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ORIGINAL PAPER

Transcriptomic analysis of global changes in cytokine expression in mouse spleens following acute *Toxoplasma gondii* infection

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Abstract *Toxoplasma gondii* is a global pathogen that infects a wide range of animals and humans. During *T. gondii* infection, the spleen plays an important role in coordinating the adaptive and innate immune responses. However, there is little information regarding the changes in global gene expression within the spleen following *T. gondii* infection. To address this gap in knowledge, we examined the transcriptome of the mouse spleen following *T. gondii* infection. We observed differential expression of 2310 transcripts under these conditions. Analysis of KEGG and GO enrichment indicated that *T. gondii* alters multiple immune signaling cascades. Most of differentially expressed GO terms and pathways were downregulated, while immune-related GO terms and pathways were upregulated with response to *T. gondii* infection in mouse spleen. Most cytokines were upregulated in infected spleens, and all differentially expressed chemokines were upregulated which enhanced the immune cells chemotaxis to promote recruitment of immune cells, such as neutrophils, eosinophils, monocytes, dendritic cells, macrophages, NK cells, basophils, B cells, and T cells. Although IFN-γ-induced IDO (Ido1) was upregulated in the present study, it may not contribute a lot to the control of *T. gondii* because most differentially expressed genes involved in tryptophan metabolism pathway were downregulated. Innate immunity pathways, including cytosolic nucleic acid sensing pathway and C-type lectins-Syk-Card9 signaling pathways, were upregulated. We believe our study is the first comprehensive attempt to define the host transcriptional response to *T. gondii* infection in the mouse spleen.

Keywords *Toxoplasmas gondii* · Spleen · Cytokines · Transcriptome · RNA-seq

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Introduction

Toxoplasma gondii is a widespread parasite that can infect all warm-blooded vertebrates, including pigs, dogs, birds, and cats. Cats are very common human pets and are the definitive hosts of *T. gondii*. This presents a significant health risk, as one infected cat can shed millions of chemical-resistant *T. gondii* oocysts. The broad host range and strong chemical resistance of the *T. gondii* oocyst render it an important zoonotic pathogen that threatens human health on a global scale. Previous reports showed that the global *T. gondii* seropositivity rates in humans range from 0 to over 90 % (Pappas et al. 2009; Tolba et al. 2014). Clinical signs of *T. gondii* infection range from fever to severe complications, such as severe fever in infants, abortion or stillbirth in pregnant women, and death

in immunodeficient patients (especially in the case of human acquired immunodeficiency syndrome). The host immune system and immune factors (e.g., cytokines) play critical roles in the response to *T. gondii* infection. At the time of writing, several immune factors involved in controlling *T. gondii* infection or host immunopathology have been researched. Such as IL-10 (Gazzinelli et al. 1996), IL-6 (Mirpuri and Yarovinsky 2012), CCL3 (Schulthess et al. 2012), PD-L1 (Wu et al. 2014), CXCL9 (Ochiai et al. 2015), IL-33 and ST2 (Tong and Lu 2015), IL-12 and IFN-γ (Yarovinsky 2014).

IFN-γ is a highly pleiotropic cytokine that controls *T. gondii* through upregulation of indoleamine 2,3-dioxygenase (Ido1), inducible nitric oxide synthase (iNOS), interferon inducible immunity-related GTPases (IRGs), and guanylate-binding proteins (GBPs) (Yarovinsky 2014). After *T. gondii* invades the host cell, it builds a parasitophorous vacuole (PV) in order to avoid some components of the host immune system. However, some IFN-γ-induced IRGs and GBPs, such as Irgm1, Irgm3, Gbp1, and Gbp2, are able to recognize the PV and induce its disruption (Haldar et al. 2013). *T. gondii* is a tryptophan and arginine auxotroph (Laliberte and Carruthers 2008). IFN-γ induces Ido1 converts tryptophan into N-formylkynurenine and iNOS using L-arginine as substrate which produces nitric oxide (NO) and also causes arginine downregulation (Kang et al. 2013). In addition, NO has potent microbicidal effects on *T. gondii* (Chao et al. 1993). Due to multiple effects, IFN-γ is therefore an important player in *T. gondii* control. IFN-γ production can be initiated by Toll-like receptor (TLR) signaling and the IL-12 pathway (Yarovinsky 2014).

However, the type I strain of *T. gondii* has evolved to manipulate IFN-γ production by inducing lower IL-12 expression comparing with type II strain (Robben et al. 2004). For now, we have known that rhoptry protein 16 (ROP16) of type I *T. gondii* can be localized in the host cell nucleus and activate Stat3 to reduce production of IL-6 and IL-12 (Butcher et al. 2011). ROP18 of type I *T. gondii* also modulates the host immune response through phosphorylating ATF6β, which triggers the ATF6β proteasomal degradation. ATF6β is an endoplasmic reticulum (ER)-localized transcription factor that regulates gene expression once activated by ER stress responses. The interaction between ROP18 and ATF6β can disturb the production of ER stress-responsive genes such as IFN-γ (Yamamoto et al. 2011; Yamamoto and Takeda 2012). In addition, ROP18 of type I *T. gondii* can also mediate degradation of NF-κB (Du et al. 2014) and phosphorylating IRGs and GBPs to avoid PV disruption as described previously (Haldar et al. 2013). Thus, molecular markers of pathway activation may not always correlate with changes in the expression of all downstream genes, and transcriptomic analyses to detect more nuanced variations in gene expression are required.

Previously, technical bottlenecks and research costs restricted many research groups to the study of a handful of genes or selected pathways (Aguilar et al. 2001; Fischer et al. 1997; Mun et al. 2005). With the advent of RNA sequencing, infectious disease researchers now have a high throughput and low cost resource for the study of global gene expression changes. RNA sequencing has been applied to study the immune response in the mouse brain and macrophages (Melo et al. 2013; Tanaka et al. 2013). However, no similar report on the mouse spleen, which plays important roles in the regulation of the crosstalk between innate and adaptive immunity (Bronte and Pittet 2013), has been published. In addition, over immune response in acute *T. gondii* infection usually are lethal and negative regulation is very important for host survival (Gazzinelli et al. 1996). Studying the spleen response to *T. gondii* infection will provide more details that intersecting the innate and adaptive immunity, furthering the understanding of how *T. gondii* manipulates the host immune system and, conversely, how the host eventually reacts in order to avoid over immune response for its survival. To our knowledge, this is the first report in which RNA sequencing has been applied to describe the global gene expression profiles in the mouse spleen before and after *T. gondii* infection.

Materials and methods

Ethical statement

All animals were handled strictly according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was reviewed and approved by the Animal Ethics Committee of Lanzhou Veterinary Study Institute, Chinese Academy of Agricultural Sciences.

Parasites and mice

The RH strain of *T. gondii* was maintained in our own laboratory and cryopreserved in liquid nitrogen. RH strain activity and virulence were recovered by passaging three generations in Kunming mice before use. All female Kunming mice were purchased from Laboratory Animal Center, Lanzhou University (Lanzhou, China). Twelve Kunming mice were randomly divided into two groups (six in the infected group and six in the non-infected group). Each mouse of the infected group was peritoneally infected with 200 tachyzoites. Six days post-infection, all twelve mice spleens were individually harvested within 3 min post-euthanasia, rinsed extensively in saline, and then frozen immediately in liquid nitrogen.

Table 1 Primers used for Q-PCR validation

Primer name	Primer sequence (5′ to 3′)	Length of Q-PCR products (bp)
Igtp-F1	ACATCTGGGCCAACATAGAT	209
Igtp-R1	TAGGCCTTGCACTCTTTACT	
Gbp2b-F	AAATGGCCTCAGAAATCCAC	107
Gbp2b-R1	TTGGATAGCAGACAGGATGT	
Tlr11-F1	TCTTAGGCATACAACTTCAGG	110
Tlr11-R1	TTCTAAGCTAAGGAGTGTTGG	
Nfkb1-F1	ACTCGAACTACGGATTTCCT	137
Nfkb1-R1	GTCATCACTCTTGGCACAAT	
IL12-α-F1	GCCGGCTATCCAGACAATT	119
IL12-α-R1	AGGTAGCGTGATTGACACAT	
IL-12-β-F1	CAGCAAAGCAAGGTAAGTTC	330
IL-12-β-R1	GAGCCTATGACTCCATGTCT	
Cat-F1	GCAGTGATTTCACATAGGAT	131
Cat-R1	GAGAGCTGGTAATCTCTACT	
Rnh1-F1	AGTCAGGCTCAAAGAAGTG	110
Rnh1-R1	CCTTTCACTTTGCCTACTGG	
Maoa-F1	AGATTTCTAAGCCTACCTGT	139
Maoa-R1	GAGCCCTAATTTCATTCTGT	
Tap1-F1	GGCAGAAGTTGGAAGAAATG	186
Tap1-R1	GAAGCTGGTAGAGAACGAAT	
IFN-γ-F1	CCTGAAAGAAAGCAGTGTCT	85
IFN-γ-R1	TTTGTCATTCGGGTGTAGTC	
IL-1β-F1	ACAGTGATGAGAATGACCTG	332
IL-1β-R1	GTAGTGCAGTTGTCTAATGG	
β-actin-F1	GCTTCTAGGCGGACTGTTAC	100
β-actin-R1	CCATGCCAATGTTGTCTCTT	

T. gondii infection of the spleen was confirmed by PCR as previous report described (Jiang et al. 2013).

Transcriptome sequencing and read alignment to the reference mouse genome (mm10)

Total RNA was prepared individually from the cryopreserved mice spleen samples using TRIzol Reagent according to the manufacturer's protocol (Invitrogen Co. Ltd). All RNA samples were treated 20 units of RQ1 RNase-Free DNase (Promega) to remove residual genomic DNA according to the manufacturer's protocol. Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and Nanodrop 2000 (Thermo Scientific, Wilmington, DE) were used for integrity and quantification and purity of all RNA samples, respectively. After the test as described above, all good RNA templates (28 s/18 s≥1.5, RNA integrity number ≥8.0) of biological replicates of the same group were equally pooled as one template for mRNA sequencing, according to previously reported standards (Williams et al. 2014). Five micrograms of the pooled templates were used for the transcriptome libraries constructions with the Illumina TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, USA), and sequenced by 200-bp pair-end strand-specific RNA-sequencing performed on an Illumina HiSeq2000 according to the manufacturer's instructions. In our study, we used Q20 as a sequencing quality

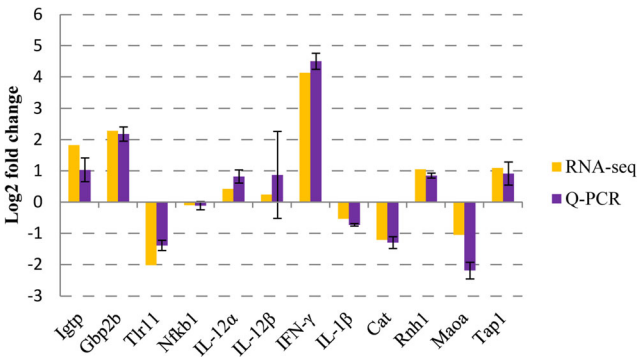

Fig. 1 Data of RNA-seq verified by Q-PCR. Twelve genes were selected randomly for evaluation of RNA-seq data

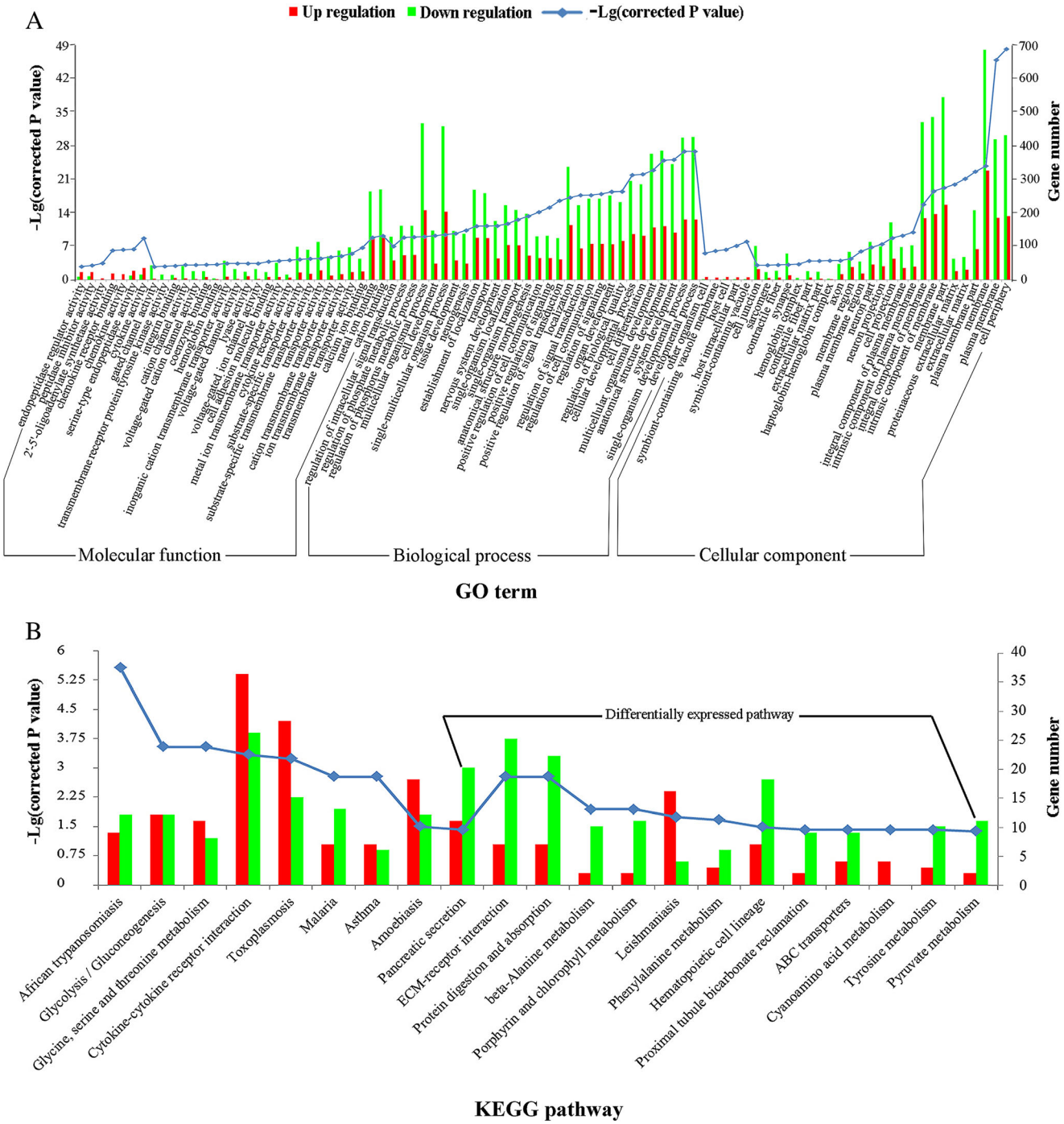

Fig. 2 Differentially expressed GO terms and enriched KEGG pathways. **a** Top 30 differentially expressed molecular functions, biological processes, and cellular components. **b** Enriched KEGG pathway. *Red bar* represent upregulation gene count, *green bar* represent downregulation gene count, *point plot* represent the—Lg (corrected *p* value) of GO term or pathway

control standard to filter low quality sequencing reads. After sequencing quality control and adapter filter, high quality clean reads were aligned to the mouse genome (mm10) with SOAPaligner/SOAP2. No more than five mismatches were allowed in the alignment. All RNA isolations, library constructions, RNA sequencing, and alignment, were performed at BGI-Shenzhen, China.

Identification and functional analysis of differentially expressed genes (DEGs)

The gene expression level was calculated by using the RPKM method (Mortazavi et al. 2008). DEGs were identified by using twofold change (log2 fold change ≥1 or ≤−1), statistical test referring to the significance of digital gene expression

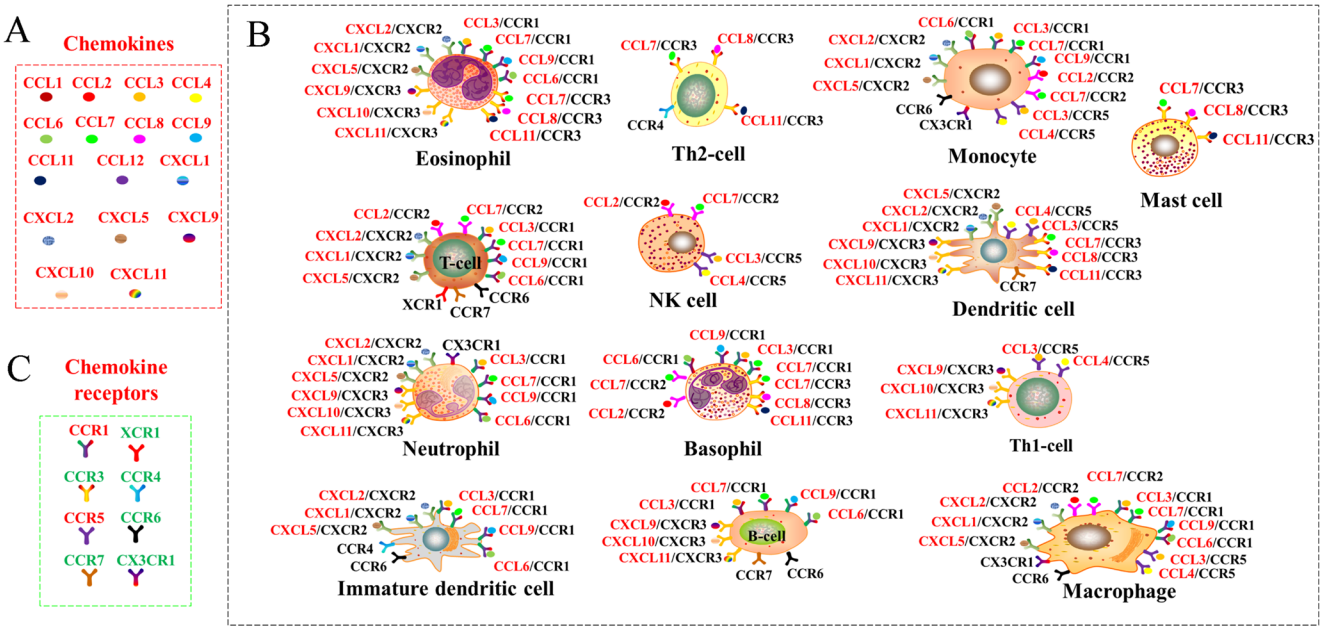

Fig. 3 Global chemokine and its receptor changes in mouse spleen infected by *T. gondii*. **a** Global chemokines altered by *T. gondii* infection in mouse spleen. **b** Predicting chemotaxis of immune cells altered by *T. gondii* infection in mouse spleen. **c** Global chemokine receptors altered by *T. gondii* infection in mouse spleen. *Red characters* represent upregulation, *green characters* represent downregulation, *black characters* represent no significantly change. The colors of chemokine receptors do not represent the receptor change in immune cells but show the RNA-seq result in present study only

profiles (Audic and Claverie 1997), and a 0.001 Benjamini and Hochberg False Discovery Rate (FDR) correction (Benjamini and Hochberg 1995) cutoff for the thresholds. GO database (http://geneontology.org/) was used to analyze the molecular function, biological process, and cellular component changes in the spleen that were associated with *T. gondii* infection. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) was used for pathway analysis. Enrichment analysis was performed to filter the DEGs that correspond to specific biological functions. This method firstly maps all DEGs to GO terms or pathways of KEGG database, calculating gene numbers for every term or pathway, then uses hypergeometric test to find significantly enriched GO terms or pathways in the input list of DEGs. The *p* value calculation method used is described as follow:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

N is the number of all genes with GO annotation or pathway; *n* is the number of DEGs in *N*; *M* is the number of all genes that are annotated to certain GO terms or pathways; *m* is the number of DEGs in *M*. The calculated *p* value goes through FDR correction (Benjamini and Hochberg 1995), taking the correction *p* value less than 0.05 as threshold. Differentially expressed GO terms or pathways were defined due to their significant enrichment which the ratio (upregulated gene count/downregulated gene count) was greater than 2 or less than 0.5.

Validation of gene expression by quantitative real-time PCR (Q-PCR)

Gene expression data was further verified by Q-PCR. The equally pooled RNA templates were reverse transcribed to cDNA using M-MLV Reverse Transcriptase (Promega) according to the manufacturer's instructions. Twelve genes were randomly selected for verification using Q-PCR and all reactions were performed on the ABI PRISM 7500 Sequence Detection System (Applied Biosystems) using SYBR Green Q-PCR SuperMix (Invitrogen). β-Actin was chosen as the endogenous reference and nucleotide-free water controls for each primer set were assayed to confirm no contamination or primer dimer present. Three replicates were analyzed for all selected genes. The qRT-PCR cycle conditions were as follows: 50°C 2 min; 95°C 2 min; 95°C 15 s, 60°C 32 s, 40 cycles; melt curve analysis was range from 60 to 95°C to ensured that a single product was amplified in each reaction. The primers used in the Q-PCR reactions are shown in Table 1. The relative expression calculation method was used to calculate gene expression as described by Livak and Schmittgen (Livak and Schmittgen 2001).

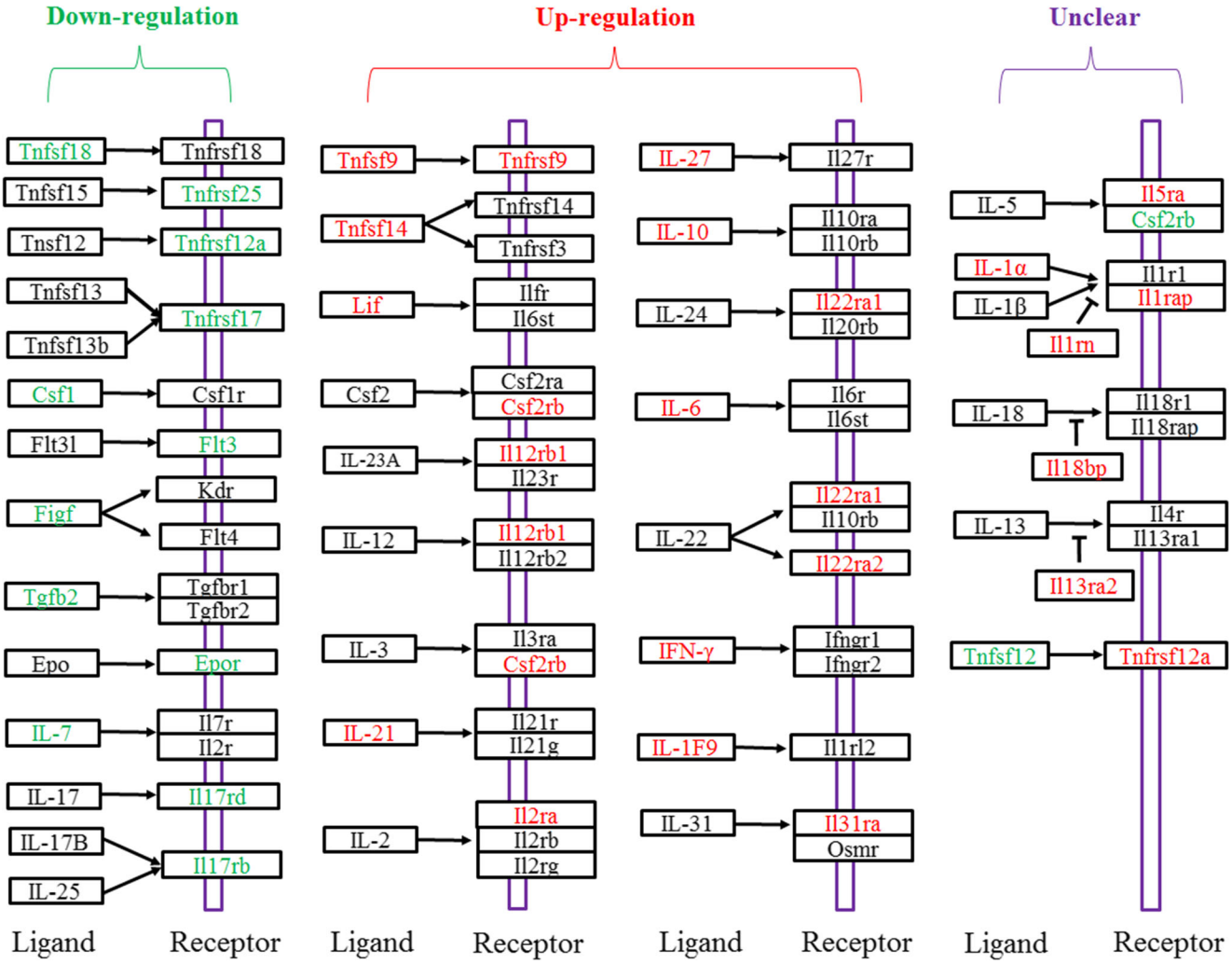

Fig. 4 Other cytokines and their associated receptors affected by *T. gondii* infection in the mouse spleen. *Red characters* represent upregulation, and *green characters* represent downregulation

Results and discussion

Transcriptomic features of mouse spleens following *T. gondii* infection

In our study, all RNA templates showed good quality (28 s/18 s≥1.5, RNA integrity number ≥8.8) for RNA-seq. Totally, 52,754,728 clean reads were obtained from infected mouse spleen (with 44,733,990 clean reads mapped to reference genome of mm10) and 51,313,176 clean reads (with 44,084,185 clean reads mapped to reference genome of mm10) obtained from the control. Although pool RNA strategy was used for RNA-seq, differentially expressed transcripts can be identified according to the statistical test and the Benjamini and Hochberg false discovery rate (FDR) correction as described in materials and methods section. The pooled RNA strategy may lose the information of biological variations among individual samples; however it is acceptable and used widely in scientific research (Kendziorski et al. 2005; Kommadath et al. 2014; Li et al. 2012; Williams et al. 2014; Xiao et al. 2010). In the present study, 2310 transcripts were differentially expressed between the uninfected and infected spleens. The RNA-seq results showed a significant host response to *T. gondii* infection compared with the uninfected controls which confirmed by Q-PCR experiments (Fig. 1).

The DEGs were functionally annotated based on gene ontology (GO) enrichment analysis (www.geneonttology.org). The top 30 differentially expressed GO terms of molecular function, biological process, and cellular component are shown in Fig. 2a. Most differentially expressed GO terms were downregulated, while GO terms related to immunity, such as chemokine and cytokine activity, were upregulated. To further elucidate the function of the DEGs in the mouse spleen after *T. gondii* infection, KEGG enrichment analysis was performed. Twenty-one pathways were significantly enriched, and 13 of them were differentially expressed (Fig. 2b). Most of the enriched pathways were downregulated, while the immunity-related pathways were upregulated.

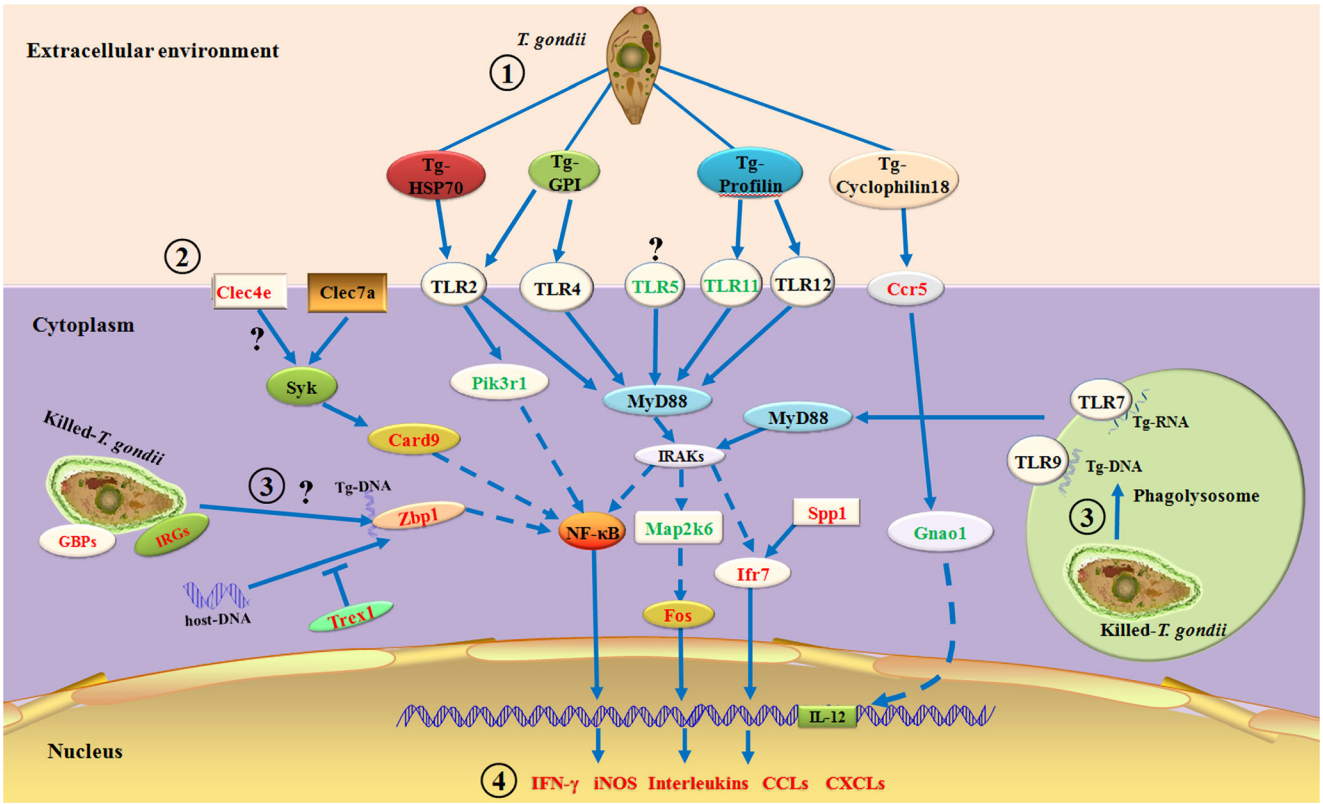

Fig. 5 Innate immunity pathways affected by *T. gondii* infection in mouse spleen. (*Circle digit one*) *T. gondii* proteins recognized by TLRs or Ccr5 on the cell surface. (*Circle digit two*) Upregulation of the Syk-Card9 signaling pathway during *T. gondii* infection. (*Circle digit three*) *T. gondii* is killed by interferon-inducible GTPases or phagolysosome. (*Circle digit four*) Cytokines were upregulated by TLR signaling and NF-κB pathway activation. *Red characters* represent upregulation, *green characters* represent downregulation, *dashed arrows* represent indirect interactions, and the *solid arrow* represents direct interactions, *question mark* represents whether involved in anti-*T. gondii* needs further study

Interestingly, most of the downregulated genes in the immunity-related pathway (cytokine–cytokine receptor pathway) were cytokine receptors. Due to the import role of cytokines in the response to *T. gondii* infection, we further investigated their regulation in more detail.

Alterations in chemokines and their receptors in mouse spleens after *T. gondii* infection

Chemokine signaling pathways play important roles in pathogen control and are responsible for attracting immune cells to infected sites (Moser and Loetscher 2001). In the present study, we found that all of the differentially expressed chemokines were upregulated, while most differentially expressed chemokine receptors were downregulated (Fig. 3). One chemokine can usually transfer the signals to several chemokine receptors expressed by immune cells (Onuffer and Horuk 2002). With this in mind, we retrieved the expression pattern of chemokine receptors based on a previous report (Onuffer and Horuk 2002) and used them to predict which immune cell types were more likely to be affected by the upregulation of chemokines. Most of the differentially expressed chemokine receptors were downregulated in present study, we could not identify whether the downregulation happened in immune cells for whole spleen RNA-seq strategy, while it seems to influence the chemotaxis weekly for multiple chemokines/chemokine receptor pairs expressed on immune cells. As shown in Fig. 3, the chemotaxis of neutrophils, eosinophils, monocytes, dendritic cells (including immature dendritic cells), macrophages, NK cells, basophils, and B and T cells were enhanced due to upregulation of chemokines. This implies that these signaling pathways and immune cells play important roles in protecting the mouse spleen from *T. gondii* infection.

Other cytokines and their receptors were classed into three subsets (up, down, and unclear subsets) according to their expression status (Fig. 4). Most tumor necrosis factor (TNF) signaling pathways were downregulated; this included the Tnfsf18, Tnfrsf25, and Tnfrsf17 signaling pathways. By contrast, the Tnfs9 and Tnfsf14 signaling pathways were upregulated. Tnfs9 signaling controls memory $CD8^+$ T cell generation in a pathogen-dependent manner (Zhao and Croft 2012). Tnfsf14 signaling can activate the NF-κB pathway through binding to Tnfrsf3 or Tnfrsf14 that leads to upregulation of

Th1 cytokines (Shaikh et al. 2001). Although experimental validation is required, these results imply that Tnfs9 and Tnfsf14 signaling may be important for the defense against *T. gondii*, which has previously not been reported. In addition, Tnfsf14-inducing IFN-γ is IL-12-independent (Brown et al. 2005) which can avoid the depressing IL-12 activity of type I *T. gondii* strain.

During *T. gondii* infection, a large fraction of IFN-γ is IL-12-dependent (Gazzinelli et al. 1994). In the present study, IL-12 was not significantly differentially expressed (the log2 of fold changes of IL-12p35 and IL-12p40 were 0.4167 and 0.2414, respectively). However, IL-12p35 or IL-12p40 was reported to be upregulated in mice spleen in previous reports (Gazzinelli et al. 1993; Nguyen et al. 2003). For example, in previous reports, the authors test IL-12p35 and IL-12p40 expression in BALB/c mice spleens by reverse transcription-polymerase chain reaction with electrophoresis analysis (Gazzinelli et al. 1993). PCR can amplify its template greatly and electrophoresis analysis is a kind of relative quantification method. So, the inconsistencies may be the results of using different mouse strain, technology, and differential expression standard.

Although IL-12 was upregulated slightly in the present study, according to our transcriptomics data, one key component of IL-12 signaling pathway, IL-12 receptor (Il12rb1), was upregulated. Upregulation of Il12rb1 enhances the binding affinity between IL-12 and its receptor (Wu et al. 1997) and then may improve IFN-γ production, even in the condition that IL-12 was slightly upregulated. In human being, Il12rb1 is associated with malaria susceptibility (Sortica et al. 2012); however, the report about the Il12rb1 in *T. gondii* susceptibility is few. So, the study of Il12rb1 expression or gene polymorphisms could be promising for the research of genetic resistance against acute toxoplasmosis (Suzuki et al. 1995).

That IFN-γ exerts its activities through inducing upregulation of IDO, iNOS, IRGs, and GBPs has been described previously. Although one IDO (Ido1) was upregulated in the present study, we do not believe that IDO-induced tryptophan degradation contributes significantly to the control of *T. gondii* infection. This is because our transcriptomic data revealed that the DEGs involved in tryptophan metabolism pathways (according to KEGG database) were downregulated (Fig. S1). In contrast with Ido1, IFN-γ-induced iNOS (Nos2) and interferon-inducible GTPases were upregulated (Fig. S2). It suggested that IFN-γ induced iNOS (Nos2) and interferon-inducible GTPases contribute more than IDO to the defense against *T. gondii*.

An excessive inflammatory response is detrimental to host health and should be regulated by an immune system balancing mechanism (Gazzinelli et al. 1996). Consistent with this, we observed that negative regulators of the inflammation process were upregulated. IL-27 exhibits anti-inflammatory activity by stimulating $CD4^+$ T cells to produce IL-10, and then subsequently inhibits the functions of Th1, Th2, and Th17 subsets of T cells (Stumhofer and Hunter 2008). In the present study, both IL-27 and IL-10 were upregulated. In contrast, some positive regulators of inflammation decreased, as shown in the "downregulation" subset in Fig. 4. For example, downregulation of IL-17 signaling pathway is known to contribute to the protection against fatal inflammation caused by *T. gondii* infection (Guiton et al. 2010).

Innate immunity pathways alterations in the mouse spleen affected by *T. gondii* infection

In order to analyze the alterations of innate immunity pathways in the infected spleen, we retrieved the altered innate immunity pathways from KEGG database as shown in Fig. 5.

Once *T. gondii* attaches to host cell membranes, TLRs recognize parasite proteins such as Tg-GPI (Debierre-Grockiego et al. 2007), Tg-HSP70 (Mun et al. 2005), and Tg-Profilin (Raetz et al. 2013) that activate the NF-κB or other pathways and then initiate the expression of cytokines such as chemokines, interleukins, and IFN-γ. Interestingly we found that TLR11, which recognizes Tg-Profilin (Raetz et al. 2013), was downregulated; TLR5 was also downregulated, but its role in *T. gondii* infection is unclear. When the parasite was killed by IFN-γ inducible GTPases or phagolysosomes, components of *T. gondii*, including Tg-DNA or Tg-RNA, were released (Andrade et al. 2013). These exogenous *T. gondii* nucleic acids can be recognized by host exogenous nucleic acid sensing receptors, such as TLR7 and TLR9 (Andrade et al. 2013). In present study, we did not find altered expression of TLR7 or TLR9. However, another nucleic acid sensing pathway (the Zbp1 DNA-sensing pathway) was upregulated (Fig. 5). Zbp1 can initiate innate immune responses by binding cytosolic DNA (Wang et al. 2008). However, there are few reports regarding the contribution of Zbp1 to NF-κB activation in the spleen of mice infected by *T. gondii*. Trex1 can prevent autoimmunity caused by host DNA damage (Stetson et al. 2008), and host DNA damage induced by *T. gondii* infection has been confirmed (Ribeiro et al. 2004). Thus, upregulation of Trex1 could prevent an excessive inflammatory response that would otherwise result from the DNA damage induced by *T. gondii* infection (Ribeiro et al. 2004).

The C-type lectins-Syk-Card9 signaling pathway is a pattern recognition pathway that links the innate and adaptive immune systems (Brown 2005; LeibundGut-Landmann et al. 2007; Rogers et al. 2005). In the present study, we found that this pathway was altered by upregulation of Card9 and Clec4e (Fig. 5). CARD9 is a key downstream component of C-type lectins-Syk-Card9 pathway and required for innate and adaptive immunity (Hara and Saito 2009). Clec4e (also known as Mincle) is a C-type lectin receptor that induces inflammatory cytokine production via activation of the NF-κB pathway and then attracts immune cells to sites of pathological cell death (Yamasaki et al. 2008). Clec7a (also known as Dectin1),

another C-type lectin receptor, was not differentially expressed in our study, while its function in anti-*T. gondii* infection in Kunming mouse has been confirmed (Yan et al. 2014). Very recently, another report showed that BpLec, a C-type lectin isolated from *Bothrops pauloensis* snake venom, can reduce the parasitic activity of tachyzoites of the RH strain (Castanheira et al. 2015). In light of these data, we recommend that upregulation of C-type lectins-Syk-Card9 signaling contributes to anti-*T. gondii* infection in mouse spleen.

In summary, this is the first study of global cytokine expression alterations in the mouse spleen following acute infection by *T. gondii*. Dozens of cytokine pathways of mouse spleen were altered by *T. gondii* infection. Most cytokines were upregulated in infected spleens and immune cells chemotactic chemokine activities were enhanced to promote recruitment of immune cells to infected spleen. We also found that IFN-γ-induced iNOS (Nos2) and interferon-inducible GTPases may contribute more anti-*T. gondii* activity than IDO does. Host innate immune signaling pathways were not affected dramatically by *T. gondii* infection, while the cytosolic DNA sensing pathway and Sky-Card9 signaling pathway were upregulated in infected spleens. Our study will provide useful clues to find out the crossroads of innate and adaptive immunity during *T. gondii* infection.

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Compliance with ethical standards

Ethical statement All animals were handled strictly according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was reviewed and approved by the Animal Ethics Committee of Lanzhou Veterinary Study Institute, Chinese Academy of Agricultural Sciences.

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