanner 3000.

\subsection*{Microarray gene expression data analysis}

For each Affymetrix array, the CEL file was loaded into Genespring (Agilent Technologies). Data were normalized on a Per Chip basis (normalized to the 50th percentile) and on a Per Gene basis (normalized to the mean). Finally, data for \textit{Candida}-infected kidneys were normalized on a Per Gene basis to control (saline-infected) samples.

Normalized data were analyzed by significance analysis of microarrays (http://www-stat.stanford.edu) to produce lists of genes with statistically significantly different expression levels, based on a false discovery rate value of 0. Gene lists were further trimmed to include only genes whose expression level differed at least 1.5-fold compared with uninfected controls. Gene trees were constructed in Genespring, with commonly regulated genes grouped together. Regulated genes were further divided into three large clusters with the three-cluster K-means function of Genespring.

Microarray data files [raw (CEL) and normalized files] were deposited in ArrayExpress (http://www.ebi.ac.uk/microarray-as/aer), accession number: E-MEXP-1458.

\subsection*{cDNA synthesis and quantitative reverse transcriptase (QRT)-PCR}

QRT-PCR with the Universal Probe Library system (Roche, UK) was used to verify differences in gene expression observed in microarray analysis.

Superscript II (Invitrogen) was used to synthesize cDNAs from 3 µg of each of the three independent RNA samples extracted from infected or control kidneys as per the manufacturer’s instructions.

The oligonucleotide primers (Invitrogen) and Universal probes (Roche) used for qRT-PCR are listed in Table 1. PCR
and 60
out at 95
run on a LightCycler 480 (Roche); initial activation was carried
exogenous control (PE Applied Biosystems, UK). Reactions were
probes Mastermix (Roche) and 1
updated from a number of sources.
pathway, and use knowledge databases that are continually
Both resources determine association by
tistical tests (Kruskal–Wallis and Mann–Whitney
Expression levels were compared using nonparametric statis-
three independent cDNA samples from each treatment group.
PCR reactions were carried out in triplicate for each of the
standard curve and normalized to the GAPDH control. qRT-
10 s. For each gene, the copy number was determined from a

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### Table 1. qRT-PCR oligonucleotide primers and Universal probes

| Primer name | Sequence (5′–3′) | Universal probe |
|-------------|-----------------|-----------------|
| Tlr2F | TGGCCCAATGGCTAGTG | 51 |
| Tlr2R | CAGACAGTGTTGCAGCAC | |
| Tlr4F | GGACTCTGACATGGCACTG | 51 |
| Tlr4R | CTGATCCATGGGTAGGT | |
| Tlr13F | CTAATTGCTAGGGCCCTTGAGAG | 6 |
| Tlr13R | TTTCTCTGCAAGAGCTACGGA | |
| LIF | GCCACAATCTGATGATAAATCA | 6 |
| IL6R | CAGGCTGACTGGTTACCG | 81 |
| Clec4nR | GCAGGCAGAAGTTTCCTTGGG | 78 |
| Clec4nF | CAGTGAAGGGACTATGGTGTCA | |
| Myd88R | GAATCAGTCGCTTCTGTTGGA | 80 |
| Myd88R | GAATCAGTCGCTTCTGTTGGA | |
| Il1bF | TGTAATGAAAGACGGCACACC | 78 |
| Il1bR | TCTCCTTGGTATTGCTTGGG | |
| Tlr13F | CTTCTCTGCAAGAGCTACGGA | |
| Tlr13R | TTTCTCTGCAAGAGCTACGGA | |
| Clec4nF | TCTCCTTGGTATTGCTTGGG | 81 |
| Clec4nR | TCTCCTTGGTATTGCTTGGG | |
| Cxcl12F | TCTCCTTGGTATTGCTTGGG | |
| Cxcl12R | TCTCCTTGGTATTGCTTGGG | |

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reactions (20 μL) contained 3 μL cDNA, 0.2 μL Universal
probe, 0.2 μM forward primer, 0.2 μM reverse primer, 1 ×
probes Mastermix (Roche) and 1 × murine GAPDH endo-
genous control (PE Applied Biosystems, UK). Reactions were
run on a LightCycler 480 (Roche); initial activation was carried
out at 95 °C for 5 min, 50 amplification cycles of 95 °C for 10 s
and 60 °C for 30 s and then a final cooling step at 40 °C for
10 s. For each gene, the copy number was determined from a
standard curve and normalized to the GAPDH control. qRT-
PCR reactions were carried out in triplicate for each of the
three independent cDNA samples from each treatment group.
Expression levels were compared using nonparametric statisti-
cal tests (Kruskal–Wallis and Mann–Whitney U-tests) due to
unequal variances for the different groups.

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**Bioinformatics: biological function and pathway analysis**

Biological functions and pathways associated with the gene
lists obtained from expression analysis were identified by
means of Ingenuity Pathway Analysis (Ingenuity Systems;
http://www.ingenuity.com) and the Database for Annotation,
Visualization and Integrated Discovery (DAVID) (http://david.
abcc.ncifcrf.gov) (Dennis et al., 2003) bioinformatic resources.
Both resources determine association by P-values, found by
comparing genes on a list with the total number of genes for a
pathway, and use knowledge databases that are continually
updated from a number of sources.

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**Histology**

Sections (5 μm) were cut from paraffin wax blocks of
formalin-fixed kidney portions. Sections were stained by
periodic acid-Schiff to show the fungal cells clearly and
poststained with hematoxylin to illustrate kidney morphology
and immune infiltrates.

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**Results**

**Reduced virulence of strain NGY355 is evident at 24 h postinfection**

In the murine model of systemic candidiasis, *C. albicans*
strain NGY152 is virulent, attaining high renal fungal burdens and rapidly causing fatal infection (Brand et al., 2004; MacCallum & Odds, 2005). The second *C. albicans*
strain, NGY355 (*pmr1Δ*), a mutant that lacks *N*- and *O-
linked mannann side chains in the cell wall, has been shown to be severely attenuated in virulence, both in terms of
mouse survival times and of fungal organ burdens (Bates et al., 2005; MacCallum et al., 2009).

Mice infected with either NGY152 (virulent) or NGY355
(attenuated) were sampled 24 h postinfection, when a rapid
increase in kidney fungal burden is known to occur in
kidneys infected by the virulent strain (MacCallum & Odds,
2005). In contrast, little change in kidney fungal burden was
found at this time for the attenuated (*pmr1Δ*) strain
(MacCallum et al., 2009).

In this study, virulence differences between the two
strains were evident at this early time, in terms of signifi-
cantly higher burdens in the kidney, brain and lung (Fig. 1a)
in mice infected with the virulent strain, with the greatest
difference (approximately 100-fold) observed for the kidney.

The kidneys of virulent strain-infected mice contained
filamentous fungal cells associated with leukocyte infiltrates
(Fig. 1b), but there was little evidence of similar fungal
lesions or immune cell infiltration in the kidneys of mice
infected with the attenuated fungal strain. Control kidneys
from saline-infected mice showed no evidence of fungal cells
or immune cell infiltrates (data not shown).

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**Renal gene expression changes associated with *C. albicans* infection**

Because infection by the different *C. albicans* strains led to
different renal phenotypes, i.e. immune infiltrate and lesion
development, global transcript profiling of kidneys 24 h
postinfection was used to reveal the local host responses
underlying these different responses. Whole genome DNA
microarrays were used to compare expression profiles of
kidneys infected with either the virulent or the attenuated
*C. albicans* strain relative to control, uninfected kidneys.

Results demonstrated that 2099 (5.4%) murine genes were
upregulated in the kidneys of mice infected with NGY152
(virulent strain), compared with only 557 (1.4%) genes in the
kidneys of mice infected with NGY355 (attenuated strain).
The numbers of downregulated genes were similar to those
upregulated, with 2069 (5.3%) for the virulent strain.
infection and 393 (1.0%) for the attenuated strain. There were 273 common upregulated and 286 common downregulated genes, representing the majority of those with altered expression levels in NGY355 infection. These commonly regulated genes are suggested to represent a core transcriptional response of the kidney to *C. albicans* infection.

All infection-regulated genes were grouped into three large sets (Fig. 2) using clustering analysis based on K-means. Regulated genes common to both infections, and representative of the core response, were found in cluster 2, while genes more upregulated in the virulent strain infection formed cluster 1. Cluster 3 represented genes that were commonly downregulated or downregulated specifically in virulent strain infection.

Biological functions and pathways associated with *C. albicans* infection

To identify biological functions and/or pathways associated with genes with altered expression in infected kidneys, results were analyzed by Ingenuity Pathway Analysis (http://www.ingenuity.com) and the DAVID (http://david.abcc.ncifcrf.gov) resource.
Results demonstrated that both the attenuated and the virulent strains induced changes in gene expression associated with the acute-phase response (a generalized response to disturbances in physiological homeostasis) and complement and coagulation cascades (Fig. 3a and b). These responses represent the core response of the kidney to *C. albicans* infection, with their expression regulated in response to infection with either strain (cluster 2, Fig. 2). In addition to the core response, infection with the attenuated strain induced regulation of genes in some metabolic pathways, whereas infection with the virulent *C. albicans* strain was strongly associated with regulation of genes involved in induced immune responses (Fig. 3a and b). Functions most strongly associated with virulent strain infection included cytokine–cytokine receptor interactions and a number of immune signaling pathways, such as interleukin (IL)-6, IL-10 and nuclear factor κB. In addition, gene expression changes linked to leukocyte movement were also associated with infection by the virulent strain. There was also evidence for initiation of an adaptive immune response with gene expression changes associated with antigen processing and presentation, and also signaling from both T-cell and B-cell receptors in the kidneys of mice infected with the virulent strain (Fig. 3b). Genes associated with the above functions were found in cluster 1 (Fig. 2), with little change in the expression of these same genes in kidneys infected by the attenuated strain.

Genes downregulated during infection, found in cluster 3 (Fig. 2), were associated with pyruvate metabolism, fatty acid metabolism, degradation of valine, leucine and isoleucine, as well as tryptophan metabolism. However, these associations were based on only one-fifth of the genes, with the majority of downregulated genes not yet assigned to functions or pathways.

**Cytokine and pattern recognition receptor (PRR) gene expression during infection**

Because of the differences in renal gene expression associated with cytokine interactions and signaling in the different infections, a more detailed examination of cytokine gene expression was performed (Fig. 4). Results demonstrated a clear induction of the proinflammatory...
cytokine genes Il1a and b, Il6 and Tnf, which have also been linked to the acute-phase response, in the kidneys of mice infected with the virulent strain, whereas little induction of these genes was seen in kidneys infected with the attenuated strain. Among 54 cytokine genes examined, 32 were significantly altered in virulent strain infection compared with control kidneys (Fig. 4). The majority of these genes were upregulated, but a single cytokine gene, Cxcl12, was downregulated in the renal response to the virulent C. albicans strain and unchanged in attenuated strain infection.

In the kidneys of mice infected with the attenuated strain, the only cytokine/chemokine genes upregulated were Cxcl10, Cxcl11, Kc, Mcp1, Mig, Mip1a, Mip2a and Rantes. These genes, along with Il6, were also the most upregulated in virulent strain infection, with expression levels at least 10-fold higher than those of mice infected with the attenuated strain (Fig. 4).

The large differences in cytokine gene expression in response to C. albicans infection prompted an examination of the expression of PRR genes, the signaling from which influences cytokine production. PRRs known to be involved in the recognition of C. albicans include Toll-like receptors (TLRs) (Netea et al., 2006a, b; Carpenter & O’Neill, 2007) and C-type lectins (Willment & Brown, 2008).

There was little change in PRR gene expression in kidneys infected with the attenuated strain (Fig. 5), but in the kidneys of mice infected with the virulent strain there was an increased expression of genes encoding TLRs (Tlr1, Tlr2, Tlr4, Tlr6 and Tlr13) and their associated adaptor molecules: Myd88, Cd14 and Md2 (Fig. 5). The greatest increase in TLR gene expression was seen for Tlr2. In addition, a mouse-specific TLR gene, Tlr12, was downregulated during virulent strain infection, but was unaltered in attenuated strain infection.

Although the majority of non-TLR PRR genes examined showed increased expression in the kidneys of virulent strain-infected mice, the expression of mannose receptor genes was reduced, and the greatest increase in non-PRR gene expression was seen for dectin-2 (Fig. 5). Similarly, expression of FCγ receptor genes, whose products are

**Fig. 4.** Cytokine/chemokine gene expression in infected kidneys. For each cytokine, the gene expression levels obtained by microarray analyses are shown relative to control, where A represents infection by the attenuated strain and V represents infection by the virulent strain. Expression level (-fold) change is colored as shown on the scale.

**Fig. 5.** PRR gene expression in infected kidneys. The expression levels of various pattern recognition receptors and some adaptor molecules are shown, where A represents infection by the attenuated strain and V represents infection by the virulent strain. Expression levels are normalized to controls. Expression level (-fold) change is colored as shown on the scale.
required for dectin-2 signaling (Sato et al., 2006), were also upregulated in response to virulent strain infection.

**Confirmation of transcript profiling results by qRT-PCR**

In order to confirm the gene expression differences found by microarray analysis, qRT-PCR was used to analyze expression levels of several genes of interest. Immune response-associated genes, with altered expression in virulent strain infection, were selected for further investigation: Tlr2, Tlr4, Tlr13, Myd88, Il1b, Il6, dectin-2 and Cxcl12.

Results obtained by qRT-PCR for the individual kidney RNA samples showed good correlation with the transcript profiling results for RNA pools (r = 0.93, data not shown), with statistically significant differences found for all genes when renal responses to the virulent strain were compared with those of mice infected with the attenuated strain or of control mice (Fig. 6). These results confirmed the changes in innate immune response-related gene expression seen by microarray analysis in kidneys in response to the virulent strain, but that only occurred to a limited extent in response to the attenuated strain.

Surprisingly, qRT-PCR also demonstrated a small, but significant, increase in Tlr4 gene expression in response to infection by the attenuated strain that was not seen by microarray analysis (Fig. 6).

**Discussion**

The mouse intravenous challenge model is frequently used to characterize the virulence potential of *C. albicans* isolates and strains and is a good model of severe human disseminated infection. From previous studies, we have shown that kidney burdens in virulent strain-infected mice continue to increase until the mice become severely ill (MacCallum & Odds, 2005). In contrast, in infection initiated by the attenuated strain, kidney burdens remain roughly constant over 28 days of infection (Bates et al., 2005; MacCallum et al., 2009). These results demonstrate that the virulent strain initiates a progressive infection, while infection initiated by the attenuated strain is controlled. Analysis of organ burdens at 24 h postinfection demonstrated virulence differences between the two strains, even at this early time in infection. Kidney burdens were significantly higher for mice infected with the virulent strain and infiltrating immune cells were already evident in the kidneys of these mice. These results clearly indicate that early renal responses to the two *C. albicans* strains must differ.

In this study, the transcriptional responses of the kidney at 24 h postinfection were analyzed to determine how responses to known virulent and attenuated *C. albicans* strains differ. Microarray gene expression patterns were confirmed by qRT-PCR, confirming that the use of RNA pools for transcript profiling is a valid approach for this experimental infection model.

Results clearly demonstrated that the kidney responds to *C. albicans* infection by inducing a core response: the acute-phase response and complement and coagulation cascades. Induction of the acute-phase response was not surprising as this reaction is a response to physiological alterations and it is usually activated during inflammation. Although previously associated with the liver (Kushner, 1982), renal proximal tubular epithelial cells have also been shown to generate a strong acute-phase response (Luyckx et al., 2009), suggesting that this cell type may be involved in the core response.

While the core response appeared sufficient to prevent overgrowth of the attenuated strain within the first 24 h of infection, reducing fungal burdens to levels similar to those found at 28 days postinfection (Bates et al., 2005), it was insufficient to control the virulent strain. Deficiencies in the core response to *C. albicans* infection have previously been shown to lead to increased susceptibility to systemic candidiasis (Gelfand et al., 1978; Odds, 1988; Mullick et al., 2004b; Held et al., 2008), and may allow normally attenuated strains to initiate progressive infections.

In progressive renal infection initiated by the virulent strain, the fungus proliferated in the kidneys, accompanied by a massive transcriptional change, mostly genes associated with innate and adaptive immune responses. This demonstrates a clear link between induction of host immune
responses in the kidney and progression of 	extit{C. albicans} infection. Immune responses occurring in the kidney have previously been linked to 	extit{C. albicans} infection outcome (Brieland 	extit{et al}., 2001; Spellberg 	extit{et al}., 2003; Cao 	extit{et al}., 2005; MacCallum 	extit{et al}., 2009).

The majority of cytokines and chemokines genes examined showed increased expression in response to infection by the virulent strain, their products thus leading to attraction and activation of immune cells. In contrast, the attenuated strain showed little induction of proinflammatory gene expression in the kidney, correlating with the lack of infiltrating immune cells in the kidney (Fig. 1b) and also a previous study where the attenuated strain induced much lower levels of tumor necrosis factor (TNF-\(\alpha\)) and IL-6, due to its defect in cell wall protein mannosylation (Netea 	extit{et al}., 2006b). However, although the defect in TNF-\(\alpha\) and IL-6 induction is due to the lack of mannosylation in the attenuated pmr1\(\Delta\) mutant1 (Netea 	extit{et al}., 2004), we have recently demonstrated that attenuated clinical isolates, in general, induce lower renal levels of chemokines and proinflammatory cytokines compared with virulent isolates (MacCallum 	extit{et al}., 2009). This confirms that upregulation of cytokines and chemokines is a general response of kidneys exposed to virulent 	extit{C. albicans} strains (MacCallum 	extit{et al}., 2009).

Proinflammatory cytokines, for example TNF-\(\alpha\) and interferon (IFN)-\(\gamma\), are required for normal responses to 	extit{C. albicans} infection (Steinshamn \& Waage, 1992; Kullberg 	extit{et al}., 1993; Louie 	extit{et al}., 1994; Brieland 	extit{et al}., 2001). However, previous studies have demonstrated that the fatal outcome of 	extit{C. albicans} infection is linked to the development of T-helper type 2 (Th2) and IL-10 responses in the kidney (Brieland 	extit{et al}., 2001; Spellberg 	extit{et al}., 2003; Cao 	extit{et al}., 2005). IL-10 is known to have strong inhibitory effects on defense against 	extit{C. albicans} (Romani 	extit{et al}., 1994), with IL-10 knockout mice more resistant to 	extit{C. albicans} infection (Vazquez-Torres 	extit{et al}., 1999). We found, in agreement with others (Brieland 	extit{et al}., 2001; Spellberg 	extit{et al}., 2003), that renal expression of Il10 was induced in progressive infection. Cytokine profiles, however, showed evidence of both Th1 and Th2 responses in infected kidneys, similar to the results obtained by Bellochio 	extit{et al}., (2004). Differences between this study and the study of Cao 	extit{et al}., (2005), who clearly found that a Th2 response in the kidney was linked to a lethal outcome, may be due to the use of different 	extit{C. albicans} strains, or due to different kinetics of infection progression, as mice in this study had lower survival times than those in the study of Cao 	extit{et al}., (2005).

The renal transcriptional response to 	extit{C. albicans} progressive infection also included regulation of PRR gene expression. Previously, a number of PRRs have been demonstrated to be involved in host recognition of 	extit{C. albicans} (Netea 	extit{et al}., 2006a; Willment \& Brown, 2008), particularly TLR2, TLR4 (Netea 	extit{et al}., 2002, 2008; Villamon 	extit{et al}., 2004b; Blasi 	extit{et al}., 2005) and dectin-1 (Brown 	extit{et al}., 2003; Gantner 	extit{et al}., 2005; Gow 	extit{et al}., 2007; Ferwerda 	extit{et al}., 2008). In this study, the kidney cells upregulating expression of the PRRs were not identified but, in response to renal injury, TLR2 and TLR4 levels are increased on tubular epithelial cells (Wolfs 	extit{et al}., 2002; Kim 	extit{et al}., 2005a; Leemans 	extit{et al}., 2005; Shigeoka 	extit{et al}., 2007; Wu 	extit{et al}., 2007). This again suggests that these cells may play a role in renal responses to 	extit{C. albicans} infection, especially as these cells are capable of producing numerous cytokines and chemokines (reviewed in Daha \& van Kooten 2000), and have been shown to respond to other infections with induction of inflammatory cascades (Ross 	extit{et al}., 2006). However, the microarray results in this study also show good agreement with those found for endothelial cells (Mulder 	extit{et al}., 2007), indicating that transcriptional changes may be due to multiple cell types in the kidney. Examination of the transcriptional responses of kidney cell subsets to 	extit{C. albicans} infection will be the subject of future studies.

In response to infection by the attenuated strain, there was little change in PRR gene expression in the kidneys by microarray analysis. However, qRT-PCR indicated a small significant increase in Tlr4 expression in these kidneys. This demonstrated that, as shown previously (Allanach 	extit{et al}., 2008), qRT-PCR is more sensitive in detection of subtle changes in gene expression and is more suitable for investigating small gene expression differences.

The PRR genes most highly upregulated in response to infection by the virulent strain were Thr2 and a C-type lectin gene, dectin-2, which is involved in the recognition of 	extit{C. albicans} hyphae (McGreal 	extit{et al}., 2006; Sato 	extit{et al}., 2006). TLR2 has also been implicated in the recognition of 	extit{C. albicans} hyphae (van der Graaf 	extit{et al}., 2005) and plays an important role in 	extit{C. albicans} infection (Netea 	extit{et al}., 2002; Blasi 	extit{et al}., 2005; Gil \& Gozalbo, 2006; Murciano 	extit{et al}., 2007). Many of the cytokine/chemokine genes involved in the response to virulent strain infection have been shown to require signaling from TLR2 in combination with dectin-1 (Brown 	extit{et al}., 2003; van der Graaf 	extit{et al}., 2005; Netea 	extit{et al}., 2006b; Ferwerda 	extit{et al}., 2008). However, in this study, although dectin-1 was upregulated in response to infection by the virulent strain, dectin-2 was much more highly upregulated. Differences in the expression of C-type lectins between the different studies are most likely due to the host cells analyzed, with whole kidneys analyzed in this study and isolated immune cells analyzed in others.

One of the major questions arising from this study is why infection by the virulent strain in the kidney is not controlled when there is an obvious inflammatory response occurring. This may be due to induction of regulatory responses in the kidneys. Although TLR2 signaling can induce the production of proinflammatory mediators (macrophage inflammatory protein-2, KC (keratinocyte- derived cytokine), TNF-\(\alpha\), IL-1\(\beta\) and IFN-\(\gamma\)) (Netea 	extit{et al}., 2009).
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2002, 2004; Bellocchio et al., 2004; Villamon et al., 2004a, b; van der Graaf et al., 2005; Gil & Gozalo, 2006; Murciano et al., 2007; De Filippo et al., 2008), it can also induce IL-10 production, suppressing immune responses to C. albicans (Romani et al., 1994, 2004; Netea et al., 2004). TLR2 is also involved in controlling the expansion and function of regulatory T cells (Netea et al., 2004; Suttmuller et al., 2006), with fewer regulatory T cells found in TLR2 knockout mice (Netea et al., 2004). Therefore, increased expression of Tlr2, leading to increased levels of IL-10 and increased survival of regulatory T cells, may dampen the immune response against C. albicans, even in the presence of immune infiltrates in the kidney, and allow infection to progress.

Amplification of immune responses to infection in the kidney may also occur, as the proinflammatory cytokines TNF-α and IFN-γ have been shown to induce renal expression of Tlr2 and Tlr4 (Wolfs et al., 2002). This may lead to continued production of both proinflammatory and immunosuppressive mediators in response to fungal cells. Continued inflammatory responses, where the fungal growth is not controlled, can be regarded as inappropriate, and have previously been shown to contribute to development of sepsis (reviewed in Sriskandan & Altmann, 2008), which leads to host deterioration and eventually death in the mouse model of systemic C. albicans infection (Spellberg et al., 2005).

This study has several limitations, including study of a single time point during infection and the use of single examples of virulent and attenuated C. albicans strains; however, it does serve as a basis for future expansion in this area of research, examining the renal responses temporally during infection progression, in different mouse strains (including mouse strains with defined gene knockouts) and responses in organs other than the kidney, where infection by C. albicans is known to be controlled.

Conclusion

This study has characterized the transcriptional profiles of kidneys responding to infection by virulent and attenuated C. albicans strains. A core response was identified, which consisted of the acute-phase response, including the complement and coagulation cascades. Progressive infection initiated by the virulent C. albicans strain was characterized by a strong inflammatory transcriptional response, with massive induction of cytokine and chemokine gene expression, accompanied by an influx of immune cells into the infected kidney. The induced renal immune response did not control growth of the virulent C. albicans strain in vivo, but possibly contributes to the development of sepsis, causing host death. In the future, therapies targeting the immune response to progressive infection may be effective in treating systemic C. albicans infection.

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