Dynamics of Gene Expression in Mice Infected with Different Genotypes of *Toxoplasma gondii*

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Abstract

Background: *Toxoplasma gondii* is genetically diverse and different genotypes differ markedly in phenotype. The present study aims to define transcriptional patterns and biological processes that characterize host response to distinct strains of *T. gondii*.

Methods: We conducted a time course study of gene expression microarray in mice during acute infection (days 1 to 7) with the highly virulent type I (GT1 strain), intermediately virulent type II (PTG strain) and non-virulent type III (CTG strain) parasites.

Results: Overall, the number of genes affected increased from day 1 to day 5, and decreased on day 7. However, type III and type II infections up-regulated more genes than did type I at the very early phase, whereas type I infection up-regulated more genes at the late phase. Gene ontology (GO) analysis showed that the genes related to inflammatory and immune response were mostly affected and the majority were up-regulated, with type III infection inducing a higher degree of change and affecting more genes than did type I at the early phase. However, this pattern was reversed at the late phase. The change of expression during type II infection was between that of types I and III. Many genes associated with inflammatory and immune responses showed bimodal effects, with the first peak expression mostly at day 3 and then a second peak expression mostly at day 5. Several differentially expressed genes, including INF- γ , iNOS, CXCL10/IP-10, and numerous immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs) were previously experimentally confirmed important host factors in controlling *T. gondii* infection. Bioinformatic analysis of biological pathways enriched during infection revealed upregulation of pathways relating to cell-mediated immunity and the inflammatory response during all three infection types, though such enrichment was most expansive and pronounced during type I infection, and much less pronounced during type III infection.

Conclusions: The findings in our study revealed dynamic differences of gene expression and different pathways of immune response in mice infected with three distinct strains of *T. gondii*.

Background

Toxoplasma gondii is one of the most successful protozoan parasites infecting mammals and birds [1]. It is estimated that up to one-third of the world's human population is chronically infected with this parasite [1, 2]. Genetic analyses showed that the majority of *T. gondii* strains in Europe and North America belong to three distinct lineages, namely types I, II, and III [3, 4, 5]. Type 12, a fourth clonal lineage prevalent in wild animals from North America was recently described [6, 7]. Though genetic variation between these clonal lineages is about 1–2 % [8], the type I, II and III strains differ widely in virulence, persistence, migratory capacity, attraction of different cell types, and induction of cytokine expression [9]. Type I strains are highly virulent in mice with an LD₁₀₀ as low as a single parasite, while strains of type II and III are less virulent (LD₁₀₀>1000), and as such are able to establish chronic infections in mice readily [10, 11]. The variability of disease outcome in human infections may also be linked to the type of strains that

cause the infection. Type I strains were often found in severe acquired toxoplasmosis and congenital infection [12, 13]. Type II strains caused the majority of human cases of toxoplasmosis in North America and Europe, whereas type III strains were found largely in animals, and in general are not associated with disease [3, 14].

To understand how these distinct *T. gondii* lineages lead to different outcomes in infected host, strain-specific host responses are of interest for investigation. A previous study of macrophage cytokine production after *in vitro* infection with parasites of virulent type I and intermediate type II genotypes showed that a type II strain induced nearly 200-fold higher level of IL-12 than a type I strain after 24-h of infection [15]. Macrophages were activated through the classical pathway by type II infection, but the alternative pathway by type I and III infections [16]. The *Toxoplasma* rhoptry kinase ROP16 (type I and III alleles) was reported to be responsible for alternative activation through activating STAT6 [17]. The type II dense granule protein GRA15 is able to promote classical activation of macrophage through activating NF- κ B pathway [16]. Strain-dependent host response was also found in human foreskin fibroblasts [18, 19], chicken embryonic fibroblasts [20], human neuroepithelial cells [21], and hamster kidney cells [22]. Our previous study also showed that overall transcription levels in mice were similar in type I and type II infections, and both had greater changes than the infection with type III on day 5 post-infection [23].

Based on these studies, it is clear that there are significant differences in the host response to different *T. gondii* genotypes. However, the knowledge of host response to these different genotypes is confined to *in vitro* model and/or limited time points sampled. To provide a broad spectrum of host response to different *T. gondii* strains, we therefore conducted a time course study in mice to reveal dynamics of gene transcription, and expression networks in mice infected with different lineages of *T. gondii*. Through gene ontology and network analysis, major cause-effect relationship among a number of transcripts of immune and inflammatory responses are proposed and discussed.

Methods

Parasite strains and infection in mice

T. gondii type I strain GT1 (high virulence), type II strain PTG (intermediate virulence), and type III strain CTG (non-virulence) were propagated in confluent human foreskin fibroblast (HFF) monolayers as described previously [23]. The tachyzoites were harvested by filtering through 3- μ m-pore-size polycarbonate filters (catalog # 420400; Fisher Scientific, Hanover Park, IL) and counted with a hemocytometer. Five hundred tachyzoites were inoculated into 6–8-week old female outbred CD-1 mice (ICR-Harlan Sprague) by intraperitoneal injection. Four mice were euthanized on day 1, 2, 3, 4, 5 and 7 post infection, respectively. To collect peritoneal cells, 5 ml of ice-cold phosphate-buffered saline (PBS) was injected into the peritoneal cavity, and then peritoneal lavage was collected. The cells were collected by centrifugation at 1,000 $\times$ g for 10 min at 4 °C, resuspended in 1 ml of RNeasy lysis solution (Qiagen, Austin, TX), incubated in 4°C for 1 h, and then stored at –70°C before RNA extraction. The mouse spleens were

collected, weighed, homogenized, and homogenized in PBS to 0.1 g/ml for DNA extraction and subsequent determination of parasite burden. Four uninfected mice were used as negative controls.

Microarray analysis

Microarray analysis was performed by the method described previously [23]. In brief, total RNA from peritoneal cells was extracted using Qiagen RNeasy Plus Mini Kit (catalog #74131; Qiagen, Valencia, CA) following the manufacturer's instructions. The quality of RNA was tested by agarose gel electrophoresis, and the concentration of RNA was determined by reading the absorbance at 260/280 nm. Affymetrix Mouse Exon 1.0 ST array containing 35,556 transcripts was used. RNA samples were processed according to the Affymetrix protocol for one-cycle DNA synthesis using a Message Amp II-Biotin Enhanced Kit (P/N AM1791; Ambion, Austin, TX). Five micrograms of fragmented cRNA was hybridized to the Affymetrix GeneChip. Arrays were processed using the Affymetrix Hyb/Stain kit PN900720. Arrays were immediately scanned using the Affymetrix 7G scanner. Array images were visually inspected for anomalies. The individual chip scans were quality checked for the presence of control genes and background signal values. All microarray procedures were done at the University of Tennessee Affymetrix Core Facility.

Analysis of differential gene expression

Gene expression analysis was performed using Partek Genomics Suite software 6.6 (Partek Incorporated, Saint Louis, MO.) as described previously [23]. The raw data in the form of Affymetrix CEL files from the arrays were normalized with the robust multichip algorithm with GC content correction (GC RMA). A two-way analysis of variance (ANOVA) was used to detect genes with statistically significant expression levels between *T. gondii*-infected mice and the uninfected control mice. Gene transcripts were considered to be differentially expressed when there was at least a 2-fold change (up or down) from the uninfected controls with an ANOVA P value of ≤ 0.05 .

Analysis of biological pathways enriched during *T. gondii* infections.

In order to identify enriched biological pathways, the Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7 bioinformatics software was employed (Huang et al, Nature Protocols, 2009). This online software allows for analysis of large gene lists based on functional annotations from multiple databases. In our analysis, lists of Affymetrix exon gene IDs for significantly up- or downregulated genes ($p \leq 0.05$ based on FDR analysis) from each of the three infection types across all time points were uploaded into the DAVID functional annotation tool. Functional Annotation Clustering analysis was then performed using annotation from the KEGG PATHWAY and BioCarta databases. This analysis identified blocks of functionally related pathways that were enriched among the listed transcripts, meaning that genes belonging to these pathways were represented significantly more frequently than would be expected in a random set of *M. musculus* transcripts. Determination of pathway

relatedness was based on the number of shared genes among pathways. An enrichment score was generated for each cluster, calculated by averaging the negative logarithms of the modified Fisher Exact P-values (EASE scores) of the individual pathways in the cluster.

Microarray data validation by quantitative real-time RT-PCR (qRT-PCR)

To validate the microarray data, a few up- and down-regulated transcripts at different time points were selected for qRT-PCR analyses using the High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (catalog #4374966; Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. One microliter of RNA was transcribed into cDNA, and Quantitative PCR was performed using TaqMan Gene Expression Assays and Mouse ACTB Endogenous Control (Assay ID 4352341E; Applied Biosystems, Foster City, CA) in the iCycler thermal cycler (iQ5, Bio-Rad Laboratories, Inc. Hercules, CA). The TaqMan Gene Expression Assays consist of pre-formulated PCR primers and a FAM dye-labelled TaqMan probe for selected target transcripts. The Mouse ACTB Endogenous Control contained pre-formulated PCR primers and a VIC dye-labelled TaqMan probe of β -actin. Multiplex PCR reaction mix had a volume of 25 μ l containing 9 μ l of H₂O, 12.5 μ l of 2 $\times$ TaqMan Fast Advanced Master Mix (catalog #4444964; Applied Biosystems, Foster City, CA), 1.25 μ l of 20X TaqMan® Gene Expression Assay buffer, 1.25 μ l of ACTB Endogenous Control, and 1 μ l of cDNA. The reaction was carried out using the following cycling conditions: 50°C for 2 min, 94°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 60 sec. The comparative CT method ($\Delta\Delta$ CT) was chosen for the relative quantification of gene expression [24].

Parasite load determination by quantitative PCR (qPCR)

Parasite load in mouse spleen tissue was determined as described previously [23] with modifications. Briefly, 100 μ l of homogenized spleen tissue at a concentration of 0.1 g/ml in PBS was diluted with 100 μ l of PBS, then 50 μ l of 10 mg/ml proteinase K was added, and the mixture was incubated at 55°C for 2 h. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (catalog number 69504; Qiagen, Valencia, CA). For qPCR standard controls, the spleen of an uninfected mouse was homogenized and diluted in PBS, and *T. gondii* tachyzoites were spiked into the homogenized spleen to make a series of concentrations of 1×10^7 to 1×10^3 tachyzoites/ml. All homogenized spleen samples were adjusted to a volume of 0.1 g/ml in PBS, and genomic DNA was purified using the same procedure as detailed above. The concentration of parasites in mouse spleen was estimated by qPCR using a TaqMan probe targeting the ITS1 sequence (GenBank accession number AY143141). The primers for PCR amplification were ITS1-Fx (GAAGGGGCTCAATTTCTGG) and ITS1-Rx (TGTTCTCAGATTTGTTGTTTGA), which amplify a 117-bp sequence. The ITS1 probe was 5'-5-FAM-CGTGTCTCTGTTGGGATACTGATTTCCAGG-BHQ-1-3', with the 5' end labeled with 6-carboxyfluorescein (FAM) and the 3' end labeled with Black Hole Quencher-1 (BHQ-1) (Integrated DNA Technologies, Inc., Coralville, IA). The qPCR mixture had a total volume of 25 μ l containing 10.9 μ l of H₂O, 12.5 μ l of 2 $\times$ TaqMan Fast Advanced Master Mix (catalog number 4444964;

Applied Biosystems, Foster City, CA), 0.15 µl of 50 µM ITS1-Fx and ITS1-Rx primers, 0.3 µl of 50 µM ITS1 probe, and 1 µl of purified DNA. The reaction was carried out using the iCycler thermal cycler (iQ5, Bio-Rad Laboratories, Inc. Hercules, CA) under the following conditions: 94°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 60 sec. The CT value of each sample was compared to values of the standard controls to estimate the concentration of parasite per gram of spleen.

Results

Microarray analysis

Two to four high-quality RNA samples from mice infected with each of the three *T. gondii* strains at each of the six time points were used for microarray experiments. A total of 45 mouse peritoneal cell samples were processed and analyzed—including two samples for each strain at each time point with exception of three samples for CTG on day 5 and day 7, three for GT1 on day 7, four samples for GT1 on day 5 and four for uninfected mice. Differentially expressed genes (DEGs) were identified based on signal intensity values and fold changes compared to values from the uninfected mice (Fig. 1). For GT1 infected mice, 0, 0, 9, 75, 1830, and 1307 genes were significantly changed on days 1, 2, 3, 4, 5, and 7, respectively. For PTG infected mice, 0, 58, 71, 124 1029, and 0 genes were significantly changed on days 1, 2, 3, 4, 5 and 7 respectively. For CTG infected mice, 0, 112, 52, 111, 64 and 158 genes were significantly changed on days 1, 2, 3, 4, 5 and 7, respectively. Changes in DEG number for each strain type over the course of the experiment are shown in Fig. 1A. In both PTG and CTG infected mice, the number of DEGs increased markedly from day 1 to day 2. However, in the case of CTG, the number of DEGs then remained relatively constant through day 7, while in the case of PTG, DEGs increased again nearly ten-fold on day 5, then had dropped completely by day 7. The pattern of gene expression observed for GT1 infection was quite distinct, as no significant change was observed until day 3, followed by roughly ten-fold increases in DEGs on days 4 and 5. This peak number of DEGs was then approximately maintained through day 7. Thus, each of the three strain types elicited a unique response in terms of the temporal patterns of gene expression, with CTG and PTG eliciting an earlier response than GT1, which then remained constant for CTG while spiking on day 5 and then dropping on day 7 for PTG. GT1, on the other hand, elicited a delayed response which increased steadily to reach a peak level similar to that of PTG on day 5, but which unlike PTG was maintained through day 7. The change of up-regulated genes in all three infections followed the same trend of total number of DEGs. On day 2 and day 3, clearly there are higher numbers of genes up-regulated in PTG and CTG infection than in that of GT1 (Fig. 1B). Down regulated genes were considerably lower in all strains, but increased in GT1 and to a lesser extent PTG on day 5, followed by decreases on day 7 (Fig. 1C)

Gene Annotation Enrichment Analysis

In order to identify biological functions of the DEGs, the lists of differentially expressed transcripts and the associated fold changes at each time point in each infection group were created and uploaded to

Partek Genomic Suite software. The top 20 categories of enriched biological processes for each infection group, and their numbers of transcripts at six time points are shown in Tables S1, S2, and S3 for *T. gondii* GT1, PTG and CTG strains, respectively. Inflammatory and immune response, cell cycle, cell division, and mitosis were the functional groups mostly affected by *T. gondii* infection. However, the patterns of responses were different among the three types of infections. Because inflammatory and immune responses are closely related, we combined the two groups together for analysis (Fig. 2A). The results indicated that the number of DEGs in GT1 infection increased gradually from day 1 and reached the peak on day 5, and then decreased on day 7, whereas the number of transcripts in PTG and CTG infection peaked between day 3 and day 5, and decreased dramatically between days 5 and 7 (Fig. 2A). Analysis of functional categories revealed that most transcripts were up-regulated in inflammatory and immune responses. For the cell cycle functional group, the number of differentially expressed transcripts in GT1 infection increased gradually from day 1, reached the peak on day 5, and maintained the level on day 7 (Fig. 2B). However, for PTG and CTG infection, the number of differentially expressed transcripts increased quickly to reach peaks on day 2, and then decreased at different rates from day 3 to day 5. On day 7, the number of differentially expressed transcripts for cell cycle maintained at a medium level for CTG infection, but it almost decreased to zero in PTG infection. Similar to inflammatory and immune response, more transcripts were up-regulated than down regulated for cell cycle.

Inflammatory and immune response genes

In the GT1 infection, chemokine (C-X-C motif) ligand 10 (CXCL10/IP-10), interferon inducible GTPase 1 (ligp1/Irga6) and chemokine (C-C motif) ligand 12 (CCL12) and chitinase 3-like 3 (Chi3l3/ECF-L/Ym1) and chemokine (C-C motif) ligand 3 (CCL3) were the top five up-regulated genes, and transcript proteoglycan 4 (Prg4), chemokine (C-X-C motif) ligand 13 (CXCL13), platelet factor 4 (Pf4), selectin of platelet (Selp) and thrombospondin 1 (Thbs1) were the top five down-regulated genes (Fig. 3). In PTG infection, the top five up-regulated genes were CXCL10/IP-10, ligp1/Irga6 and Chi3l3/ECF-L/Ym1, killer cell lectin-like receptor subfamily G, member 1 (Klrg1) and interleukin 1 beta (IL-1b), and the top five down-regulated genes were CXCL13, Prg4, Pf4, CCL24 and Selp. In CTG infection, ligp1/Irga6, CXCL10/IP-10 and serine peptidase inhibitor, clade G, member 1 (Serping1), Chi3l3/ECF-L/Ym1 and immunity-related GTPase family M member 1 (Irgm1) were the top five up-regulated genes, and Prg4, Pf4, CXCL13, Selp and CCL24 the top five down-regulated genes. Among the highly up-regulated genes, ligp1/Irga6, CXCL10/IP-10 and Chi3l3/ECF-L/Ym1 are shared by all three types of infections, the time course expression of these genes is presented in Fig. 3 (A, B and C). IFN- γ and iNOS are considered important factors to control *T. gondii* infection, their time course expression is presented in Fig. 3 (D, E). Pf4, Prg4, CXCL13 and Selp were the top four down-regulated genes in inflammatory/immune response shared by the three distinct strains infection (Fig. 3F-I). Other genes responsible for pro-inflammatory response (CCL2, CCL4, CCL7, CCL12, CCL22, CXCL9, CXCL11, IL-1b, Myd88 and TNF) were highly expressed with distinct time-course expression patterns. These are also summarized in Fig. S1.

To compare the strength of inflammatory and immune responses induced by the three types of parasites, fold change in absolute values of the 102 genes were calculated and presented in Fig. 4. On day 2 post-infection, the average fold changes in the CTG group was 8.42 ± 28.70 , significantly higher ($P < 0.05$) than those in the PTG (4.54 ± 9.57) and GT1 (3.69 ± 8.59) group. No significant differences were found on day 1, day 3 and day 4 among the three groups. However, on day 5, the average fold changes in GT1 infection (23.24 ± 50.72) was significantly higher than that in the CTG (6.86 ± 13.78), and on day 7, it (15.30 ± 42.87) was significantly higher than those in CTG (5.02 ± 10.95) and PTG (3.46 ± 5.43) infection.

Immunity-related GTPase gene family upregulation

Among the highly upregulated genes, a number of representatives of the immunity-related GTPase (IRG) family were identified. In addition to *Irga6*, mentioned above, *Irgb6*, *Irgm1* and *Irgm3* were also significantly upregulated during infections with each of the three strain types (Fig. S1), though the specific temporal patterns were varied. *Irga6* was shown to be highly upregulated during CTG infection from day 2 through the end of the study, with a dramatic peak on day 2 followed by more moderate upregulation during subsequent time points. In the case of PTG infection, upregulation was maintained from days 2–4, followed by a peak on day 5 and a marked decline on day 7. For GT1 infection, upregulation declined during days 2–4, then increased on days 5 and 7. In the case of *Irgb6*, all three strains also caused early upregulation starting on day 2, though this was more marked in PTG and CTG infections than in GT1 infection. Upregulation was maintained steadily during CTG infection, increased over time for GT1 infection, and dropped to zero by day 7 for CTG infection. The regulatory IRGs *Irgm1* and *Irgm3* also displayed significant levels of upregulation. For both genes, this upregulation persisted from days 2 to 7 during CTG infection, though declining overall with time. PTG infection resulted in strong upregulation from day 2 to 5, followed by a drop to zero on day 7. GT1 caused a relatively constant level of upregulation from days 2 through 7.

In addition to the IRGs, numerous genes encoding members of the p65 guanylate-binding protein (GBP) family were found to be upregulated during infection with all three strain types including the chromosome 3 GBPs *Gbp1*, *Gbp3*, *Gbp5* and *Gbp7*, and the chromosome 5 GBPs *Gbp4*, *Gbp6*, *Gbp8* and *Gbp9* (Fig. S1). The chromosome 3 GBP genes all followed similar patterns of upregulation, showing strong upregulation on day two for CTG and PTG infections, with comparatively lower upregulation for GT1 infection. Upregulation associated with PTG and CTG infections dropped on day 3 and then rose through day 5, while levels associated with GT1 infection remained relatively constant during this period. Upregulation remained relatively high on day 7 of CTG and GT1 infections, but dropped to zero for PTG infection. For chromosome 5, *Gbp4*, *Gbp6*, *Gbp8* and *Gbp9* also all showed similar temporal patterns of expression, with upregulation observable for all strains on day 2, highest during CTG infection and lowest during GT1 infection. Upregulation then declined through day 4 for CTG infection, then increased gradually through day 7. In the case of PTG infection, upregulation was maintained relatively constantly until dropping completely or to a low level on day 7. GT1 infection, by contrast, resulted in a gradual and

somewhat steady increase in upregulation over the course of the experiment, ultimately reaching levels comparable to those generated by CTG infection.

Chemokine gene upregulation

In addition to the IRG and GBP genes, other immunity-related genes also showed significant upregulation. The chemokine gene CXCL10 was strongly upregulated on day 2 of CTG infection, then declined steadily through day 7. PTG and GT1 resulted in lower level upregulation on day 2, fluctuating and dropping to a minimal level on day 7 for PTG, and increasing steadily during the course of the experiment for GT1 (Fig. 3B). The CXCL11 gene showed a sharp rise in upregulation during PTG infection between days 1 and 3, followed by an equally sharp decline to zero between days 5 and 7. For CTG infection, only a single sharp spike on day 3 was observed, followed by a decline to zero upregulation by day 5. GT1 showed no upregulation until day 3, followed by maintenance of fairly constant upregulation for the duration of the experiment. CXCL9 showed an early spike in upregulation on day 2 of CTG infection, followed by a steady decline to a minimal level by day 5. Upregulation of this gene increased steadily from days 2 through 5 for both PTG and GT1 infections, then dropped for both strain types on day 7, completely so for PTG and only moderately for GT1 (Fig. S1). CCL2, CCL3, CCL4, CCL7, CCL8, CCL12 and CCL22 all showed similar patterns of expression (Fig. S1), with a sharp peak in expression on day 3 of CTG infection, followed by a decrease to near zero by day 7. PTG infection resulted in fairly strong upregulation between days 3 and 5, followed by a sharp decline on day 7. GT1 infection resulted in a sharp increase in upregulation from day 3 to 5 (with a moderate dip on day 4 in most cases), followed by a more gradual drop on day 7. In the case of CCL22, the increase from day 4 to 5 during GT1 infection was especially dramatic relative to the expression levels associated with infection by other strain types.

Upregulation of other immunity-related signaling molecule genes and related effectors

The IFN γ and iNOS genes both displayed similar patterns of expression (Fig. 3D-E), with weak upregulation during CTG infection peaking at day 3, somewhat stronger upregulation during PTG infection peaking at day 5, and weak upregulation during GT1 infection starting on either day 3 or 4, followed by a steady increase to high levels by day 7. TNF α also was moderately upregulated, with all three strains producing a similar increase in expression on day 3, which then dropped to zero by day 5 for CTG infection and fluctuated before dropping to zero by day 7 for PTG infection. GT1 infection produced a similar pattern to PTG infection through day 5, but dropped only slightly on day 7, so that unlike in the cases of PTG and CTG infection, upregulation was still high at the end of the experiment during GT1 infection. The gene encoding TLR signaling protein Myd88 was upregulated at low but significant levels for PTG and GT1 infection (Fig. S1U), but dropped to zero on day 7 during the former. Only a small spike in upregulation of this gene on days 3 and 4 was observed during CTG infection. The IL-1 β gene showed increases in upregulation during all infection types from days 2 through 4, followed by a decline during

CTG infection, and sharper increases in GT1 and PTG infection followed by a small decrease during GT1 infection and a very sharp decrease during PTG infection on day 7 (Fig. S1).

Other upregulated genes

Other genes were also upregulated, including the chitinase-like gene *Ym1*, which spiked on day three during CTG infection, then declined rapidly, while spiking and gradually declining on day 5 for PTG and GT1 infections, with a stronger spike in the latter than in the former case. The killer cell lectin-like receptor gene *Klrg1* showed marked upregulation between days 3 and 5 for all strain types, followed by declines on day 7 (Fig. S1). Both the strength of upregulation on the subsequent decline were considerably more pronounced during infection with PTG compared to the other strain types. Lipocalin2 gene (*Lcn2*) expression was upregulated to a low degree for all three infection types, but in GT1 infection the level increased dramatically between days 4 and 7 (Fig. S1). *Serpin1* showed moderate upregulation on days 2 and 3 during PTG infection, followed by a gradual decline to zero by day 7 (Fig. S1). GT1 infection did not produce upregulation until day 3, but this was maintained fairly consistently through day 7. CTG infection produced much stronger upregulation than the other two strains on day 2, which then dropped precipitously on day three and then rose back to the maximal level on day 5, maintaining this high upregulation through day 7. The high-affinity IgG receptor gene *Fcgr1* showed similar patterns of upregulation for all three strains in the earlier stages of infection (Fig. S1), increasing on days 2 and 3, followed by a plateau or small decline on day 4. After this point, upregulation showed a consistent but slow decline for GT1 infection, a sharp drop followed by a plateau for CTG infection, so that both GT1 and CTG infections produced similar expression levels on day 7, and a continued increase followed by a sharp decline to zero on day 7 for PTG infection. The IgG/IgE receptor gene *Fcgr4* showed stronger upregulation on day 2 for CTG and PTG infections than for GT1 infection (Fig. S1), followed by declines associated with the former strains and an increase associated with the latter, so that all three strains produced similar expression on days 3 and 4. However, while GT1 and PTG infections both showed declines from days 5 to 7, upregulation associated with CTG infection increased considerably on day 5, followed by a moderate decline that still resulted in relatively strong upregulation on day 7.

The lymphocyte antigen 6 complex, locus c1 gene (*Ly6c1*) showed a dramatic increase in upregulation on day three during infections with all three strains types, followed by declines associated with PTG and CTG infections, while upregulation during GT1 infection remained relatively constant (Fig. S1). The *Ly6* locus 1 gene (*Ly6i*) showed a similar pattern of upregulation (Fig. S1), except that upregulation was much higher overall, and during GT1 infection it increased consistently from day 3 to day 7, rather than plateauing as in the case of *Ly6c1* expression. The peptidase inhibitor *Serpinb6b* displayed a similar pattern of upregulation to *Ly6i* (Fig. S1), though like *Ly6c1*, this was also on a smaller scale. Upregulation of the IFN γ -induced Z-DNA-binding protein gene *Zbp1* followed a pattern similar to that of *Ly6c1* (Fig. S1), and on a similar scale, though upregulation dropped to zero during day 7 of PTG infection.

Downregulated genes

In addition to upregulation, many genes were also found to be downregulated during *T. gondii* infection, including platelet factor 4 (Pf4), proteoglycan 4 (Prg4) chemokine (C-X-C motif) ligand 13 (CXCL13), platelet selectin (Selp), arachidonate–15 lipoxygenase (Alox15), apolipoprotein E (ApoE) and thrombospondin 1 (Thbs1). These patterns of downregulation are displayed in figure 3F-I and figure S1AA, AB, AI. Expression of these genes showed a general trend of strong downregulation on day 3 of infection with each strain type, followed by a return to control or near-control levels of expression on day 5 for CTG infections and day 7 for PTG infections. During GT1 infections, downregulation of these genes generally fluctuated between days 4 and 7, though always ending up with significant downregulation on day 7. In the case of Prg4, severity of downregulation continued to increase from day 4 to 7, resulting in a nearly 275-fold downregulation by the end of the observation period.

Annotated pathway enrichment analysis

Bioinformatic analysis using the DAVID software revealed that sets of up- and downregulated genes associated with infection by each of the three *T. gondii* strains were enriched for genes associated with markedly different biological pathways.

CTG infection was associated with enrichment of the smallest number of pathways annotated in the KEGG PATHWAY and BioCarta databases. These comprised clusters of genes involved in cell-mediated immunity and inflammation (Including MHC Class I genes, perforin 1, IFN γ , TNF α and IL–1) and cell cycle regulation (cyclins and cyclin kinase inhibitors).

For PTG infection, 7 enriched clusters were identified. All of these comprised cell-mediated immunity-related pathways such as those relating to CD4 $^{+}$ T Cell activation and Th1 differentiation (IL–12, IL–12 receptor, CD3, IFN γ , IFN γ receptor, TNF α), proinflammatory cytokine production and cytotoxicity (IFN γ , TNF α , IL–10, CCL3, CCL4, IL–1, granzyme B, Nos2), T-Cell and other leukocyte signaling and surface molecules (selectins, ICAM–1, Lck, Zap70) and apoptosis (Fas, FasL, Caspase 7, I κ Ba).

GT1 infection was associated with enrichment of the largest number of identified clusters, 15 in all. These included many immunity-related gene clusters similar to those enriched during PTG and CTG infection, but in general the number of DEGs per cluster was higher, and comprised a greater diversity of pathways. These included immune pathways relating to CD4 $^{+}$ T-Cell differentiation and proliferation (CD28, CD4, CD3, IL–2, IL–12, IL–12 receptor, IL–18 receptor, IL–4 receptor, CXCR3, colony-stimulating factor 2), inflammation (CCL3, CCL4, CCR2, CCR5, IFN γ , IFN γ receptor, iNOS2), apoptotic pathways (fas, fasL, fas death domain associated protein (DAXX), MAPKAPK2, caspase 3, 7, cytochrome C, TNF α , TNF α receptor, mapk13, I κ Ba, PKC), the complement pathway (complement component 1, 6, C1, C3 prepropeptide, complement component factor H, factor B, complement receptor 2) and T Cell signaling (CD3, CD4, CD8, CD28, IL–6, fas, fasL, fyn proto oncogene, lymphocyte protein tyrosine kinase, zap kinase, ctl associated protein4, inducible T-cell co-stimulator). Pathway clusters not directly related to the

immune response were also enriched, including those associated with DNA replication and damage response (dna primase p49 subunit, DNA replication helicase 2 homologue (yeast), ATP-dependent DNA ligase I, DNA polymerase subunits, replication factors, exonuclease I, checkpoint kinase 1 homologue (*S. pombe*), polo-like kinase, Brca1), carbohydrate metabolism (galactose, starch, sucrose, amino sugar and nucleotide sugar metabolic enzymes), muscle functioning and Calcium channel regulation (voltage dependent calcium channel subunits, insulin-like growth factor 1, integrins, AAPK gamma2 subunit, cardiac ryanodine receptor, solute carrier family proteins, TGFb, actinin A1, gap junction protein alpha 1, junction plakoglobin, cardiac RyR2), cell-cycle regulation (cell division cycle protein homologues (*S. pombe*), cyclins, CDPK6, P21 CDPK inhibitor, dihydrofolate reductase, IkbA), transcriptional regulation and cellular signaling (calmodulin, jun oncogene, phospholipase C, PKC, vav1 oncogene, endothelin1, Fyn protooncogene, STAT1, Igf1, inositol monophosphatase, phosphoinositide-3-kinase, neuregulin 4, jun oncogene, MyD88). This latter category also included genes related to B-cell response and humoral immunity (B-Cell Linker protein, CD79B, high affinity Ige Fc receptor (alpha polypeptide), membrane spanning 4 domains subfamily A2).

Microarray data validation by quantitative real-time RT-PCR (qRT-PCR)

To validate the microarray data, four up-regulated transcripts (IFN- γ , iNOS, IL-12 β and TNF) and one down-regulated gene (CXCL13) at five time points (days 1, 2, 3, 4, and 5) were used for qRT-PCR analysis. RNA samples from day 7 were accidentally lost during storage and could not be included in analysis. TaqMan Gene Expression Assays (Applied Biosystems) were applied for IFN- γ (assay ID: Mm01168134_m1), iNOS (Mm00440502_m1), IL-12 β (Mm00434174_m1), TNF (Mm00443260_g1), and CXCL13 (Mm04214185_s1). As shown in Fig.5, the qRT-PCR expression data of five transcripts at five time points revealed a good agreement with the microarray data ($R^2 = 0.7964$).

Determination of parasite load by qPCR

Parasite loads in the spleens of mice infected with 500 tachyzoites on day 1, 2, 3, 4 and 5 post-infection were quantified. Since the parasite was not detectable in some samples on day 1, the data generated from day 1 were excluded in the following analysis. Parasites load on day 2 through day 5 in three infection groups are displayed in Fig.6. No significant differences in parasite loads were observed from day 2 to day 4 among the three groups; however, the parasite loads on day 5 in the GT1 group was significantly higher than that in the PTG and CTG groups. The mean value of the parasite loads in GT1, PTG and CTG were 2.05×10^7 , 4.89×10^6 , and 3.63×10^6 parasites per gram of spleen, respectively.

Discussion

The purpose of this study was to reveal how the host responds to infection by distinct *T. gondii* strains using the murine model. Our results showed that the host responses were dynamic and different among the three *T. gondii* strains tested. At the early stage of acute infection (i.e., from day 1 through day 3), the

number of DEGs in the type I strain GT1 infection was lower than those of the type II PTG and type III CTG infections. However, by day 4, all three strain types showed a comparable number of DEGs. While DEGs associated with CTG infection then reached a plateau, the numbers associated with GT1 and PTG continued to increase on day 5 to an approximately equal level, which is maintained by GT1 infection through day 7, while dropping to zero for PTG infection at this point. The dynamic changes are more prominent in the up-regulated genes (Fig. 1B). Due to the lack of data in day 6, it is not clear whether the number of DEGs associated with PTG infection started to decline on day 5 or day 6, but likely peaked between day 5 and day 6. For this strain type, it seems that the period of day 5 to day 7 is an important window when the host response underwent drastic changes to *T. gondii* infection. These changes may be the consequence of negative feedback of host responses to dampen detrimental responses of *T. gondii* infection. The degree of expression and composition of genes affected may play a key role in determining different outcomes of infection with three distinct strains.

Though a wide range of functional groups were found to be significantly changed by *T. gondii* infection, inflammatory and immune response (Tables S4, S5, and S6), and cell cycle (Tables S7, S8, and S9) are the major groups affected (Fig.2). Regarding inflammatory and immune response, the number of genes affected follow the same trend for all genes counted (Figure 1A and Figure 2A). However, the majority of genes for inflammatory and immune response are up-regulated from day 1 to day 7 (Tables S4, S5 and S6). GT1 strain infection induced a remarkable up-expression of the inflammation and immune response associated genes compared with PTG and CTG strains infection on day 7 (Fig. 2A). Clinically, the mice infected with the GT1 strain had a significantly higher parasite load than that of PTG and CTG strains on day 5 (Fig. 6), and the animals started to show severe symptoms of infection on day 7, while those infected by the type II or III did not have obvious symptoms of disease. In accordance with our previous data [23], many genes such as CCL2, CCL3, CCL4, CCL7, CCL8, CCL12, CCL22, CXCL10, CXCL11, Myd88 and TNF, were highly expressed in GT1 infection (Fig. S1) at the later stage of acute infection, which may contribute to a lethal inflammatory response to this highly virulent strain.

To identify the intensity of inflammatory and immune response induced by the three types of parasites for each day, we calculated the average fold changes of the 102 DEGs (Fig. 4) and found that CTG induced significantly stronger immune response at the early stage (day 2) than GT1 and PTG. This response in CTG infection coincided with a higher number of genes affected in inflammatory and immune response categories (Fig. 2A) and a high number of genes up-regulated in cell division (Fig. 2B). These early responses to CTG infection may be of importance to alert the host immune response, which can control parasite infection and prevent over-reaction of the inflammatory response, dampening immunopathology in the host.

Many of the genes of inflammatory and immune response, such as *ligp1/Irga6*, *Irgm1*, *CXCL10/IP-10*, *INF-γ*, *iNOS*, *CXCL13*, *CCL2*, *CCL3*, *CCL7*, *CCL8*, *CCL12*, *CCL22*, *CXCL11*, *IL-1b*, *Myd88*, and *TNF*, showed bimodal responses with the first peak expression at day 3 (except *ligp1/Irga6* at day 2), and then a second peak expression mostly at day 5. These responses may reflect oscillations which are common in the immune system [25]. In addition to bimodal responses, expression levels of these genes vary widely

among the infections of the three different *T. gondii* strains (Fig. 3 and Fig. S1), which may contribute to different outcome in infected hosts.

One of the genes found to be most highly upregulated during infections with all three strain types was *ligp1/lrga6*, a member of the interferon-inducible immunity-related GTPases (IRG proteins/p47 GTPases), which are key factors in murine defense against *T. gondii* infection [26, 27, 28, 29, 30]. In INF- γ -stimulated cells, the IRGs have been found to accumulate on *T. gondii* parasitophorous vacuole membranes (PVM) in a stepwise manner, resulting in constriction and ultimate disruption of the PVM, followed by parasite clearance. *Mus musculus* expresses at least 19 of these genes, which are clustered on chromosomes 11 and 18. Several other members of this class were also found to be highly upregulated in this study, including *lrgb6*, *lrgm1* and *lrgm3*. *lrgb6* and *lrga6* have been identified as two of the earliest IRGs recruited to the PVM, and their presence is also necessary for localization of subsequent IRGs, making them highly important to this defense mechanism. *lrgm1* and *lrgm3* also play a key role by associating with host intracellular membranes, specifically those of the lysosomes and the golgi apparatus in the case of *lrgm1* and the endoplasmic reticulum in the case of *lrgm3*, protecting them from disruption by effector IRGs like *lrga6* and *lrgb6* and thus preventing deleterious autoimmune effects. Deletion of either *lrgm1* or *lrgm3* has previously been shown to result in strongly increased susceptibility of mice to *T. gondii* infection [31]. Parasite defense against the IRG immune response involves the virulence factors ROP18 and ROP5, which in Type I strains inactivate IRG proteins in a collaborative manner. During infection by virulent *T. gondii* strains including Type I strains such as GT1, the rhoptry kinase ROP18 is secreted into the host cytoplasm where, with catalytic activity enabled by interaction with the rhoptry pseudokinase ROP5, it phosphorylates IRGs at specific conserved threonine residues, thereby preventing their colonization of the PVM. Type III strains such as CTG do not express ROP18 and thus are unable to prevent parasite clearance in this manner, while Type II strains such as PTG lack the proper allele or alleles of ROP5 needed to enhance the kinase activity of ROP18, and thus are also deficient in protecting against clearance [32]. In the current experiment, both effector and regulatory IRGs showed similar patterns of expression, with upregulation occurring early (day 2) in the course of PTG and CTG expression and later (days 3 through 5) during GT1 infection. Then upregulation was found to diminish to insignificant levels between days 5 and 7 in PTG infection while being maintained through day 7 for CTG and GT1 infections.

In addition to the IRG proteins, *Mus musculus* also expresses a second family of IFN γ -activated GTPases known as the p65 guanylate-binding proteins (GBPs), the 11 active genes encoding which are clustered in tandem on chromosomes 3 and 5 [33]. Loss of the chromosome 3 GBPs has been shown to dramatically increase susceptibility of mice to *T. gondii* [34, 35]. Like the IRGs, GBPs also accumulate on the PVM shortly after invasion by *T. gondii*, an action which is inhibited by virulent strains of the parasite. Unlike the IRGs, which cause direct disruption of the PVM, GBPs act by recruiting protein complexes to the membrane, such as the inflammasome, autophagy apparatus, and NOX2 NADPH oxidase, which have antimicrobial functions [35, 36, 37, 38, 39]. In the current study, the majority of known GBPs from both the chromosome 3 and 5 clusters were found to be upregulated during infection with all three strain types. It is noteworthy that all of the GBPs clustered on chromosome 5 followed essentially the same pattern of

expression as was observed for the IRGs, whereas the chromosome 3 GBPs were only found to be upregulated starting at day 5 for all strain types, with the exception of Gbp1, a chromosome 3 GBP that followed the chromosome 5 expression pattern closely. Thus far, only the contribution of chromosome 3 GBPs to defense against *T. gondii* infection has been assessed experimentally, so that the importance of the chromosome 5 GBPs in this regard is not certain. The data presented here would appear to suggest a more central role for the chromosome 5-encoded GBPs in the immune response to *T. gondii* than their chromosome 3 counterparts, underscoring the necessity of more thorough investigation into this potentially important group of proteins.

It is likely that these differential patterns of IRG and GBP expression reflect the varied pathogenic strategies of the Type I, II and III lineages. By avoiding the IRG defense system in early-infected host cells, Type I strains may be able to replicate more freely within PVs, without the strong activation of the inflammatory immune response resulting from the PVM disruption, parasite clearance and programmed host cell death associated with the earlier stages of Type II and III infections. Thus, it is not until the later stages of acute infection, when host cells are lysed by replicating tachyzoites and the parasite burden begins to expand exponentially as subsequent rounds of infection occur, that strong activation of the host immune response occurs. IFN- γ itself was not found to be strongly upregulated during the early stages of any infection type, but the upregulation of many IFN- γ -responsive genes suggests functionally significant increases in the activity of this cytokine. The reduced upregulation of IRGs and GBPs observed towards the end of the experimental period for PTG infections in contrast to CTG and GT1 infections may be related to unique mechanisms of host gene regulation employed by Type II strains. For example, Type II strains are known to express different alleles of at least two important regulators of host transcription, ROP16 and GRA15, which act on the JAK-STAT3/6 and NF- κ B pathways, respectively, and have been shown to alter the expression of a number of host cytokines during infection. More investigation into these possible mechanisms is of interest.

In addition to Irga6, CXCL10/IP-10 was another of the most highly upregulated immunity-related genes in all three infection types. A similar expression pattern was observed for CXCL10/IP-10 as for the IRG genes. On day 2 post infection of CTG strain, its expression level was 6-fold higher than that in the GT1 infection, and 3-fold higher than that in the PTG infection (Fig. 3B). CXCL10/IP-10 is an IFN- γ -induced chemokine with potent chemoattractant properties that acts on activated T cells, NK cells, monocytes, and neutrophils [40]. Previous work has demonstrated that the blockade of CXCL10/IP-10 during acute toxoplasmosis prevented T-cell trafficking to liver and spleen, led to high parasite burdens in the CNS, and caused mice to succumb to infection [41]. Recent works have also found that this chemokine was required to maintain T cell-mediated resistance during chronic ocular toxoplasmosis and toxoplasmic encephalitis [42, 43]. Taken together, high expression of Irga6 and CXCL10/IP-10 at the early stage of CTG infection may help host control parasite growth and minimize inflammatory responses. In contrast, the number of DEGs of inflammatory and immune response to GT1 infection lagged behind from day 1 to day 4, but exceeded PTG and CTG infection on day 5 and maintained a relatively high level on day 7 (Fig. 2A), this coincide with the significantly higher parasite burden in GT1 infection on day 5 and severe symptom of infection in mice on day 7. Combined with the different parasite loads found in the spleen of

three infection groups (Fig. 6), the data show the high level of *ligp1* in the CTG infection, especially at early stage of infection, playing an important role in the control of parasite growth, and therefore resulting in a low parasite burden.

INF- γ and iNOS are well documented for their roles in controlling *T. gondii* infection. In this study, the transcription level of IFN- γ increased continuously from day 2 to day 7 in GT1 infection, while it started to decline in the PTG and CTG infection groups on day 5. INF- γ in the GT1 infection group was about 8 and 14-fold higher than that in the PTG group and CTG group, respectively (Fig. 3D). INF- γ is considered the most critical mediator of immunity against *T. gondii* in early infection, and it is also an important immunopathogenic mediator of toxoplasmosis [44, 45, 46]. Similar expression trend was also found in another important immunopathogenic mediator, inducible nitric oxide synthase (iNOS) (Fig. 3E).

Pf4, Prg4, CXCL13 and Selp were the top four down-regulated genes in inflammatory/immune response shared by the three distinct strains infection. They were all largely down-regulated as early as day 3 post-infection in three infection groups; however, at the late stage of acute infection, the expression level was in a strain-specific manner, much lower in highly virulent strain (GT1) than those in the intermediately virulent strain (PTG) and non-virulent strain (CTG) (Fig. 3F to 3I). Since Pf4 was reported to be released from [alpha-granules](#) of activated [platelets](#) that could fight some parasite infections [47, 48, 49], the data imply that platelet may play an important role in host defense to the infections with different *T. gondii* genotype; however, the detailed mechanism needs to be further studied. The roles of three other down-regulated genes (Prg4, CXCL13 and Selp) in *T. gondii* infection also need to be identified in the future study.

Consistent with our previous data, type I and type II induced higher number of DEGs than type III did on day 5 post-infection [23]. A previous study have showed that ROP38, a member of *T. gondii* rhoptry kinase (ROPK) localizing to the rhoptries and parasitophorous vacuole, was highly expressed in the type III strain VEG with up to 64 times higher expression than in RH (type I), and this kinase was responsible for the relatively lower transcriptional changes in type III in the infected cells [50]. To test if high expression of ROP38 in CTG results in fewer DEGs in the mice infected with CTG, transgenic parasites that lack ROP38 expression should be employed in the future study.

Finally, to better understand the broader patterns of gene expression and their implications for the pathogenic strategies of the different *T. gondii* strains, we used DAVID bioinformatic analysis to identify biological pathways that were enriched during infection with each strain type. This analysis revealed stark differences between each of the responses, both in regard to the number and types of pathways affected. CTG infection resulted in enrichment of the fewest pathways, with only two clusters identified, relating to cell-mediated immunity and cell cycle regulation. The cell-mediated immunity cluster comprised four individual pathways. This relatively low pathway enrichment is consistent with the expression of fewer genes being altered overall by CTG infection compared to the other two strains. PTG infection resulted in substantially more pathways being enriched, 7 in total and all relating directly to the immune response. The pathways affected were mainly related to the T-Cell function, and more frequently

the Th1 response. Several apoptotic pathways were also enriched, suggesting an important role for this process, possibly mediated by CTL activity, in the control of PTG infection by mice. For GT1 infection, more than twice as many enriched pathway clusters were identified compared to PTG infection. This included 15 clusters total, with 8 being directly associated with the immune response. The immunity related clusters included pathways relating to T-cell activation, differentiation and function, underscoring the importance of this cell type for defense against *T. gondii*. Several pathways relating specifically to the Th1 response were also enriched, further supporting a major role for cell-mediated immunity and inflammation as opposed to humoral immunity in combating *T. gondii* infection. A few pathways relating to B-cell function were also among those upregulated, but the individual genes involved were not unique to these particular pathways, and so it is unclear whether their upregulation is actually a reflection of an enrichment of the B cell response. Even more extensively than for PTG infection, apoptotic pathways were notably enriched during GT1 infection. These included genes and pathways involved in Fas signaling, the caspase cascade, and apoptosis triggered by mitochondrial damage. The strong enrichment of apoptotic pathways in response to both PTG and GT1 infections suggests strong importance as a mechanism of defense against *T. gondii* infection. That such enrichment is considerably more pronounced during GT1 infection may be simply reflective of greater overall changes in gene expression during infection with this strain type or may be indicative of activation of unique defense mechanisms in response to the alternative pathogenic strategies of Type I strains. Other pathway clusters that were not significantly affected by PTG infection were enriched during GT1 infection, including those relating to the complement system. The upregulation of numerous complement components and related genes during GT1 infection may indicate a greater importance of this defense mechanism to Type I infections compared to the other types. Other unique pathway clusters enriched during GT1 infection included those relating to stress responses, including the NF- κ B/AP-1 stress response and multiple clusters relating to DNA damage repair. The enrichment of these clusters may reflect the greater parasite burden and corresponding increase in tissue damage towards the end of the acute phase of infection. While Type II and III infections are generally being suppressed at this point, Type I infections are most often still proceeding unchecked, resulting in cell death and tissue damage, and as such it is not surprising that GT1 infection would lead to upregulation of pathways aimed at controlling such damage and responding to the associated stresses. Other clusters enriched during GT1 infection, such as those involved in muscle function and Ca²⁺ channel regulation, as well as numerous and varied signal transduction and transcriptional regulation pathways, may also be a result of the host responding to the rampant proliferation of the parasite and damage to host tissues. It is interesting to note that clusters relating to cell cycle regulation were enriched during both GT1 and CTG infection, but not during PTG infection. This may again be a reflection of the relative genetic distance of type II strains compared to types I and III, which manifests in part as unique alleles of a number of effector proteins secreted into host cells, resulting in unique patterns of host transcriptional activation and suppression. Overall, we see the greatest concerted pathway activation during type I infection, followed by type II and then type III, which corresponds to the severity of disease caused by each of these infection types based on previous observations. The immunity related clusters enriched during each infection tend to be centered around the Th1, cell-mediated and inflammatory response rather than the humoral response. Apoptosis also

appears to be a primary reaction to type I and II infections, possibly as part of CTL-mediated killing. Other responses are also induced during type I infection, including the complement activation and multiple stress-response pathways.

Conclusions

In summary, using a mouse model and cDNA microarray technology, we found that mice infected with type I, II and III strains of *T. gondii* displayed distinct gene expression profiles. In late stage of acute infection, the differentially expressed genes and affected function groups had a remarkable difference between the type I infection and the type II / III infection, which may partly explain the different outcomes after infection with three distinct lineages of *T. gondii*. We also identified several potential target genes that may be critical in regulating the inflammatory and immune response to type I and type II infections. The results here present an overall picture of host response to three distinct lineages of *T. gondii*. Future experiments, such as using gene-knockout mice, are needed to validate the roles of the candidate regulatory genes in the pathology of toxoplasmosis caused by distinct *T. gondii* strains.

List Of Abbreviations

Alox5	arachidonate 5-lipoxygenase
CCL	chemokine (C-C motif) ligand
Chi313	chitinase 3-like 3, also known as ECF-L and Ym1
Clec4e	C-type lectin domain family 4, member e
Colec12	collectin sub-family member 12
Ctla4	cytotoxic T-lymphocyte-associated protein 4
CXCL	chemokine (C-X-C motif) ligand
C1qb	complement component 1, q subcomponent, beta polypeptide
C1ra	complement component 1, r subcomponent A
C1rb	complement component 1, r subcomponent B
C4b	complement component 4B (Childo blood group)
Hfe	Hemochromatosis
IL-6	interleukin 6
IFN-γ	interferon gamma
Iigp1	interferon inducible GTPase 1, also known as Irga6
iNOS	inducible nitric oxide synthase
Lcn2	lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL)
Lbp	lipopolysaccharide binding protein
Marco	macrophage receptor with collagenous structure
Ms4a2	membrane-spanning 4-domains, subfamily A, member 2
Myd88	myeloid differentiation primary response gene 88
Olr1	oxidized low density lipoprotein (lectin-like) receptor 1
Pf4	platelet factor 4
Prg4	proteoglycan 4
Selp	selectin, platelet
TNF	tumor necrosis factor
TLR	toll-like receptor
Thbs1	thrombospondin 1
Tnfsf	tumor necrosis factor (ligand) superfamily

Declarations

Ethics approval and consent to participate

The use of mice for this study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Knoxville.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CS designed the work; LY, KS and RH collected the data; LY, KS, RH and JG analyzed the data; LY and KS drafted the article; CS, XZ and JS did critical revision of the article. All authors contributed to discussion of the results followed by writing read and approved the final manuscript.

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Figures

Figure 1

Differentially expressed genes (DGEs) at six time points. (A) Total number of DEGs ; (B) The number of up-regulated genes; (C) The number of down-regulated genes.

Figure 2

The number of DEGs in inflammatory and immune response, and cell cycle. A: inflammatory and immune response; B: cell cycle.

Figure 3

Time-course expression of *ligp1/lrga6* (A), *CXCL10/IP-10* (B), *Chi3l3/ECF-L/Ym1* (C), *IFN- γ* (D), *iNOS* (E), *Pf4* (F), *Prg4* (G), *CXCL13* (H), and *Selp* (I).

Figure 4

Comparison of fold changes of 102 DEGs involved in inflammatory and immune response at days 1, 2, 3, 4, 5, and 7 past infection. Absolute values of both positive and negative fold changes were used for the calculation of average fold change. Mann-Whitney U Test was used for the statistical analysis. CTG and PTG infections induced significantly higher expression change than that of GT1 on day 2. GT1 infection induced significantly higher change than that of CTG on day 5, and than both PTG and CTG on day 7.

Figure 5

Comparison of microarray and qRT-PCR measurements of gene expression levels. The scatter plot compares the mean expression level of five genes in mice infected with *T. gondii* GT1, PTG and CTG strains. Each point represents the average signal strength for one infection group at a time point with microarray (y axis) and qRT-PCR (x axis).

Figure 6

Parasite burdens in the spleen tissues of mice. Parasite load in the spleen of mice on day 2, 3, 4 and 5 post infection was determined by qPCR. GT1 has a significantly higher parasite burden than PTG and CTG at day 5 (*, $P = 0.004$, by ANOVA).

Supplementary Files

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